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FOREWORD

I am pleased to put into the hands of readers Volume-4; Issue-2: Mar-Apr 2019 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: May, 2019

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Biology of Cotton Mealybug, *Phenacoccus solenopsis* (Tinsley) on some Vegetable Crops in the Gazira State, Sudan

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***Abstract*—** *The recent out-break of cotton mealybug, Phenacoccus solenopsis (Tinsley) (Hemiptera: Pseudococcidae) on many crops belong to different families making a real worry for both farmers and research scientists. The objective of this study was to determine total life cycle of the cotton mealybug from the first instar to the death of adult. The study focused on the biology of P. solenopsis (Tinsley) on tomato and eggplant under laboratory conditions at the Entomology Section, Agriculture Research Corporation (ARC), Sudan during the period from January to March 2016. The results showed that the developmental period for the 1st ,2nd and 3rd instars, adult female longevity and duration of life cycle were recorded as 4.5±1.29, 6.6±2.07, 6.0±2.16, 3.2±1.31, 3.7±1.77, 2.9±1.12 and 26.9±9.72 days respectively, on tomato and 6.0±1.56, 9.5±2.87, 10.3±3.29, 3.0±0.9, 4.33±1.72, 2.66±0.85 and 35.79±11.22 days respectively, on eggplant. That means the life cycle of mealybug on eggplant was longer more than on tomato*

***Keywords*— Biology of cotton mealybug, Eggplant, Phenacoccus solenopsis (Tinsley), Tomato.**

I. INTRODUCTION

Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera : Pseudococcidae) has been reported from 35 localities of various ecological zones of the globe [1]. It has a wide range of variation in morphological characters, biological adaptation and ecological adjustability [2]. *P. solenopsis* has been shown to be sexually dimorphic, having short life cycle winged males and longer-lived, wingless females. A survey was made in the Gezira and Khartoum states showed at least 26 host plant species belonging to 16 plant families were infested with citrus mealy bug (CMB) and reported from Gezira, Khartoum, Sennar, Gedarif, Kassala, White Nile and Blue Nile, Northern state and River Nile states, and the mealybug was identified as *P. solenopsis* (Tinsley) [3].

It was found to reproduce sexually, producing off-springs instead of laying eggs. The eggs are retained in the body until they are ready to hatch, a phenomenon known as ovoviviparity [4]. The study concluded that *P. solenopsis* occurred more commonly on the roots, stems and foliage close to the soil line in dry climates compared to settling on the upper foliage of the plant in more humid areas. The feeding habit of mealybug causes yellowing, defoliation, reduces plant growth and finally plant death at sever stage [5].The objective of this study was to determine the developmental stages, pre-oviposition, oviposition, post-oviposition and life span of cotton mealybug *P. solenopsis* (Tinsley) on tomato and eggplant.

II. MATERIAL AND METHODS

This study was conducted at Entomology Section, Crop Protection Research Center, Agriculture Research Corporation (ARC), Gezira state, Sudan during January – March 2016. The laboratory has an average temperature range between 25 - 45ºC and the relative humidity ranging between 20 - 40%. Special leaf or plant cage were used. These cages made from disposable plastic containers, in each cage a leaf of tomato or eggplant was placed with one adult female. This was replicated fifteen times. Cages were observed daily for egg-laying during the life span of the adult female.

The same individual adult in the cage was shift to another leaf in the same leaf (eggplant) or branch (tomato) cage, daily after counting the ovisacs, nymph observed. Soft camel hair brush and the eye - lens of binocular microscope (5x) were used in counting the neonates of the mealybug. First, the adult was removed gently by the brush to a new leaf and the counted individuals of neonate were excluded gently by using the brush and the magnifying lens. The number of ovisacs observed and nymphs were recorded and subjected to analysis.

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The number of eggs per ovisac for each insect was recorded using binocular microscope (5x) and replicated over one hundred times.

Five newly hatched crawlers of mealybug (first nymphal instar) were enclosed in a separate leaf cage of tomato or eggplant with 20 replicates. The nymphal period was observed every 24hrs using a magnifying hand lens (5x). The nymph was considered passing to another stage when a change in size, presence of exuvia or deposition of wax was observed.

The newly hatched crawlers were reared to the adult stage which determined by the sign, presence of exuvia or deposition of wax, then the total life span for the adult was monitored.

Collected data were subjected to descriptive analysis using Microsoft Excel.

III. RESULT AND DISCUSSION

3.1. Determination of ovisacs/female

Table (1) shows the number of ovisacs laid by each of the 20 females. The results showed that each female had formed only one ovisac during its entire life time.

Table (1) Number of ovisacs laid by each female of *P. solenopsis* on tomato and eggplant

Female experimented	Number of ovisac laid by female
1	1
2	1
3	1
4	1
5	1
6	1
7	1
8	1
9	1
10	1
11	1
12	1
13	1
14	1
15	1
16	1
17	1
18	1
19	1
20	1
Total	**20**
Mean	**1**

3.2. Number of nymphs / ovisack

Table (2) shows the number of nymphs/ovisac on both host plant i.e. tomato and eggplant. Among the 130 ovisacs tested, the highest mean number of nymphs/ovisac was found to be 459 nymphs/ovisac, while the lowest mean number was 70 nymphs/ovisac with an average of 167 nymph/ovisac i.e. 21715 nymph observed in 130 ovisacs, lowest and highest mean number/ovisac was 70, 459 and 167, respectively. Also this table illustrated that more than 38% of the examined an ovisacs contain 100–200 nymphs, while 6% of them contain 400–500 nymph. That means the nymphs laying capacity of this species was high i.e. 100-200 or 400-600 nymphs/ovisac when compared with the previous results obtained by Radadia *et al*., (2008) who found the mean 150-600 egg/ovisac and the total number of egg/female 310-625 with mean of 470 eggs/female by Tanwar *et al*., (2007).

Table.2: Number of nymphs / ovisac of cotton mealybug P. solenopsis on tomato and eggplant

Number of nymphs / ovisac	Ovisac		Total	Mean
	Number	%		
0 - 100	43	33.1	3038	70.6
101- 200	50	38.5	7252	145
201 – 300	20	15.4	4658	232.9
301 – 400	9	6.9	3091	343.4
401 – 500	8	6.1	3676	459.5
Total	**130**	**100**	**21715**	**1251.4**
Average nymphs / ovisac			**167**	

Number and life time of nymphal instars

Table (3) shows that the CMB has only three nymphal instars. The periods (days) taken by the first, second and third nymphal instars were (4.5±1.29, 6.0±1.56), (6.6±2.07, 9.5±2.87) and (6.0±2.16, 10.3±3.29) on tomato and eggplant, respectively. Also hereunder appendices and Table (4) show the pre-oviposition, oviposition, post ovipositon and total life cycle as (3.2±1.31, 3±0.90), (3.7±1.77, 4.33±1.72), (2.9±1.12, 2.66±0.85) and (26.9 ±9.97 , 35.79±11.22) on tomato and eggplant, respectively. The time required for growth and development was longer on eggplant compared to tomato. However similar results were shown by Sharma (2007) who found that the period of development from crawler to adult stage is approximately 25-30 days, depending on the weather and temperature. Mean development period were 4.9±1.4, 4.5±0.6 and 5.9±1.1 days, respectively for the first, second and third instars of female and 15.3±1 days mean development from crawler to adult stage (Mohammed, 2015). While the same trend was observed for the pre-, post- and oviposition period but with low rate. The results of this study here were similar to that was obtained by Dhawan and Saini (2009), who were found that the female of CMB have three nymphal instars, the first instar nymph lasted for 4-6 days, the second was also 4-6 days and the third instar nymph lasted 5-7 days and the adult female period lasted for 13-17 days, while

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the pre-oviposition, oviposition and post- oviposition were 3-5, 8-9 and 2-3 days, respectively.

4. Life time of the adult female

Tables 4 and 5 show that the duration of the pre-oviposition, oviposition and post-oviposition period of 31 studied adult females of cotton mealybug on tomato and 20 ones on eggplant. The pre-oviposition period was determined starting with completion of the third instar stage to the appearance of the ovisac out of the female ovipositor.

Table.3: Period (days) required for growth and development for various stages of P. solenopsis on tomato and eggplant

Stage	Days (Mean± SD)	
	Tomato	**Eggplant**
First instar	4.5±1.29	6.0±1.56
Second instar	6.6±2.07	9.5±2.87
Third instar	6.0±2.16	10.3±3.29
Pre-oviposition	3.2±1.31	3.0±0.90
Oviposition	3.7±1.77	4.33±1.72
Post-oviposition	2.9±1.12	2.66±0.85
Total life cycle	26.9±9.72	35.79±11.21

Table.4: Pre-oviposition, oviposition and post-oviposition period (days) of P. solenopsis on tomato

Number of adult female studied	Pre oviposition period (days)	Oviposition period (days)	Post ovipositon period (days)
4	2	3	2
1	2	7	2
1	2	3	7
2	2	5	3
1	2	4	2
1	2	2	3
1	2	6	2
2	3	5	2
2	3	7	3
2	3	1	3
1	3	1	4
3	3	3	2
1	4	3	2
1	4	5	3
2	4	2	2
1	4	3	4
2	5	3	2
2	5	4	3
1	5	3	5
Total	**60**	**70**	**56**
Range	1-5	1 - 7	2 – 7
Mean ± SD	3.2±1.31	3.7±1.77	2.9±1.12

IV. CONCLUSION

From the above results, the following conclusion was obtained; firstly, the cotton mealybug, *Phenacoccus solenopsis* ***(Tinsley)***, has three nymphal instars, and the developmental stages need more time on eggplant than on tomato. Secondly, each female of cotton mealybug lays only one ovisac during its entire life span. The total life span of Cotton mealybug on tomato and eggplant completed in 26.9±9.7 and 35.7±11.2, respectively.

Eventually, tomato and eggplant not be intercropped when the cotton mealybug was expected to occur during the season or even subsequent and/or overlapping each other in the cropping system.

Table.5: Pre-oviposition, oviposition and post-oviposition period (days) of P. solenopsis on eggplant

Number of adult female studied	Pre oviposition period (days)	Oviposition period (days)	Post oviposition period (days)
1	2	7	2
1	2	6	2
1	2	3	2
2	2	3	3
3	2	5	3
1	3	4	2
1	3	3	5
1	3	6	2
1	3	2	3
1	3	3	2
3	4	4	3
1	4	4	2
1	4	8	2
1	4	3	3
1	4	4	4
Total	**45**	**65**	**40**
Range	2-4	2-8	2-5
Mean ±(SD)	3±0.90	4.33±1.72	2.66±0.85

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Giving of Biochar and Biocompost to Growth, Results and N Uptake Soybean Plants (Glycyne Max (L) Merr.)

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Abstract**—** *The use of biochar and biocompost can be one solution to the addition of organic matter in soybean cultivation. This study aims to obtain a good description of the role of biochar and biocompost on growth, yield, and N uptake. The results of the study show that: (1) Application of biochar in soybean plants affects plant height, number of vacuous pods, number of pods containing, weight 100 seeds, and N uptake compared with no biochar application. (2) Biocompost application has a significant effect on the height of soybean plants aged 2,3, 4 and 5 weeks after planting, dry weight, seed weight, number of vacuous pods and number of pods filled. (3) Biocompost with a liquid form dose of 2.5 grams/plant significantly affected the number of filled pods and the number of soybean pods. (4) Liquid biocompost dose of 5 grams/plant affects dry weight and plant height of 2 MST. While the 7.5 gram/plant liquid biocompost has an effect on the weight of soybean seed plants.*

Keywords**— Biochar, Biocompost, and Soybeans.**

I. INTRODUCTION

Soybean (Glycine max L) is one of the food plants that plays an important role in Indonesia. Annual domestic soybean consumption is estimated at 2.6 million tons to 2.7 million tons. Soybean production in Indonesia alone has not been able to offset these needs (Reily, 2018).In NTB the harvest area and soybean production from 2015-2017 also decreased, which in 2015, soybean production reached 125,036 tons/ha with a planting area of 94,948 ha experiencing a drastic decrease in production to 56,097 tons/ha with an area of 43,149 ha in 2017. Soybean yield potential (tons/ha) in NTB also decreased from 1,316 tons/ha (2015) to 1.3 tons/ha (2017) (Kementerian Pertanian, 2017). This decrease in production is caused by various factors such as soil factors, climate, pests and diseases, and poor methods of land management. Therefore we need a variety of technological innovations that can increase soybean production, one of which is through land intensification. Efforts that can be made to increase the production output both the quality and quantity of soybean production are by adding organic matter in the soil.

Furthermore, the use of biochar and biocompost can be an alternative problem solver in soybean cultivation. Biochar is a material made from the pyrolysis process of organic matter and when deposited on the soil can function as a provider of soil carbon for a long time (Quiliam et al, 2013). From various research results, biochar combined with fertilizer can also increase soil nutrients in it which increases soil biota and nitrogen (N) and phosphorus (P) uptake (DeLuca et al, 2009).

Biocompost itself is compost made with the help of lignocellulolytic microbes that persist and act as controlling agents for plant diseases and decomposers of organic matter (Mastur, 2014). Sudantha (2007) reported that T.harzianum saprophytic fungi SAPRO-07 isolates and T. koningii endophytic fungi ENDO-02 isolates were local superior microbial NTB as decomposers of organic matter.

The interaction of the influence of both is not well known. To obtain a good description of the role of biochar and biocompost on growth, yield and N uptake, this research needs to be carried out in depth. Therefore, the authors are interested in conducting a study of "Biochar and Biocompost Giving Responses to Growth, Yield and Absorption of Soybean Plants (Glycyne Max (L) Merr.)".This study aims to determine the effect of biochar and biocompost administration on growth, yield and N uptake of soybean crop production on dry land.

II. METHOD

The ingredients used include: Anjasmoro variety soybean seeds, biochar ingredients, biocomposite ingredients, and Trichoderma spp. The research was carried out in the village of Montong Are, Kediri District.

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III. RESULTS AND DISCUSSION

Biochar Fermentation Trichoderma Application TestPlant height

Table.1: Average soybean plant height due to biochar administration at 2,3,4 and weeks after planting (MST).

Treatment	Plant Height (cm)			
	2 MST	3 MST	4 MST	5 MST
With biochar	15,07 a*)	19,10 a*)	25,09 a*)	31,14 a*)
Without biochar	13,96 b	17,74 b	22,49 b	28,60 b
BNJ 5%	0,44	0,51	0,53	0,41

Based on Table 1, the effect of biochar has a significant effect on the height of soybean plants. The average height of soybean plants with the application of biochar was 15.07 cm, whereas without biochar it was 13.96 cm in the parameters of plant height at 2 MST.Plant height at the age of 3 MST showed the average height of soybean plants with the application of biochar was 19.10 cm, whereas without the application of biochar it was 17.74 cm.

The parameters of plant age 4 MST showed that the average height of soybean plants with the application of biochar was 25.09 cm, whereas without the application of biochar it was 22.49 cm. The parameters of plant height 5 MST showed the average height of soybean plants with the application of biochar was 31.14cm, while those without biochar were 28.60 cm.

Biochar can act as a trigger for plant growth and retain nutrients in the soil so that nutrients in the soil are not easily lost in the washing process in the soil and will ultimately affect the increase in yields (Lehmann et al., 2003).In addition, this Trichoderma fermented biochar provides macro nutrient and micro nutrient requirements, containing humic acid (humus) increasing soil cation exchange capacity, increasing soil microorganism life and can help increase soil pH (Lelu et al., 2017). Especially in N nutrient uptake which is important in supporting the growth of soybean plant height. Where according to Lingga and Marsono (2006), that the role of N is to accelerate overall growth, especially stems and leaves.

Number of vacuous pods and contains

Table.2: Average number of soybean pods with application of biochar fermented Trichoderma spp.

Treatment	Amount of vacuous Pods (fruit)	Number of pods containing (fruit)
With biochar	6,58 a*)	32,82 a*)
Without biochar	5,37 b	30,46 b
BNJ 5%	51,13	1,912

Based on Table 2, biochar has a significant effect on the number of soybean empty pods where without the biochar application the highest number of empty pods is 6.58 pods. Whereas soybean plants applied by Trichoderma spp mushroom fermentation biochar have a low number of empty pods which are only 5,37 pods.

Giving of biochar also has a significant effect on the number of pods containing soybeans. Soybean plants with biochar have the highest number of pods, 32.82 pods. While soybean plants without the application of Trichoderma spp biochar fungal fermentation have a number of pods containing low which is 30.46 pods.

The addition of biochar itself can significantly increase plant growth and important nutrient uptake during flowering (Steiner, et al, 2008). It can also accelerate the flowering time of soybean plants faster and increase the number of filled pods (Sudantha, 2011). Biochar can spur soil biological functions by providing a growing habitat for soil microorganisms that have an effect on nutrient availability and enzymes (Gomez, et al, 2014). Because the condition around the plant becomes more porous, it is very possible the absorption of available nutrients and inorganic nutrients that affect the process of forming soybean pods (Thies and Rillig, 2009).

Biochar can spur soil biological functions by providing a growing habitat for soil microorganisms that have an effect on nutrient availability and enzymes (Gomez, et al, 2014). Because the condition around the plant becomes more porous, it is very possible for absorption of available nutrients and inorganic nutrients to influence the process of forming soybean pods (Thies and Rillig, 2009).

Weight of 100 seeds

Table.3: The average weight of 100 seeds of soybean plants with the application of fermented Trichoderma spp.

Treatment	Weight of 100 seeds (g)
With biochar	17.98 a*)
Without biochar	17,04 b
BNJ 5%	0,772

Based on Table 3, shows that the treatment of Trichoderma spp biochar fermentation has a significant effect and can improve the quality of soybean seed plants. This can be seen from the weight of 100 seeds treated with

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biochar. The average weight of 100 seeds is highest in the treatment with biochar, which is 17.85 grams and the lowest is in the control treatment (without biochar), which is 16.71 grams.

Improving the quality of soybean seeds due to the addition of Trichoderma spp fermented biochar is due to the ability of biochar to help fulfill the nutrients needed by plants for the vegetative and generative phases. Lingga (2003) explains that the nutrients needed by plants are available in sufficient quantities, the metabolic results will increase. Of course cell division, elongation and maturation of tissue becomes more perfect and fast so that the increase in volume and weight is getting faster which in the end plant growth will be better.

N Uptake

Table.4: Average N uptake of soybean plants with the application of fermented Trichoderma spp.

Treatment	N uptake (%)
With biochar	1.242 a*)
Without biochar	0.472 b
BNJ 5%	0,096

In table 4, it can be seen that biochar has a significant effect on soybean N uptake in the amount of 1.242%. Compared with without biochar application which only shows N uptake of 0.472%.N uptake in soybean plants is also strongly influenced by the addition of fermented biochar. This is consistent with the statement of Zwieten et al (2010) that the addition of biochar significantly increases N uptake with fertilizers in ferrosol soil. This is because biochar has a high absorption area, high porosity and various content of organic material which has the potential to increase cation exchange capacity, and absorption capacity when added to the soil (Rajakumar and Sankar, 2016).

Biokompos Fermentation Trichoderma Plant High Application Test

Table.5: Average height of soybean plants as a result of the influence of biocompost at ages 2,3,4, and 5 MST

Treatment	Seed weight per plant (g)
with biocompost 7,5 gram liquid/plant	10,40 a*)
with biocompost 2,5 gram liquid/plant	10,00 a
with biocompost 5,0 gram liquid/plant	9,81 a
with biocompost 7,5 gram liquid/plant	9,78 b
with biocompost 2,5 gram liquid/plant	9,02 b
with biocompost 5,0 gram liquid/plant	8,96 b
Without biocompost	7,23 c

Based on table 7, soybean plants without biocompost application have a significant effect on the weight of soybean seeds, which is weighing 7.23 grams. The application of biocompost in the form of a liquid that is a dose of 7.5 grams / plant has the largest seed weight of 10.40 grams and the lowest weight of soybean seeds obtained in biocompost treatment in granular form with a dose of 5 grams / plant weighing 8.96 grams.

Of course the administration of biocompost is very appropriate because it can increase nutrient availability, increase nutrient efficiency and take nutrients during fertilizer application (Agegnehu et al, 2015; Jeffery, et.al, 2011).

Rizqiani et al (2007) stated that absorbed elements can be used to encourage cell division and the formation of new cells to form better organ organs such as leaves, stems and roots so as to facilitate photosynthesis.

Dry Sturdy Weight

Table.8: Average dry weight of soybean plants by application of biocompost fermented Trichoderma spp

Treatment	Dry weight (gr)
with biocompost 5,0 gram granules/plants	11,55 a*)
with biocompost 7,5 gram granules/plants	10,54 a
with biocompost 7,5 gram granules/plants	10,48 a
with biocompost 2,5 gram granules/plants	10,43 a
with biocompost 2,5 gram granules/plants	10,43a
with biocompost 5,0 gram granules/plants	9,8 b
Without biocompost	7,23 c

Based on table 8, soybean plants without the application of biocompost to soybean plants have no significant effect on the dry weight of soybean plants which is 7.23 grams. The application of biocompost in the form of liquid with a dose of 5 grams / plant has the highest dry weight, which is weighing 11.55 grams and the lowest

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weight weight is obtained in biocompost treatment in granular form with a dose of 5 grams / plant weighing 9.8 grams.

The difference in dry weight between treatments without biocompost and given biocompost application shows that biocompost is able to provide sufficient amounts of nutrients needed. Lingga (2003) explains that if nutrients are sufficient for plants, metabolic synthesis will increase.

Addition of soil organic matter such as biocompost from Trichoderma spp. this is very influential on the absorption of nutrients in soybean plants. This Trichoderma spp mushroom serves as a source of nutrients for plants and energy sources for soil organisms and helps plants to grow and develop better (Sudantha, 2010a).

IV. CONCLUTION

1. The application of biochar on soybean plants affects plant height, number of empty pods, number of pods containing, weight of 100 seeds, and N uptake compared with without biochar application.
2. The biocompos application has a significant effect on the height of soybean plants aged 2.3, 4 and 5 weeks after planting, dry weight, seed weight, number of empty pods and number of pods filled.
3. Biocompost with a liquid form of 2.5 grams/plant has a significant effect on the number of filled pods and the number of soybean pods. Liquid biocompost dose of 5 grams / plant has an effect on dry weight and plant height at 2 MST. While the 7.5 gram/plant liquid biocompost has an effect on the weight of soybean seed plants.
4. Biocompost granules of 7.5 gram dosage / plant plants have an effect on plant height aged 3 and 5 MST. While the dose of 2.5 grams of granules / plants affects the height of soybean plants aged 4 MST.

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Trinuclear Oxovanadium Complexes of Doxycycline: Synthesis, Characterization and Antiplasmodial Studies

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Abstract*—** *Variable oxidation states of vanadium and strong binding ability of doxycycline have been exploited to synthesize three new oxovanadium complexes of doxycycline. The structures of the new complexes were validated by elemental analysis (C, H, N) and FTIR spectroscopy. The ratio of oxovanadium to doxycycline is 1:3 in all the three complexes. Doxycyclinecoordinates tothe first and second vanadium using phenolic oxygen and nitrogen atoms at ring A as well as the amide and keto oxygen atoms of ring A. The third vanadium binds to phenolic and keto oxygen atoms on rings B and C to form complex* ***1*. 2,2-bipyridine and 1,10-phenanthroline coordinate to the third oxovanadium replacing the two aqua ligands attached to it in complexes* ***2*** *and* ***3*** *respectively. Oxovanadium forms 5-coordinate complexes in all the three complexes.Antiplasmodial studies showed that complex* ***2*** *have comparable activity with the parent drug, doxycycline, while all three complexes have higher activities than lincomycin.*

***Keywords*— *doxycycline; iron(III); diimine; antibacterial; DNA Binding; antiplasmodial*.**

I. INTRODUCTION

Vanadium is a trace element which is beneficial and possibly essential in humans[1] but certainly essential for some organisms.[2-8] Vanadium exists in many oxidation states: –3, –1, 0, + 1, + 2, + 3, + 4, and +5 although thermodynamically and kinetically possible oxidation states under physiological conditions are + 5, + 4, and +3. The most notable action of vanadium ion and vanadium compounds is their insulin-mimetic activityand ability to reduce blood sugar levels from high to normal though the oxidation state most relevant to insulin action has not been established.[9]

A monoperoxovanadate(V) complex, oxoperoxopicolinatovanadium(V) dehydrate [mpV(pic)] (**Figure 1**), has been shown to achieve a 20% decrease in plasma glucose in STZ-diabetic rats when administered by intraperitoneal or subcutaneous injection.[10] Vanadium has also been shown to have some ability to lower cholesterol levels and blood pressure at low doses in humans.[9]

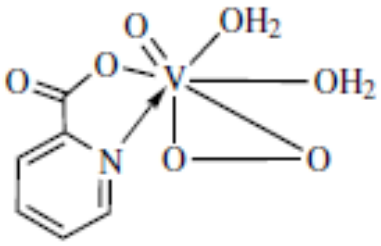

Fig.1: Oxoperoxopicolinatovanadium(V) [mpV(pic)] with pentagonal bipyramidal geometry

Vanadium ions have many structural roles shown by its structural and electronic analogy to phosphorus.[8,10] It is an enzyme co-factor[1] and is found in certain tunicates[9,10,11]and possibly mammals. $VOSO_4$ has been reported to be a potent inhibitor for *Escherichia coli* alkaline phosphatase[12,13] and the aqueous V^{IV} chemistry has been described in detail to explain this phenomenon.[14] The most well-known V^{IV} species is the vanadyl cation (VO^{2+}-cation, $[VO(H_2O)_5]^{2+}$(**Figure 2**).[15-17]

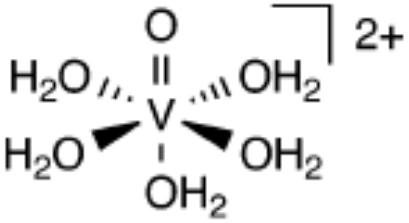

Fig.2: Vanadyl cation, the most common in aqueous solution

The CT DNA, protein binding (bovine serum albumin), DNA cleavage and cytotoxic activities of chiral V(V) schiff base complexes, (S)-[VO(OMe)L] and (R)-[VO(OMe)L] (**Figure 3**), have been reported. Both enantiomers of the same complexshowed efficient groove or surface binding with DNA, the (R)-[VO(OMe)L] enantiomer exhibiting stronger DNA binding affinity ($5 \pm 1 \times 10^5$ M^{-1}) than its S, enantiomer ($8 \pm 1 \times 10^4$ M^{-1}). The

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R enantiomer efficiently cleaved the DNA in the presence of white fluorescence light via mechanistic pathway that involves the formation of singlet oxygen. The R enantiomer also displayed stronger BSA binding and cytotoxic activity.[18]

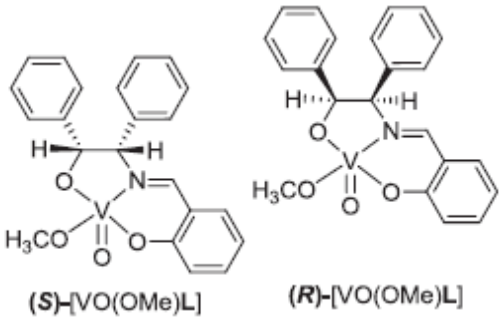

Fig.3: Vanadium(V) schiff base complexes

Chakravarty and co-workers have shown that heteroleptic oxovanadium(IV) compounds with salicylidene and N,N-heterocyclic ligands like phen, dpq, or dppz coordinated to the metal e.g. [VO-(sal)(phen)] (**Figure 4**) bind to double-stranded DNA with a K_b value in the 10^5 M^{-1} range.[19]Although the chemical nuclease activity in the dark was poor, light-induced double-strand cleavage was observed upon excitation at both 365 and 750 nm through a singlet oxygen formation pathway, whereas neither the ligands or vanadyl sulfate alone showed any activity.

Both singlet oxygen and hydroxyl radical formation were identified in mechanistic studies with various quenchers and radical scavengers of the bis-dppz complex $[VOCl(dppz)_2]^+$ (**Figure 4**) when activated with near-IR light at 750 nm.[20]

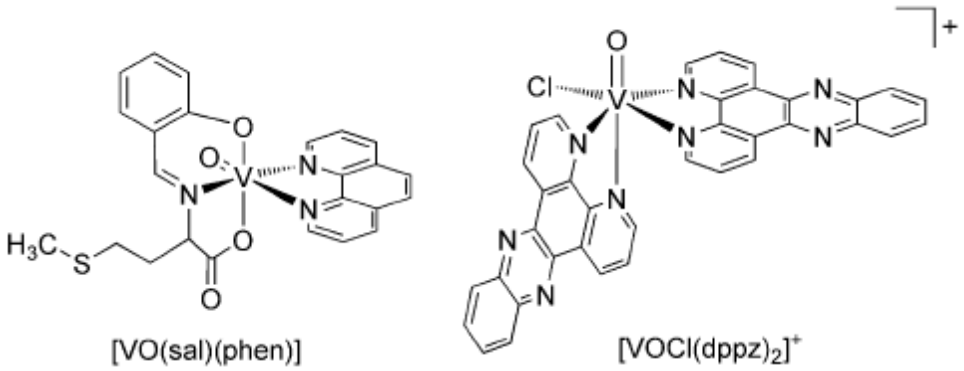

Fig.4: Oxovanadium(IV) complexes with diamine ligands

A recent review on the applications of vanadium-based compounds in industrial processes such as in catalysis, batteries, metal-organic frameworks as well as possible pharmacological applications has been published.[21]

II. EXPERIMENTAL

Materials and methods

All reagents and solvents were of analytical grade and used without further purifications. Doxycycline hyclate was a gift from Neimeth International Pharmaceuticals Plc, Lagos, Nigeria. Fresh solutions were prepared to ensure stability; 1,10-phenanthroline monohydrate and vanadyl sulphate were obtained from S. D. Fine Chemicals Ltd., India and used as received. Chloroquine diphosphate was obtained from Sigma. UV/Vis spectra were recorded on a Jasco UV-vis spectrophotometer. Infrared spectra were recorded on samples pressed in KBr pellets. Elemental analyses were taken on ElementarAnalysenSystemeVario ® MICRO VI 6.2 GmbH. Melting points were taken on Jenway digital melting point apparatus and were uncorrected.

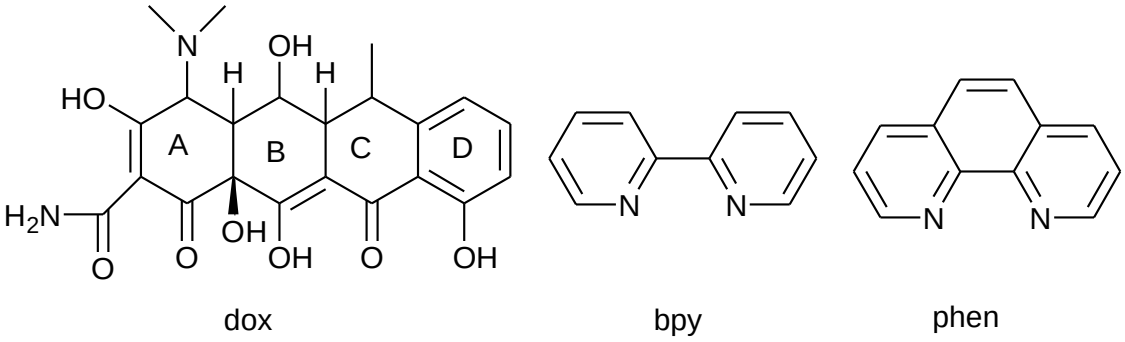

Fig.5: Structures of ligands used.

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Synthesis of the complexes

Synthesis of $[(VO)_3Dox(H_2O)_4(OH)_2]$ (1)

0.126 g (0.5 mmol) of $VOSO_4$ was added to 0.256 g (0.5 mmol) doxycycline hyclate in water-methanol and the solution stirred for 2 hours. The resulting solution was set aside at room temperature to obtain green solid which was redissolved in methanol and purified by column chromatography using alumina as stationary phase and acetone and methanol as eluent. Calculated: C, 35.22; H, 4.43; N, 3.73. Found: C, 34.82; H, 3.75; N, 3.57. FT-IR (KBr, ν/cm^{-1}): 3367, 1582, 1443, 1216, 1127, 953.

Synthesis of $[(VO)_3Dox(H_2O)_4bpy]$ (2)

0.126 g (0.5 mmol) of $VOSO_4$ was dissolved in 3 ml water and 0.256 g (0.5 mmol) doxycycline hyclate and 5 ml methanol added. The solution was stirred at ambient conditions for 2 hours and 0.078 g (0.5 mmol) of 2, 2-bipyridine was added as solid and stirring continued for another 1 hour. The resulting solution was allowed to stand at room temperature and the green solid obtained was redissolved in methanol and purified by column chromatography using alumina as stationary phase and acetone and methanol as eluent. Calculated: C, 44.05; H, 4.51; N, 6.42. Found: C, 44.99; H, 4.10; N, 6.65. FT-IR (KBr, ν/cm^{-1}): 3366, 1584, 1495, 1442, 1313, 1242, 1158, 1061, 1025, 952, 885, 765, 732.

Synthesis of $[(VO)_3Dox(H_2O)_4phen]$ (3)

0.126 g (0.5 mmol) of $VOSO_4$ was dissolved in 2 ml water and 0.260 g (0.5 mmol) doxycycline hyclate and 10 ml methanol added. The solution was stirred at ambient conditions for 2 hours and 0.1100 g (0.5 mmol) of 1,10-phenanthroline was added as solid and stirring continued for another 1 hour. The resulting solution was allowed to dry by setting aside at room temperature. The dark green glassy solid obtained the next day was redissolved in methanol and purified by column chromatography using alumina as stationary phase and acetone and methanol as eluent. Calculated: C, 45.55; H, 4.38; N, 6.25. Found:C, 45.48; H, 3.70; N, 6.32. FT-IR (KBr, ν/cm^{-1}): 3342, 1738, 1582, 1519, 1445, 1426, 1326, 1217, 1107, 1038, 963, 848, 805, 723, 620, 606, 572, 549, 530, 523.

Antiplasmodial study

Samples of all compounds were tested in triplicate against chloroquine-sensitive (NF54) strains of *Plasmodium falciparum*. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified procedure of Trager and Jensen.[22] Quantitative assessment of *in vitro* antiplasmodial activity was determined with the parasite lactate dehydrogenase assay using a modified method of Makler and Hinrichs.[23] 20 mg mL^{-1} stock solution in 100% DMSO of the test samples were prepared to and stored at −20 °C. Further dilutions were prepared on the day of the experiment. Chloroquine diphosphate (CQDP) was used as the reference drug. A full dose–response experiment was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50}-value). Samples were tested at a starting concentration of 100 μg ml^{-1}, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 μg mL−1. Reference drug (CQDP) was tested at a starting concentration of 1000 ng mL^{-1}. The highest concentration of the solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC_{50}-values were obtained using a non-linear dose response curve fitting analysis via Graph Pad Prism v.4.0 software.

III. RESULTS AND DISCUSSION

Synthesis and characterizationof complexes 1-3

Trinuclear oxovanadium complexes of doxycycline have been synthesized and characterized. The complexes were obtained in good yield and are stable in solid state and in solution at ambient conditions. Data obtained from FT-IR and elemental analysis are in agreement with the proposed molecular formulae for the complexes.

Amide II absorption at 1520 is absent in all the complexes except in VODoxphen (**3**) which appears at 1519 cm^{-1}(weak). Strong band at 1678 cm^{-1} due to carbonyl stretching of ring A, ν(C=O), is absent in all the complexes indicating its coordination to VO^{2+}. Carbonyl stretching ν (C=O) on ring C at 1616 cm^{-1} in doxycycline shifted to 1582, 1584 and 1582 cm^{-1}in VODox (**1**), VODoxbpy (**2**) and VODoxphen (**3**) respectively. ν (COH) + δ (CH_3) of BCD chromophore at 1558 cm^{-1} also shifted to 1443, 1442 and 1445 cm^{-1} for VODox (**1**), VODoxbpy (**2**) and VODoxphen (**3**) respectively. This suggests there is considerable change in the structure and probably conformation in the doxycycline ring of the formed complexes due to the trinuclear nature of the complexes formed. Similar coordination modes proposed for the new complexes have been previously reported for dinuclear Vanadium-tetracycline complexes.[24]

Two bands at 1244 and 1219 cm^{-1}in FTIR spectrum of doxycycline assigned to δ (NH_2) and ν(C-NH_2) are essentially unchanged in the new complexes indicating that NH_2 is not involved in coordination in the complexes. The stretching frequency of the V=O group expected in the range 960 ± 50 cm^{-1} are the new strong absorptions at 953, 952 and 963 cm^{-1}in VODox(**1**), VODoxbpy(**2**) and VODoxphen(**3**) respectively. ν(C-N-C) of diimine appeared at 765, 732 for VODoxbpy(**2**) and 723 VODoxphen(**3**) while C=N of diimine appeared at 885 and 848 for VODoxbpy(**2**) and VODoxphen (**3**)respectively.

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Table.1: Diagnostic bands in the FT-IR Spectra of complexes 1-3 (wavenumber in cm^{-1})

VODox (1)	**VObpyDox (2)**	**VODoxphen (3)**	**Assignment**
3367 str, br	3366 str, br	3342 br	υ-O-H
			Amide I C=O absent in the complex
1582	1584	1582, 1519	Amide II absorption δNH_2 +υC-NH_2
1443	1495, 1442	1445, 1426	δ(COH)+ δ(CH_3) of BCD chromophore
1216	1242	1217	δNH_2 andυC-NH_2 (no change)
953	952	963	ν(V=O)
	885	848	C=N of diamine
	765, 732	723	υ(C-N-C) of diamine
		572, 549, 530, 523	O=V-Ligand bond

str: strong; br: broad

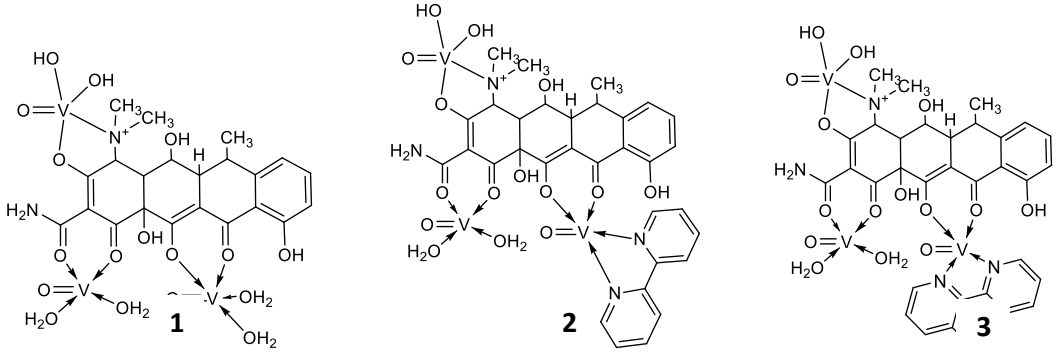
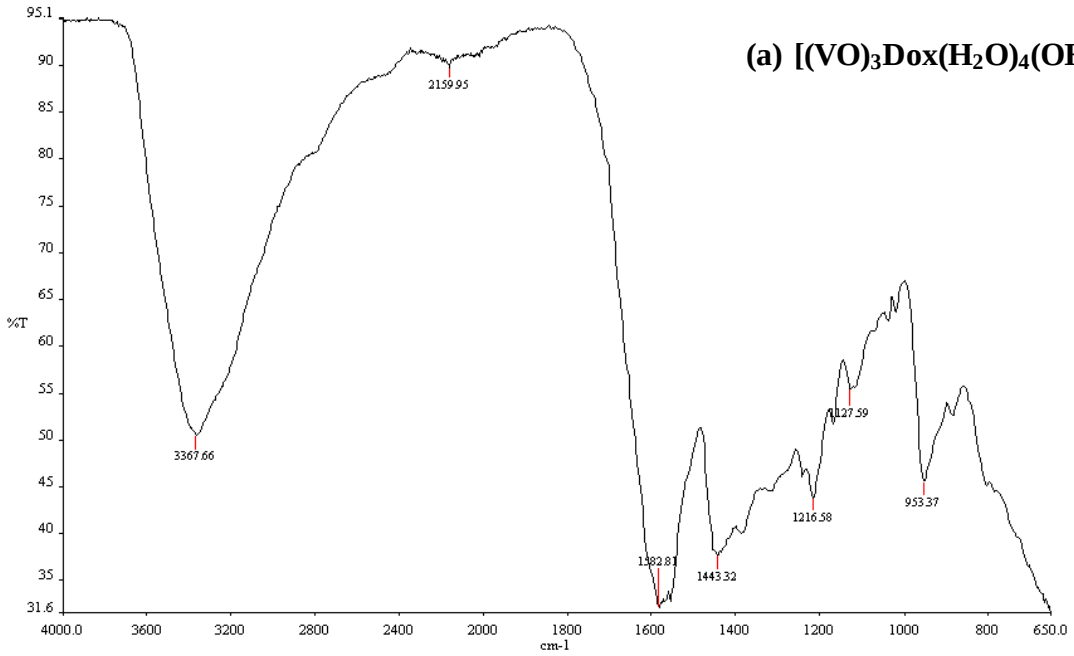


Fig.7: proposed structures of the complexes $[(VO)_3Dox(H_2O)_4(OH)_2]$ (1), $[(VO)_3Dox(H_2O)_4bpy]$ (2) and $[(VO)_3Dox(H_2O)_4phen]$ (3)



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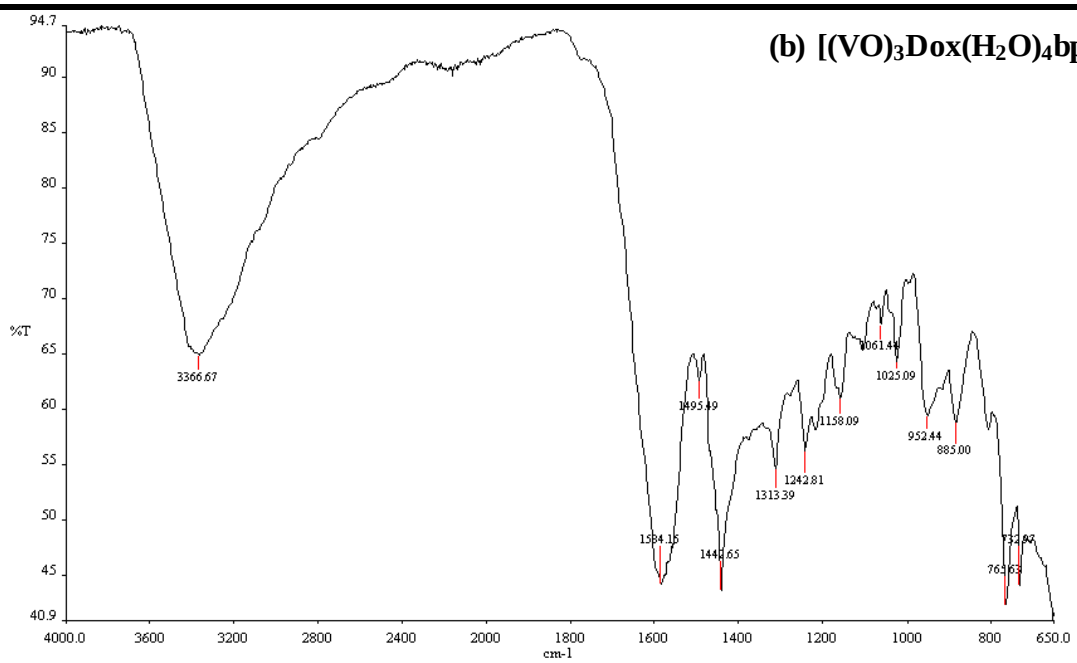

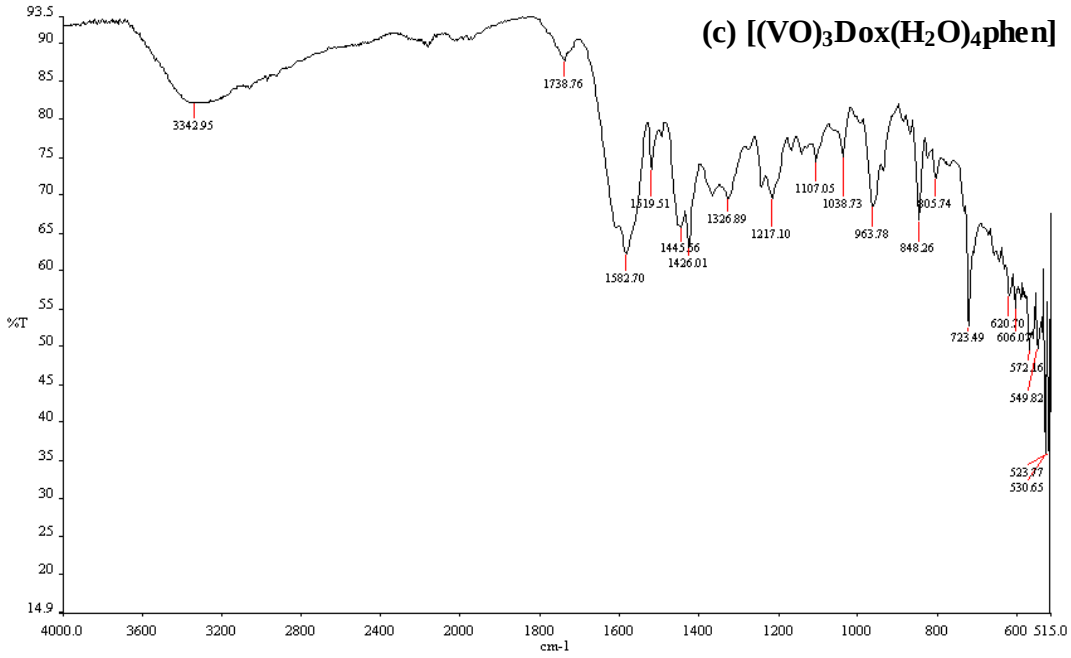

Fig.6: FTIR spectra of (a) 1, (b) 2 and (c) 3

Antiplasmodial activity study

The minimum inhibitory concentration and relative activity— IC_{50} (parental compound/IC_{50} (metal complex) of doxycycline and the vanadyl complexes **1-3** are presented in **Table 2.**

Table.2: Antiplasmodial activity of doxycycline and vanadium complexes

S/N	COMPLEXES	CONCENTRATION (µg/ml)	RELATIVE ACTIVITY TO DOX
12.	VODox **1**	20	0.5
13.	VODoxbpy **2**	13	0.77

14.	VODoxphen **3**	30	0.33
A	Dox Hyclate	10	
B	Lin Hydrochloride	>100	
C	CQ diphosphate	0.02	

For the vanadium complexes, the bpy complex (**2**) was seen to have similar activity to the parent ligand doxycycline while the binary complex (**1)** and phenanthroline complex(**3)** were found to be two and two-and-half fold less effective than the parent ligand respectively. This shows that the planarity of the polypyridyl ligands is not a criterion for the activity of these complexes. Though the activities of all these complexes against chloroquine sensitive (CQS) strain of *Plasmodium falciparum* (NF54) are lower than the parent drug, doxycycline and chloroquine, theyall have higher activities than lincomycin.

IV. CONCLUSION

Three new oxovanadium complexes of doxycycline have been synthesized and structurally characterized. FTIR and elemental analyses data confirmed the formation of both single ligand doxycycline complex (**1**) and mixed ligand doxycycline complexes with 2,2-bipyridine (**2**) and 1,10-phenanthroline (**3**). Complex **2** possess comparable antiplasmodial activity against chloroquine sensitive *NF54* and higher activity than lincomycin. These complexes are quite soluble in water and very stable at ambient conditions. This work has shown that formation of single ligand and mixed ligand complexes hold promise to finding stable drugs with equal therapeutic efficacies.

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The Effect of Product Knowledge and Risk Perception on Consumers' Online Word-of - Mouth Search Behavior for the Bio-Agricultural Products–in the Case of Ganoderma Dietary Supplements

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Abstract— *Many consumers have adopted the food of dietary supplements. However, since the threshold of professional knowledge for dietary supplements is usually high, most of the consumers do not possess sufficient knowledge for making an appropriate purchase decisions as they concern buying the products of dietary supplements. As a result, many consumers rely on word-of-mouth as they need to make purchase decisions on buying dietary supplements. It causes health risk to consumers since the word-of-mouth may be wrong and thus to make consumers take wrong dietary supplements. To address this deficiency, this study explores how consumers' knowledge for dietary supplements and risk perceptions influence their search behavior for word-of-mouth in the purchase of dietary supplements. With a two-stage cluster sampling strategy, 370 questionnaires were distributed to thecustomers of retail stores for selling Ganoderma dietary supplements, while 314 questionnaires were valid for statistical analysis. With the analysis of linear structural equation modeling, the causal structural relation among consumers' product knowledge, risk perception and word-of-mouth search behavior was examined. According to the statistical results, consumers' subjective product knowledge has greater overall impacts on the degree of word-of-mouth search behavior, compared with consumers' objective product knowledge. Namely, if consumers have more subjective knowledge for products, they are less likely to rely on word-of-mouth for the purchase of dietary supplements. For promoting the concept of food safety to consumers, consumers should be prepared with more correct product knowledge, in order to develop trust in self product knowledge in the publicity of safe consumption of dietary supplements, and reduce their dependence on the reputation of the dietary supplements without clear sources.*

Keywords— ***word-of-mouth, product knowledge, information search, risk perception, food safety.***

I. INTRODUCTION

Due to the emergence of health-oriented lifestyle, dietary supplements have become a major line of product in the food biotechnology industry. According to the statistics, the transaction value of the global dietary supplement market reached USD 195.8 billion in 2011 (Liu, 2012), and the scale of dietary supplement market in Taiwan was NT$ 62.5 billion, accounting for 10.4% of the entire food consumption market and becoming the largest food industry in Taiwan (Chen,2008; Chen, 2007). In fact, many consumers have accepted the idea of using dietary supplements to strengthen bodily functions. According to the consumer survey by the International Food Information Council Foundation, over 70% of the interviewed people agree that consuming particular food could enhance bodily functions and ensure health (Liu, 2007). Nevertheless, dietary supplements often involve complicated constituents and effective ingredients, physiological mechanism or manufacturing procedure. Therefore, the threshold of product knowledge for dietary supplements is high. According to the theory of consumers' purchase decision process, consumers with inadequate internal knowledge would seek more external information so as to reduce purchase risk in highly risky purchases. Consumers can obtain information through diverse channels, among which word-of-mouth (WOM) is nearly the most reliable external information source for consumers. Hence, WOM is often regarded as the main external information for consumers (Bansal &Voyer, 2000; Mattila&Wirtz, 2002). According to the results of Nielsen's online questionnaire survey on 47 countries in

2007, WOM is the most trustworthy information source for purchase decision (Nielsen, 2007). The consumer studies on dietary supplements in recent years have also shown that dietary supplements are consumer goods with high risk perception and high involvement of information search, while WOM play an important role as reference to the consumers for purchasing dietary supplements (Pillai & Hofacker, 2007; Raju et al., 1995; Slone, 2006).

From the perspective of foods and drugs safety, consumers' excessive dependence on WOM may lead to much anxiety in the safety offood and drug consumption. Despite that health foods must pass the entirereview and testing procedure of the government before it is launched in the market, consumers often misuse dietary supplements or trust the WOM without clear sources due to the lack of adequate professional knowledge, thus suffer from physical and mental harms(Hsieh, 2008). Moreover, some manufacturers purposely adopt buzz marketing to avoid the regulation on prohibiting the publicity with medical effects. In buzz marketing, bloggers, shopping experts and experienced users share their experiences to attract consumers. The Ministry of Health and Welfare implemented the "Five DO NOTs in Safe Medicine Consumption," namely "Do not trust,""Do not listen,""Do not buy,""Do not take" and "Do not recommend" to warn consumers on mistrust of inappropriate advertisement or WOM on dietary supplements. However, as the threshold of the professionalism on these products is high, many consumers still make purchase decisions based on the commercial advertisement of manufacturers or WOM of friends and family(Shih et al., 2010; Ministry of Health and Welfare, 2010).

As discussed above, studies on consumers' WOM search behavior are valuable to the publicity of foods and drugs safety. However, most of previous studies on the consumption of dietary supplements focused on consumers' acceptance (Frewer et al., 2003;Siró et al., 2008), attitude and understanding of dietary supplements(Childs &Poryzees, 1997; Verbeke, 2005), as well as use evaluation and purchase choice (Hailu et al., 2009; Teratanavat& Hooker, 2006; Urala&Lähteenmäki, 2003; Wandel & Bugge, 1997; West et al., 2002), while seldom on consumers' WOM search behaviors. Therefore, this study attempts to make up for this deficiency. The purposes of this study are as follows: (1) to explore the effects of consumers' product knowledge on their WOM search behavior on dietary supplements; (2) to analyze the mediating role of risk perception on consumers' product knowledge and WOM search behavior in their purchase of dietary supplements.

II. LITERATURE REVIEW

1. Product knowledge

Product knowledge refers to the degree to which a person knows about a certain product (Beatty & Smith, 1987; Solomon, 2007). It can be divided into three types according to the knowledge content: (1) Subjective knowledge: referring to consumers' measurement of their product knowledge, or their subjective understanding or self-evaluation of their product knowledge, implying the confidence that consumers show in their product knowledge; (2) Objective knowledge: referring to the amount, type and structure of information a person stores in his/her long-term memory or the degree to which a consumer knows a product in reality; (3) Experience-based knowledge: referring to the product experience that a person gradually accumulates through the purchase or use of a product as his/her product knowledge (Brucks, 1985;Park et al., 1994). As the processing of the product information differs from person to person and is difficult to measure, scholars often took objective and subjective product knowledge as the benchmark of the measurement of consumers' product knowledge (Brucks, 1985; Mattila&Wirtz, 2002;Park et al., 1994). In the purchase decision process, consumers' product knowledge plays an important role in consumers' information search and processing(Pillai &Hofacker, 2007; Raju et al., 1995).

2. Perceived risk

Perceived risk refers to psychological uncertainty. Bauer (1967) defined perceived risk as the uncertainty and negative results that consumers have to face when they cannot predict the results of purchase decision. For instance, the dietary supplement companies tend to label the effective ingredients of their products and emphasize on the health-enhancement effects, so as to intrigue consumers' purchase intention. However, as consumers do not have adequate professionalism on dietary supplements, they may not fully understand or be able to evaluate the reliability of the product information provided by the manufacturers. As a result, they become uncertain about the health-enhancement effects of dietary supplements and develop perceived risk (Solomon, 2007).

Previous studies have suggested that consumers' perceived risk has a multi-dimensional structure. Adopting the concept of time risk proposed by previous researchers suggested that consumers need to spend time and energy in the purchase decision-making, and thus face the risk of uncertainty in time and energy (Peter &Tarpey, 1975; Roselius, 1971). Jacoby and Kaplan (1972) indicated that consumers' perceived risk consists of six dimensions, namely, financial risk, psychological risk, efficacy risk, health risk, social risk, and time risk. These six risk dimensions have been cited in numerous studies as the basis for measuring consumers' perceived

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risk (Brooker, 1984). Dietary supplements are classified as food products, but as they can enhance health and need sophisticated processing technologies to ensure the functions, they are often sold at a high price. Moreover, if they are consumed in an inappropriate way, consumers may not obtain desired health-enhancement effects, or even suffer from physical health problems, in worse cases. Hence, a wrong decision on the purchase of dietary supplements would cause financial risk, efficacy risk, and health risk to consumers. The threshold of the product knowledge on dietary supplements is high and the product functions are specialized, so the purchase decision of dietary supplements requires high involvement, and may lead to high time risk. Additionally, the doubt over the functions and possible side effects of dietary supplements would also result in the psychological pressure on consumption. Consumers may face social risksdue to disagreement from their friends and family.

3. Correlation among product knowledge, risk perception and WOM search

Bansal and Voyer(2000) conducted experiments on the purchase decisions of consumers in the service industry. The research results showed that consumers' subjective product knowledge was in a significant negative correlation with perceived risk. In other words, if consumers have more subjective product knowledge, they will feel less perceived risk. Klerck and Sweeney(2007) explored the effects of consumers' subjective and objective product knowledge on their perceived risk of genetically-modified food in Australia. According to the research results, consumers' objective product knowledge has a significant negative correlation with the functional risk and psychological risk of products. As consumers gain more objective product knowledge, their perceived risk is reduced significantly. Lusk and Rozan(2005) found that if the consumers in the U.S. and Europe have less objective product knowledge on genetically-modified food, they would have greater perceived risk of the food and then have a weaker intention of purchasing it. Nevertheless, different product knowledge has different impacts on the risk perception of biological products. Sufficient objective product knowledge helps to alleviate consumers' efficacy risk perception and psychological risk perception of genetically-modified food, while sufficient subjective product knowledge increases their health risk perception of the food. However, it only happens when consumers have little objective knowledge on the food (Klerck& Sweeney, 2007). House et al.(2004) found that the type of product knowledge would influence consumers' acceptance of genetically-modified products. For them, sufficient subjective product knowledge would enhance consumers' acceptance of the products, while objective product knowledge has little impact on consumers' acceptance of the products. Previous studies on the consumption of dietary supplements seldom analyzed the correlation between product knowledge and risk perception, but the research results showed that well-educated consumers are more likely to use dietary supplements than those with lower educational level, who also seldom have positive use experience and have doubts about the consumption of dietary supplements (Landström et al, 2007; Niva&Mäkelä, 2007). According to the above literature review, this study proposes the following hypotheses:

H1a: When consumers have more subjective product knowledge on dietary supplements, they have less perceived risk of purchase.

H1b: When consumers have more objective product knowledge on dietary supplements, they have less perceived risk of purchase.

Consumers that lack product knowledge have highly complicated risk perception. If they are involved in the consumption with high perceived risk, they tend to search information to reduce the probability of risk; when they perceive higher risks, they would collect more information (Solomon, 2007). In the process of information search, consumers often face a problem -- the reliability of information sources. To lessen the risk of wrong information, consumers tend to acquire information from the most reliable information source(Goyal, 2008; Solomon, 2007). Among the information source, the most reliable one is the WOM from consumers' interpersonal network, which is often the important reference information that the consumers use to analyze risk and reduce their perceived risk (Beatty & Smith, 1987; Hirunyawipada&Paswan, 2006). According to the results of foreign studies, as consumers have high perceived risk on dietary supplements, they tend to show high involvement in information search, in particular, WOM provides reference for consumers (Pillai &Hofacker, 2007; Raju et al., 1995; Slone, 2006). Studies in Taiwan found that Taiwanese consumers without adequate professional knowledge would purchase dietary supplements according to commercial advertisements or WOM from friends and/or family (Hsieh, 2008; Ministry of Health and Welfare, 2010; Shih, 2010). Based on the literature review, this study proposes the following hypothesis:

H2: When consumers have higher perceived risk on dietary supplements, they are more likely to search for information for the purchase of dietary supplements.

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To date, no conclusion has been drawn on the impacts of consumers' product knowledge on their information search. Although previous studies have reached varied results, they could be categorized into three groups: (1) consumers' product knowledge has significant positive effect on their information search; (2) consumers' product knowledge has significant negative effect on their information search; (3) consumers' product knowledge is in a U-shaped relationship with their information search(Wang, 1999; Fiske, 1994).

Raju et al.(1995)argued that consumers' product knowledge influences their dependence on and preference for different types of information and their ability to process information in the purchase decision (Raju et al., 1995). In general, consumers with sufficient product knowledge have better information search skills and can use more appropriate clues to facilitate purchase decision-making. Moreover, they tend to evaluate their decisions according to the possible positive or negative results caused by the purchase and seldom make decisions according to actual clues (Raju et al., 1995; Cordell, 1997). Park and Lessig found that consumers with little product knowledge tend to rely on non-functional clues (e.g., price and brand) in their purchase decisions; they spend more time on decision-making than those with much product knowledge and are less confident in their decisions (Park &Lessig, 1981). Bettman and Parkfound that consumers with average knowledge have diverse methods to process available information and seldom rely on existing knowledge or experience. It is deduced that consumers with little product knowledge may not be able to process information due to their inadequate knowledge level, thus lack the motivation to analyze or process existing information. Consumers with sufficient product knowledge are capable to process information. As there is abundant internal information and rich experience in the use of products, they also lack the motivation to process existing information and tend to rely on their internal information and relevant experience (Bettman& Park, 1980). The research results also reveal that the consumers with much product knowledge feature great involvement in brand information while those with little product knowledge show more involvement in the attribute information of products than brand information.

The reference attributes that consumers rely on in the purchase decision of dietary supplements are diverse, including health benefit, efficacy, taste, sense, convenience, price and the familiarity with products, manufactures or brands. All these are essential reference factors that influence consumers' purchase decision (Urala&Lähteenmäki, 2003). Both functional and non-functional attributes of products are important clues for consumers in the purchase decision. According to the above literature review, the amount of knowledge on dietary supplements influences consumers' ability to process these reference clues and their dependence on these clues, and then affect the degree of WOM search. Hence, this study proposes the following hypotheses:

H3a: Consumers' subjective product knowledge on dietary supplements has significant effects on their WOM search for making purchase decision on dietary supplements.

H3b: Consumers' objective product knowledge on dietary supplements has significant effects on their WOM search for making purchase decision on dietary supplements.

Perceived risk may play a mediating role between product knowledge and the degree of information search. In the construction of the theoretical model of risk response strategies for consumers, Dowling and Staelinsuggested that consumers' prior knowledge is one of the antecedents that triggers their perceived risk and then influences the following-up information search (Dowling &Staelin, 1994). After that, some studies demonstrated the mediating role of perceived risk between product knowledge and information search in relevant empirical studies. For example, Klerck and Sweeney(2007) took genetically-modified food for a case study to explore consumers' product knowledge, perceived risk and information search. They found that consumers' product knowledge influences their efficacy risk and psychological risk perception of genetically-modified food, and then affects their tendency of information search before the purchase. In other words, the consumers with less product knowledge would have stronger risk perception and tendency to seek more relevant information. Scholars also found similar results in the studies on consumers' purchase of wine. Consumers' inadequate knowledge on wine would increase their risk perception of purchase, so they would seek external information to reduce possible purchase risks(Olsen et al., 2003). Atkin and Thach(2012) delved into the risk perception and information search of wine purchase of the millennium generation and found that the millennium generation often sought external information to reduce their perceived risk if they did not have adequate product knowledge. Among the relevant external information, WOM is regarded as highly reliable among consumers. Previous studies on the correlation between product knowledge and information search seldom discussed the mediating role of perceived risk. This may explain the reason why there is such great difference in the results of the studies on the correlation between consumers' product knowledge and information

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search(Fiske et al., 1994; Wang, 1999).

H4: Consumers' perceived risk of dietary supplements plays anmediating role between their product knowledge and WOM search.

According to the above research hypotheses, the research structure of this study is shown in Fig.1.

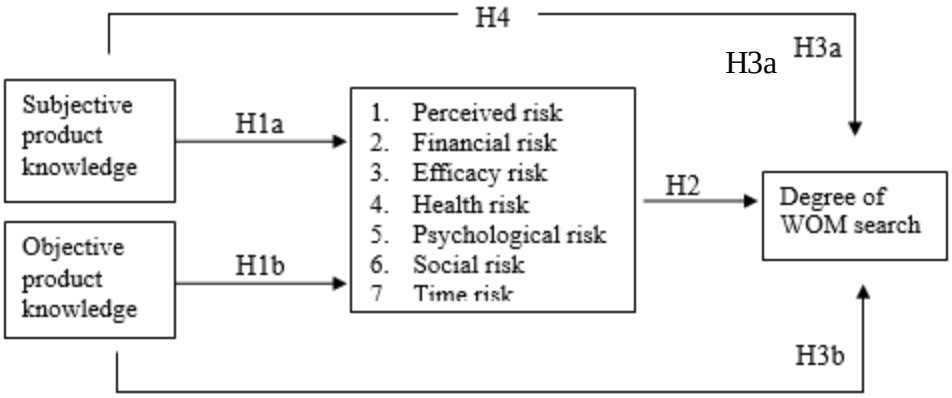

Fig.1: The conceptual work of the study

III. MATERIALS AND METHODS

1. Materials

Ganoderma is a traditional Chinese herb for health enhancement, and is highly popular in the market. The annual growth rate of the market demand for Chinese herbs with health enhancement effects is about 4.4%, and the transaction volume was predicted to reach USD 1.3 billion by 2015 (Liu, 2008). With great market potential, Chinese herbs are listed as major technological development by the government of Taiwan (Chen, 2009). This study took ganodermafor a case study, and a questionnaire survey was conducted to validate the theoretical model constructed in this study.

2. The operational definition of variables and the design of measurement items

In this study, the questionnaire was used to collect data. The official questionnaire consists of five parts. Part 1 comprises 18 items and measures consumers' perceived risks in the purchase of ganodermadietary supplements. Part 2 includes 8 items and measures consumers' WOM search on ganodermadietary supplements. Part 3 has 5 items and measures consumers' subjective product knowledge on ganodermadietary supplements. Part 4 consists of 6 items and measures consumers' objective product knowledge on ganodermadietary supplements. Part 5 includes 5 items and records the social and economic background information of the respondents. After the questionnaire was developed, 30 copies were distributed for a pre-test to ensure that the meaning of the items was conveyed accurately and appropriately. The operational definition of the variables and the design of the measurement items are as follows:

(1) Product knowledge

The measurement items for the variable"subjective product knowledge" are developed based on scholars' definition for the variable (Brucks, 1985; Park et al., 1994), so they have the content validity. In this study, five items were developed to test consumers' subjective product knowledge on ganodermadietary supplements. Additionally, this study defined consumers' objective knowledge on ganodermadietary supplements as the amount, type and structure of the information consumers store in their long-term memory or as consumers' actual knowledge on ganodermadietary supplements. Relevant measurement items were made according to the operation in previous studies (Mattila&Wirtz, 2002; Park et al., 1994; Raju et al., 1995). After the information about ganodermadietary supplements was collected and the items were developed (Ganoderma Health Foundation of Legal Persons of Consortium, 2009), three experts who specialized in the physiological development of ganoderma and the processing of ganoderma food were invited to test the validity of the measurement items. In other words, the expert validity method was used to ensure that the items were objective, definite and distinguishable, so as to test consumers' actual objective knowledge on ganodermadietary supplements.

(2) Perceived risk

According to scholars' definition of perceived risk, this study defined perceived risk as the anxiety that consumers feel when they are unsure if the dietary supplements they purchase would meet their expectation. The perceived risk is divided into financial risk, efficacy risk, health risk, psychological risk, social risk and time

risk (Bauer, 1967; Stone & Gronhaug,1993). The content validity method was also adopted to develop the questionnaire. Apart from the items of the above six risk dimensions proposed by Stone and Gronhaug(1993), this study considered other scholars' opinions on perceived risk (Brooker, 1984; Jacoby & Kaplan, 1972; Peter &Tarpey, 1975; Roselius, 1971) and adopted the items that scholars used to test consumers' perceived risk of different products (Klerck& Sweeney, 2007; Stone &Gronhaug, 1993). According to the comparison of consumers' features in the consumption of dietary supplements, the items were developed to test consumers' perceived risk ofganoderma dietary supplements.

(3) WOM search

In this study, the degree of WOM search is defined as the degree to which consumers collect others' opinions, use experience or comments in their purchase decisions of dietary supplements. The measurement items was developed based on the ones adopted by previous scholars regarding the measurement of consumers' volunteer inquiry of WOM (Bansal &Voyer, 2000;Bertrandias& Goldsmith, 2006). Based on the literature review, this study designed eight items to measure the degree to which consumers search WOM search for purchase decision of ganodermadietary supplements. Since these measurement items were based on previous definitions and scales, thus they have good content validity.

3. Sampling

The subjects of this study were existing and potential consumers of ganodermain Taipei City. Two-stage cluster sampling was adopted to develop the structural equation model for analyzing and controlling the error of the analytical results under the condition that the normalized hypothesis is valid. First, the 12 administrative regions of Taipei City were numbered from "1" to "12", and random sampling was adopted to select four administrative regions. After that, all the neighborhoods in four administrative regions were numbered, and four neighborhoods were randomly selected from each administrative region. To ensure that the selected samples were existing and potential consumers of ganoderma, samples were collected from the marketing channels of ganodermadietary supplements. The marketing channels included Chinese and Western pharmacies and chain drug stores(Chou, 2005; Hair et al., 2006).

4. Statistical Analyses

(1) Reliability and validity tests

Reliability coefficient of scales refers to the reliability of the items of a variable or the consistency or stability of the results of measurements; the validity of scales indicates the effectiveness of scales or the degree to which the adopted scales can measure the targeted subjects (Hair et al., 2006). The reliability of the measurement items of this study was analyzed with Cronbach's α. Aside from the above content validity method and the expert validity method, the maximum likelihood estimation of the confirmatory factor analysis was used to estimate parameters and calculate the composite reliability and average variance extracted (AVE) of the measurement items, and test the convergent validity and discriminant validity of the measurement items.

According to previous studies, four evaluation norms were compiled by this study to test the convergence of the items (Bagozzi& Yi, 1989; Hair et al., 2006): (1) the goodness of fit index (GFI), the normed fit index (NFI) and the comparative fit index (CFI) must be higher than 0.9, while the root mean square residual (RMR) must be lower than 0.05; (2) the factor loads of the items were significant ($\alpha = 0.05$); (3) the composite reliability (CR) of the items of the variables must be higher than 0.7; (4) the average variance extracted (AVE) must be higher than 0.5.

Discriminant validity refers to the relevance between the items describing a variable in the measurement tool and that describing other items. It is also called divergent validity. If the relevance between two variables is weak, it means that the measurement items between the two variables have discriminant validity. This study adopted the discriminant validity evaluation proposed by Fornell and Larcker(1981)to test the AVE of the items of each variable.

(2) Analysis of the theoretical model

This study employed the structural equation modeling (SEM) to validate the causal relationship among consumers' product knowledge, perceived risk and the degree of WOM search for the purchase decision of dietary supplements. According to the two-stage analysis and previous studies, this study first conducted the confirmatory factor analysis to test the goodness of fit of the model. Then, the operational SEM was analyzed to discuss the causal relationship among the potential variables (Anderson &Gerbing, 1988; McDonald & Ho, 2002). The above methods were based on the prerequisite that the observation variables are in a normal distribution. Hence, the skewness and kurtosis of the observation variables were used to test the normality of the data before the confirmatory factor analysis and the SEM analysis.

IV. RESULTS

1. Structural features of samples

This study distributed 370 copies of questionnaire, and retrieved 314 valid samples, including 128 from males (40.8%) and 186 from females (59.2%). In terms of age, most of the respondents aged from 35 to 44 (32.5%),

followed by 25 to 34 (30.6%), and lastly 18 to 24. As foroccupation, most of them work in the business/service industry (37.6%), followed by military, civil servants, and teachers (22.3%), and lastly the retirees. In terms of educational background, most of them have university or college education (68.8%). Regarding monthly disposable income of household, most of them have a monthly disposable income of NT$ 70,001 to NT$ 90,000, followed by NT$50,001toNT$ 70,000, and lastly NT$ 150,001. The social and economic background structure of the respondents is shown in Table 1.

Table.1: The socio-economics of the samples

Variable	Population	Percentage (%)	Variable	Population	Percentage (%)
Gender			***Jobs***		
Male	128	40.8	Staff of agriculture, forestry, fishery and husbandry	22	7.0
Female	186	59.2	Mining/manufacturing	28	8.9
			Business/service industry	118	37.6
Age			Soldier/civil servant/ teacher/policeman	70	22.3
18-24 years old	16	5.1	Student	16	5.1
25- 34 years old	96	30.6	Housekeeper	24	7.6
35-44 years old	102	32.5	Retirement	4	1.3
45-54 years old	64	20.4	Others	32	10.2
55-64 years old	36	11.5	***Monthly disposable income of household***		
Education			NT$30,000 or below	8	2.5
Primary school or below	2	0.6	NT$30,001 ~ $50,000	62	19.7
Secondary school	2	0.6	NT$50,001 ~ $70,000	88	28.0
High school/vocational school	32	10.2	NT$70,001 ~ $90,000	96	30.6
University/junior college	216	68.8	NT$90,001 ~ $120,000	48	15.3
Research institute or above	62	19.7	NT$120,001 ~ $150,000	10	3.2
			NT$150,001 or above	2	0.6

2. Results of the reliability and validity tests

The Cronbach's α of "subjective product knowledge" was 0.94, and those for "financial risk perception", "Efficacy risk perception", "Health risk perception", "Psychological risk perception", "Social risk perception" and "Time risk perception" were 0.83, 0.89, 0.91, 0.88, 0.82 and 0.85, respectively. The Cronbach's α of "Degree of WOM search" was 0.86. Additionally, the measurement items of "objective product knowledge" were made to test consumers' actual knowledge on ganodermadietary supplements (Mattila&Wirtz, 2002; Park et al., 1994; Raju et al., 1995; Ganoderma Health Foundation of Legal Persons of Consortium, 2009). According to the definition of objective product knowledge and the operation in previous studies, this study measured consumers' basic knowledge on ganodermadietary supplements. A higher score indicates more objective product knowledge on ganodermadietary supplements. The test items covered the source of the bitterness of ganoderma, the effective ingredients of ganoderma, the making of ganoderma products, and the immune effects of ganoderma. For that reason, the reliability of the items about objective product knowledge differed from that of the items based on the Likert's 5-point scale and thus cannot be represented through Cronbach's α. Therefore, this study adopted the expert review method, where three experts who specialize in the physiological development of ganoderma and the processing of ganoderma food were invited to test the validity of the items.

As for the validity test on the items, the items were compiled according to the content validity and the expert review method in the process, and the maximum likelihood estimation of the confirmatory factor analysis was also used to calculate the composite reliability and AVE of the items of the variables and test the convergent validity and discriminant validity of the items. The results of relevant tests are shown in the following test on goodness of fit of the measurement model.

3. Validation of the theoretical model

(1) Normal distribution of data

In this study, the statistical skewness and kurtosis were used to test the normal distribution of data. In the operation of the SEM, if the absolute value of the skewness of the data concerning variables is less than 3 and the kurtosis is less than 10, it would be regarded as normal distribution (Kline, 2011). In this study, the normal distribution of the variables is shown in Tables 2 to 4. The absolute values of the skewness and kurtosis of the variables are in the scope of evaluation values, showing that the data concerning the variables of this study met the statistical hypothetical conditions of normal distribution and were suitable for the confirmatory factor analysis and the SEM analysis.

(2) The goodness of fit of the measurement model

In this study, the maximum likelihood estimation of the confirmatory factor analysis was adopted to test the goodness of fit of the measurement models. The adopted indexes include: (a) the factor loads of the items were significant (α=0.05); (b) the measured value of composite reliability was higher than 0.7; (c) the measured value of AVE was higher than 0.5; (d) the GFI, NFI and CFI must be higher than 0.9, and the RMR must be lower than 0.05. The results are as follows:

1) The measurement model of subjective product knowledge: (a) the factor loadings of the items were all significant (P < 0.001); (b) the composite reliability was 0.941, meeting the criterion of higher than 0.7; (c) the AVE was 0.764, also meeting the criterion of higher than 0.5; (d) the GFI, NFI and CFI were 0.967, 0.982 and 0.985 respectively, all higher than 0.9, while RMR was 0.017, lower than the criterion of 0.05. The test results revealed that the measurement model of subjective product knowledge has effective goodness of fit (Table 2).

Table.2: Confirmatory factor analysis and normal distribution test of the measurement model of subjective product knowledge

Variable	Estimated Parameter of MLE				Normal Distribution Test	
	Factor Load (λx)	Measurement Error (δ)	CR	AVE	Skewness	Kurtosis
Subjective product knowledge	--	--	0.941	0.764	0.573	-0.075
1. I believe that I have sufficient knowledge on ganoderma.	0.742*	0.449	--	--	0.323	-0.333
2. It is safe to say that I am an expert on ganoderma in my social network.	0.887*	0.213	--	--	1.234	1.751
3. I have more knowledge on ganoderma than most people do.	0.928*	0.139	--	--	0.786	0.305
4. I have a good command of the knowledge on ganoderma.	0.905*	0.181	--	--	0.591	0.172
5. I am confident in selecting ganoderma products.	0.895*	0.199	--	--	0.602	-0.497

GFI=0.967, NFI=0.982, CFI=0.985, RMR=0.017
* p < 0.001
a: Composite Reliability
b: Average Variance Extracted

2) The measurement model of objective product knowledge: each item of the objective product knowledge of this study can directly indicate the amount of information of consumers. The total score of the items was taken as the observation item and added into the model. The confirmatory factor analysis was not suitable for the measurement of this variable, so in the construction of this measurement

model, this study used literature review to ensure that the measurement indexes met the theoretical foundation. Moreover, the expert review method was employed to ensure the goodness of fit of this measurement model.

3) The measurement model of perceived risk: (a) the factor loadings of the items were all significant ($P < 0.001$); (b) the composite reliability of the two-stage confirmatory factor analysis of perceived risk was 0.853, meeting the criterion of higher than 0.7; (c) the AVE was 0.509, meeting the criterion of higher than 0.5; (d) the GFI, NFI and CFI were 0.837, 0.865 and 0.890 respectively, higher than 0.8 (within the acceptable scope) though lower than the criterion of higher than 0.9. The RMR was 0.069. Although the RMR was lower than the criterion of lower than 0.05, it was also within the acceptable scope (Hair et al., 2006) (Table 3).

Table.3: Confirmatory factor analysis and normal distribution test of the measurement model of perceived risk

Variable	Estimated Parameter of MLE				Normal Distribution Test	
	Factor Load (λx)	Measurement Error (δ)	CR	AVE	Skewness	Kurtosis
Perceived risk	--	--	0.853	0.509	0.488	0.788
Financial risk	0.860*	0.260	0.844	0.648	0.317	-0.470
1. I think it is a waste of money to buy ganoderma.	0.920*	0.154	--	--	0.447	-0.272
2. I think buying ganoderma would bring me financial burden.	0.628*	0.606	--	--	-0.125	-0.733
3. I do not think it worthy to buy ganoderma.	0.839*	0.296	--	--	0.379	-0.397
Efficacy risk	0.746*	0.443	0.893	0.736	-0.400	-0.105
1. I do not think ganoderma is effective.	0.894*	0.201	--	--	-0.446	-0.452
2. I am worried that ganoderma would not bring the expected effects.	0.884*	0.219	--	--	-0.596	-0.227
3. I have no confidence in the functions of ganoderma.	0.791*	0.374	--	--	0.097	-0.672
Health risk	0.774*	0.401	0.912	0.778	0.353	-0.647
1. I am worried that ganoderma would cause side effects.	0.917*	0.159	--	--	0.179	-0.717
2. I am worried that ganoderma would cause health problems.	0.979*	0.042	--	--	0.243	-0.729
3. I am worried that ganoderma may cause potential risk to my health.	0.732*	0.464	--	--	0.189	-0.930
Psychological risk	0.843*	0.289	0.890	0.730	1.067	1.709
1. I worry about the consumption of ganoderma.	0.813*	0.339	--	--	1.091	1.286
2. I feel anxious about the consumption of ganoderma.	0.919*	0.155	--	--	1.087	2.007
3. Consuming ganoderma would impose pressure on me.	0.828*	0.314	--	--	0.996	0.999
Social risk	0.589*	0.653	0.832	0.626	0.987	1.550
1. I am worried that it is against my social	0.702*	0.507	--	--	0.946	1.033

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Table.3: Confirmatory factor analysis and normal distribution test of the measurement model of perceived risk

Variable	Estimated Parameter of MLE				Normal Distribution Test	
	Factor Load (λx)	Measurement Error (δ)	CR	AVE	Skewness	Kurtosis
status to consume ganoderma.						
2. I am worried that my friends would make joke of my consuming ganoderma.	0.913*	0.166	--	--	1.106	1.511
3. I am worried that my friends would disapprove of me on the consumption of ganoderma.	0.742*	0.449	--	--	0.780	0.444
Time risk	0.320*	0.898	0.867	0.695	-0.231	-0.816
1. I think it takes much time to collect the information about ganoderma.	0.566*	0.680	--	--	0.002	-0.781
2. I think it takes much time to search for information on choosing ganoderma products.	0.908*	0.176	--	--	-0.247	-0.723
3. I think it takes much time to search for information on how to use ganoderma products.	0.969*	0.061	--	--	-0.178	-0.708

GFI=0.837, NFI=0.865, CFI=0.890, RMR=0.069
* $p < 0.001$
a: Composite Reliability
b: Average Variance Extracted

4) The measurement model of the degree of WOM search: (a) the factor loadings of the items were all significant ($P < 0.001$); (b) the composite reliability of the two-stage confirmatory factor analysis of perceived risk was 0.887, meeting the criterion of higher than 0.7; (c) the AVE was 0.525, meeting the criterionof higher than 0.5; (d) the GFI, NFI and CFI were 0.853, 0.842 and 0.844 respectively, higher than 0.8 (within the acceptable scope) though lower than the criterionof higher than 0.9. The RMR was 0.052. Although the RMR was lower than the criterionof lower than 0.05, it was also within the acceptable scope (Hair et al., 2006) (Table 4).

This study referred to the discriminant validity criterion suggested by Fornell and Larcker (1981) to test the AVE of the items of each variable. The results showed that the AVE of each variable was higher than the variable and the squared value of relevant coefficients of other variables. This means that the variables of this study have effective discriminant validity.

Table.4: Confirmatory factor analysis and normal distribution test of the measurement model of the degree of WOM search

Variable	Estimated Parameter of MLE				Normal Distribution Test	
	Factor Load (λx)	Measurement Error (δ)	CR	AVE	Skewness	Kurtosis
Degree of WOM search	--	--	0.887	0.525	-0.205	-0.291
Active search					-0.691	0.557
1. I would consider others' suggestions in the purchase of ganoderma.	0.850*	0.245	---	---	-0.457	0.141
2. I would consult others before	0.879*	0.235	---	---	-0.606	-0.104

purchasing ganoderma.						
3. Others' opinions would make me feel assured when I purchase ganoderma.	0.833*	0.377	---	---	-0.657	0.237
4. I would collect relevant negative comments before purchasing others' opinions.	0.567*	0.577	---	---	-0.837	0.820
5. I would collect relevant positive comments before purchasing others' opinions.	0.687*	0.408	---	---	-1.117	1.893
Passive search					---	---
I seldom ask others about how to purchase ganoderma.	0.829*	0.256	---	---	0.063	-0.936

GFI=0.853, NFI=0.842, CFI=0.844, RMR=0.052
* $p < 0.001$
a: Composite Reliability; b: Average Variance Extracted

(3)The goodness of fit of theory model

Previous studies have proposed different suggestions on the test on the goodness of fit of the SEM. Following the suggestions from Bagozziet al. and Hair et al. (Bagozzi& Yi, 1989; Hair et al., 2006), this study tested three types of goodness of fit, including the absolute fit measures, the incremental fit measures, and the parsimonious fit measures. The results are shown in Table 5. The meaning and index results of the measures are as follows:

1) Absolute fit measures: it tests the degree of fit between the overall model and actual data. The indexes adopted in this study were square degree of freedom ($\chi 2$/d.f.), GFI, RMR and RMSEA. The suggested value of square degree of freedom was under 3, which indicates that the overall model and observation data haveeffective fit. The square degree of freedom ($\chi 2$/d.f.) of the overall theoretical model was 2.978, meeting thesuggested value.
Goodness fit index (GFI) refers to the ratio(Hair et al., 2006) of the variance and co-variate that can be explained by the measurement theory model. If GFI is closer to 1, the overall fit is higher and the explanatory ability of the model is stronger. In general, a GFI higher than 0.9 is taken as the threshold value, and GFI should higher than 0.8 (Bagozzi& Yi, 1989). The GFI of the overall theoretical model of this study was 0.868, which indicates that the fit of the proposed model is accepted. In terms of RMR, Hair et al. (2006) suggested that a smaller RMR is better and the best RMR is lower than 0.05. RMSEA is not influenced by either the number of samples or the complexity of model. In general, a lower RMSEA indicates a higher fit of model, and a RMSEA lower than 0.08 is regarded as the criterion(Hair et al., 2006). The RMR of the model established in this study was 0.049, and the RMSEA was 0.076, meeting the suggestion that RMSEA be lower than 0.05 and 0.08.
2) Incremental fit measures: it is used to measure the difference between the hypothetical theoretical model and the null model. The indexes adopted in this study included AGFI, NFI and CFI. AGFI is obtained by adjusting the degree of freedom of fit index (Hair et al., 2006). If it is closer to 1, the overall fit is higher and the explanatory power of model is stronger. In general, a GFI higher than 0.9 is taken as the threshold value, and GFI should be higher than 0.8 (Bagozzi& Yi, 1989). The AGFI of the overall theoretical model of this study was 0.807, which indicates that the fit of the proposed model is accepted. NFI is used to reflect the incremental fit of model, and a NFI higher than 0.9 is regarded as the criterion (Hair et al., 2006). According to the statistical results, the NFI of the hypothetical model constructed in this study was 0.914, meeting the suggestion that NFI be higher than 0.9. CFI is used to show the difference between the theoretical model and the independent model without any co-variate relation. A suggested CFI is higher than 0.9. In this study, the CFI of the proposed was 0.943, meeting the suggestion that measured CFI be higher than 0.9.
3) Parsimonious fit measures: it is used to compare the fit of the estimate coefficients of the models with different number of estimate coefficients. The adopted indexes were PNFI and PCFI. Both PNFI and PCFI should be higher than 0.5 to reach a good fit (Hair et al., 2006). The PNFI and PCFI of the proposed theoretical model were 0.616 and 0.637 respectively, both of which were higher than 0.5 and within the scope of suggested values.

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Table.5: Results of test on the fit of theoretical model

Fitness test	Evaluation index	Criterion	Results of analysis	Evaluation of Fitness test
Absolutefit measures	$\chi 2$/d.f.	<3	2.978	Effective
	GFI	>0.9 (or >0.8)	0.868	Acceptable
	RMR	<0.05	0.049	Effective
	RMSEA	<0.08	0.076	Effective
Incremental fit measures	AGFI	>0.9(or >0.8)	0.807	Acceptable
	NFI	>0.9	0.914	Effective
	CFI	>0.9	0.943	Effective
Parsimonious fit measures	PNFI	>0.5	0.616	Effective
	PCFI	>0.5	0.637	Effective

(4) Structural model of the correlation among product knowledge, perceived risk and the WOM search

The statistical results of the indexes measuring the fit of the causal relationship model of the variables of subjective product knowledge, objective product knowledge, perceived risk and the degree of WOM search was effective enough to explain the actual data. Therefore, this study adopted the structural model obtained from the SEM analysis to test the path coefficients of potential variables to determine the statistical significance of the structural relation and the causal relationship among the variables. The results are shown in Table 6, and the path analysis of the causal relation among the potential variables is shown in Figure 2.

Table.6: Results of test on the path coefficients of the structural model

Paths	Path coefficient	*t*	*p*	Research hypothesis	Test results
Subjective product knowledge→ Perceived risk	-0.42	-6.64	0.000***	H1a	Valid
Objective product knowledge→ Perceived risk	-0.01	-0.39	0.694	H1b	Invalid
Perceived risk→ Degree of WOM search	0.11	2.25	0.025**	H2	Valid
Subjective product knowledge→ Degree of WOM search	-0.39	-5.98	0.000***	H3a	Valid
Objective product knowledge→ Degree of WOM search	-0.23	-2.47	0.014**	H3b	Valid
Subjective product knowledge→ Perceived risk→ Degree of WOM search	----	----	----	H4a	Partially valid
Objective product knowledge→ Perceived risk→Degree of WOM search	----	----	----	H4b	Invalid

** p< 0.05; *** p < 0.001

The path analysis of the linear structural equation found that, the type of product knowledge has different impacts on consumers' perceived risk in their purchase of dietary supplements. Despite that consumers is in a positive correlation with their subjective and objective product knowledge of dietary supplements (β= 0.22, *P* = 0.000), the two factors have different impacts on the perceived risk of dietary supplements. Consumers' subjective product knowledge is in a negative causal relationship with their perceived risk, and the standardized path coefficient was -0.42 (*P* < 0.001); hence, H1a is supported. Consumers' objective product knowledge, however, does not have significant influence on their perceived risk (*P* = 0.694); hence, H1b is not supported. The findings are consistent with previous studies, which indicated that consumers with more subjective product knowledgehave less perceived risk of products (Bansal &Voyer, 2000; Srinivasan &Ratchford, 1991). Consumers' subjective product knowledge is correlated to objective product knowledge, the two have different impacts on consumer behaviors (Park et al., 1994; Raju et al., 1995; Teratanavat& Hooker, 2006).

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The findings of this study suggest that consumers' perceived risk of dietary supplements has significant positive impacts on the degree of WOM search in their purchase decision of dietary supplements. The standardized path coefficient was 0.11. Although the influence weight is not high, it has statistical significance (P = 0.025); hence, H2 is supported. This finding suggests that if consumers have more perceived risk of dietary supplements, the degree of WOM search is higher. This result is also consistent with previous studies (Goldsmith & Horowitz, 2006; Hennig-Thurau& Walsh, 2003).

According to the SEM in this study, consumers' subjective product knowledge has significant negative impacts on the degree of their WOM search, and the standardized path coefficient is -0.39 (P< 0.001); hence, H3a is supported. According to the analytical results, if consumers have more confidence in their knowledge of dietary supplements (or more subjective product knowledge), they are less dependent on WOM in the purchase decision of relevant products. Consumers' objective product knowledge also has similar impacts on the degree of WOM search in their purchase decision of dietary supplements. In other words, consumers' objective product knowledge has significant negative effects on the degree of WOM search, and the standardized path coefficient is -0.23 (P< 0.05); hence, H3b is supported. The results of this study are consistent with the theory on consumers' use of information. In other words, only in face of inadequate information would consumers voluntarily search for information and make greater efforts to obtain information to facilitate their consumption decision-making. According to the comparison of path coefficients, the direct impacts of subjective product knowledge are greater than that of objective product knowledge.

The analysis on the mediating role of perceived risk found that the indirect impacts of subjective product knowledge on the degree of WOM search areless than the direct impacts. The analysis of path coefficients found that the indirect impacts of subjective product knowledge on the degree of WOM search is - 0.046, which is lower than the direct impacts (- 0.390). This result is not consistent with the criterion on a complete mediating relationship that the indirect impacts of an independent variable on a dependent variable must be greater than the direct impacts (Baron & Kenny, 1986). As the direct impacts of perceived risk on the degree of WOM search exist (or H2 is valid), the perceived risk still plays a mediating role in the relationship between subjective product knowledge and the degree of WOM search, though the indirect impacts of the intermediate role are weaker than the direct impacts. In the test on the mediating role of objective product knowledge, the impacts of consumers' objective product knowledge on consumers' perceived risk are insignificant (P = 0.694). This is inconsistent with previous studies that an independent variable must have significant impacts on a mediating variable (Baron & Kenny, 1986). Therefore, it can be deduced that the mediating role of perceived risk in the relationship between objective product knowledge and the degree of WOM search is invalid. Based on the above, H4 is partially supported.

V. DISCUSSION

Due to the prevalence of chronic diseases and the problems associated with aging population, there has been an increasing demand for dietary supplements in Taiwan. With substantial market potential, dietary supplement has become a focus in Taiwan to promote the biotechnological industry. Although dietary supplements have to undergo rigorous inspection before launched into the market, they can be easily accessed and consumers can obtain them through general retailing channels without prescription. As a result, people tend to purchase dietary supplements based on their own judgment, and seldom seek for professional consultation. As the threshold on the product knowledge of agricultural and biotechnological dietary supplements is high, people without adequate knowledge would easily purchase dietary supplements according to WOM or the publicity manipulated by the manufacturers. Sometimes, consumers may mistakenly take some dietary supplements that are not legally licensed or contain prohibited ingredients, thus suffering physical problems. Despite that inappropriate WOM is a key factor that affects the drug safety, this study found that previous studies on the consumption of dietary supplements mostly focused on consumers' attitude, preference, evaluation and selection of dietary supplements, while seldom on consumers' WOM search. Therefore, this study explored the correlation between consumers' product knowledge of dietary supplements and the degree of WOM search.

This study carry both theoretical and practical contributions. The theoretical significance of this study is as follows. First, previous studies on other product fields showed that both subjective and objective product knowledge have a negative causal relationship with consumers' perceived risk. In other words, if consumers have more subjective or objective product knowledge, they have less perceived risk. This study took dietary supplements for the case study but found that there is a negative causal relationship between consumers' subjective product knowledge and perceived risk, and that the impacts of objective product knowledge on perceived risk are insignificant. Therefore, it is deduced that the impacts of consumers' product knowledge on perceived

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risk differ according to the meaning of knowledge (e.g., subjective and objective product knowledge) and the product. Second, there are three arguments on the relevance between consumers' product knowledge and information retrieval, including positive correlation, negative correlation and U-shaped correlation. Taking dietary supplements for a case study, this study found that the two are in a negative correlation and that if consumers have more subjective and objective product knowledge, they have a lower degree of WOM search. Third, the correlation between consumers' product knowledge and information search differs according to knowledge. Subjective product knowledge has direct negative impacts on the degree of WOM search, and has indirect impacts with risk perception playing a mediating role. Through the negative impacts on risk perception, subjective product knowledge can indirectly lower the degree of WOM search. However, objective product knowledge merely has direct influence on the degree of WOM search, and risk perception does not play a mediating role in the relationship between the two.

In terms of practical contribution, the findings suggest that different dimensions of product knowledge have different impacts on consumers' dependence on WOM in the purchase decision of dietary supplements and the influence mechanisms are also different. Consumers' objective or subjective knowledge has direct negative influences on the degree of WOM search. In other words, if consumers have more objective or subjective knowledge, they have a lower degree of WOM search. Aside from imposing direct impacts on WOM search, consumers' subjective product knowledge also influences the degree of WOM search through risk perception as a mediating role. If consumers have more subjective product knowledge, they have less risk perception of the dietary supplements and thus show a lower degree of WOM search. However, the impacts of objective product knowledge on risk perception are insignificant, risk perception does not have mediating effect on the relationship between objective product knowledge and the degree of WOM search. Obviously, consumers' risk perception in the purchase decision of dietary supplements comes from their inadequate confidence in their product knowledge. Therefore, they consider WOM and others' use experience to reduce possible purchase risk. Hence, the institutions in charge of drugs safety should offer correct knowledge on safe consumption of drugs and strengthen consumers' confidence in their product knowledge, so as to alleviate consumers' dependence on WOM. In particular, in the era with abundant online information, it is easy to obtain WOM. In the commercial operation model of manufacturers and through the marketing model featuring online shopping experts and WOM, the business reputation purposely manipulated by the manufacturers can easily become reference information for consumers; consequently, consumers would consume dietary supplements inappropriately, and may suffer from possible physical and mental health problems. Thus, consumers should be educated with appropriate knowledge about dietary supplement to prevent them from referring to incorrect information from WOM, and thus to danger their health.

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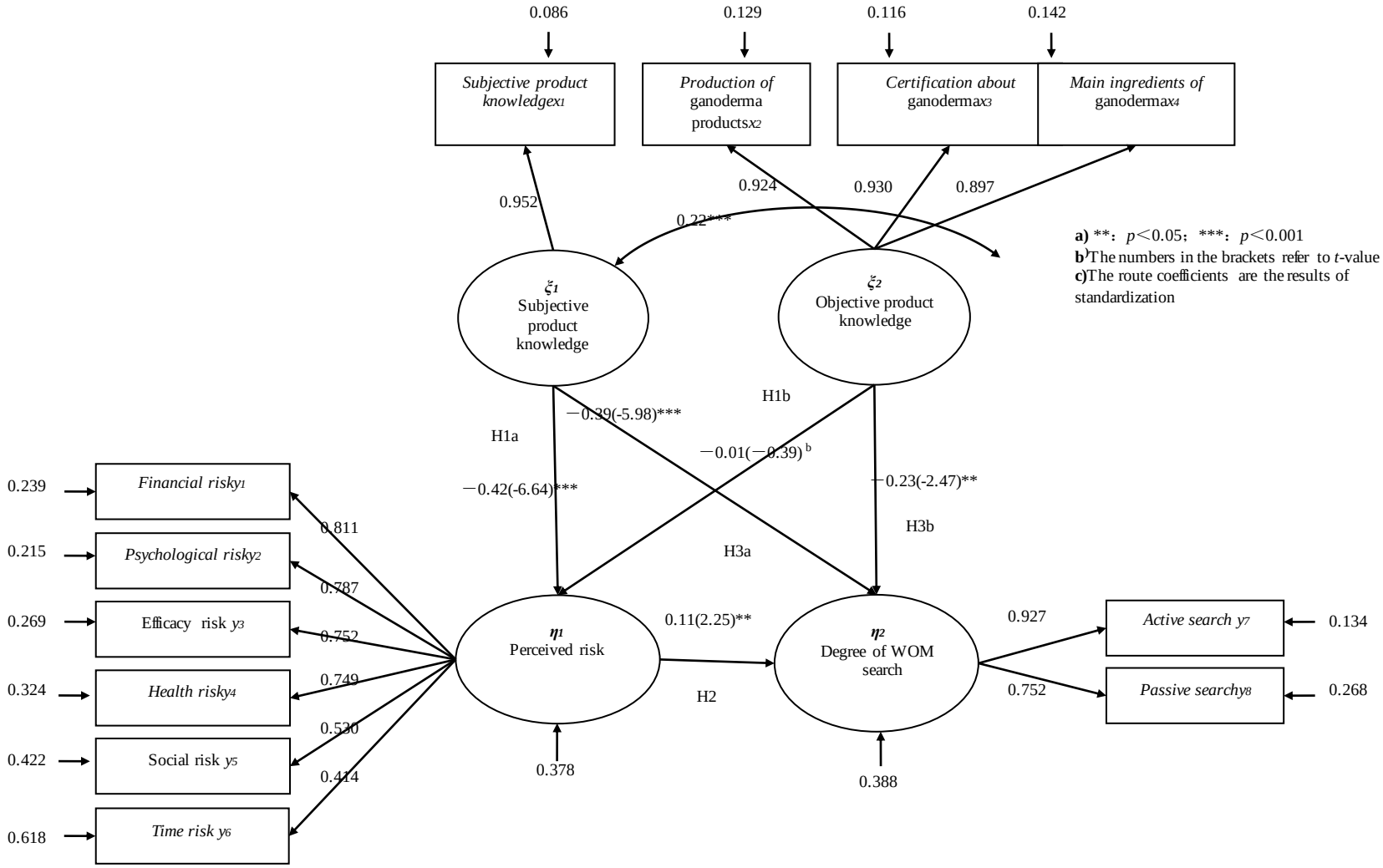

Fig.2. Causal relationship of product knowledge, risk perception and WOM search behavior

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Key Agri-Climatic and Socio-Economic Indicators for Cereal Production across the World

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Abstract**—** *This research focuses on the identification of key indicators of climate change impacting the cereal crop yields for fourteen countries across the world employing the principal component analysis (PCA) and the linear scoring technique using the World Bank Data for the period 1961 to 2013 for all indicators for all the countries. The Climate Change Crop Performance Indices (CCCPIs) are generated for each country for the first time using both the climatological and socio-economic indicators. These indices are used for comparing and monitoring the relative crop performance during the study period. The locations under study included Canada and Mexico from North America; Argentina, and Cuba from Latin America; France, and Portugal from Europe; Iran, and Israel from Middle East; Liberia and Somalia from Africa; and Mongolia, Nepal, Myanmar, and Philippines from Asia. Based on the PCA analysis and underlying assumptions, the following list of key indicators are identified for each country: Canada: temperature, CO_2e and LACP; Mexico, France and Israel: temperature, CO_2e and RF; Argentina and Cuba: CO_2e and RF; Portugal and Somalia: Temperature, CO_2e, LACP and RF; Iran: temperature, CO_2e, CY and RF; Liberia and Mongolia: CO_2e, CY and temperature; Nepal: CO_2e, CY and RF, and Myanmar and Philippines: temperature and CO_2e;. These indicators provide a signal of the desirable or undesirable changes in climatological or socioeconomic parameters that have occurred or may occur in future in the above-mentioned countries. These key indicators might help even the technology developers,* land managers and the policy makers to develop new strategies and formulate new policies.

Keywords**—*climate change, crop performance, principal component analysis, temperature, rainfall, CO_2 emissions*.**

I. INTRODUCTION

Agriculture is the key economic and sustainable development sector, sensitive [1] and vulnerable [2, 3] to climate change. It accounts to an average of 28% of the gross domestic product for many low-income countries[4] and its sustainability depends on many drivers acting at multiple scales from local to global [5].Majority of the crops are grown based on the role of climate change in agricultural productivity[6]. During the past years, due to climate change, most of the agricultural crops are reported to have a slower growth [7]. In places, where the agriculture is vulnerable to extreme climate variability like recurring droughts, floods, poor distribution of rainfall, temperature variations and carbon dioxide (CO_2) concentrations, the yields are directly affected especially during critical crop growth periods [3], which in turn has profound impact on the food security of a nation [8].Among the various factors, temperature, precipitation and CO_2 are the most influential ones affecting the crop yields directly and indirectly [7] causing a decline in the performance of most of the crops across the world by the end of the century. The broad agreement among the climate scientists is that due to an increase in CO_2 emissions under various scenarios, there will be an increase in temperatures by 1-5^{o}C to 5.8^{o}C by 2100.This will result in a shift in precipitation patterns that will cause the ecosystems to move poleward. This will also create a huge impact on the global economy and consequently decline in the quality of life [1]. For instance,

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it has been reported that there has been an approximately 40 million tonnes yield loss or $5 millions per year economic loss of the major crops such as wheat, maize, and barley, demonstrating the negative impacts of climate change [9].

To assess the effects of climate change on agriculture, literature provides an evidence of employing various impact assessment models like the climate, crop, and economic models, crop simulation models [8, 10], statistical models, process-based agro ecosystem models [11] and integrated ecological-economic modelling framework resulting in a wide range of projected climate outcomes [12]. Each of these models have their own advantages and shortcomings, presenting different levels of complexity and completeness in relation to the specific aspects considered in its analysis. In addition to the modelling approach, the impact of the climate change on agricultural sector can be assessed by selecting appropriate indicators and studying their changes in a long run. Indicators are any observations or measurable attributes that can be used to track changes or trends in any system. But the selection of appropriate indicators for climate change studies is very critical and depends on the consideration of environmental change and climate change from various perspectives. The indicators chosen should be continuous in time and space, easily measurable, monotonically increasing or decreasing, be a state variable, have a long record of observations with low natural variability [13]. In relation to agriculture, several indicators have been developed for climate smart agriculture [14] and environmental and socioeconomic indicators for measuring the outcomes of on-farm agricultural production [15]. For the Organisation for Economic Co-operation and Development (OECD) countries, which are the world's food suppliers, agri-environmental indicators have been identified related to soil water, air, and biodiversity [16]. Another study identified indicators for environmental sustainability of agriculture including waste, GHG emissions, land conversion, soil health, nutrients, and pesticides [17]. But studies involving the agri-climate change indicators are meagre and needs further research.

The current study has been aimed to identify the agro-climatological and socio-economic indicators which make an impact on agriculture, especially on the cereal yields at different geographical locations under different climatic conditions worldwide. The socio-economic indicators reflect the impacts on the current and future agricultural productivity in both developed and developing countries. However, in some of the developing countries, agriculture is purely based on the climatic conditions in addition to other factors like soil types, agricultural inputs, etc. Based on the key indicators identified, an index would be computed to show how any location would perform in terms of cereal production under the influence of the key indicators. This index would be termed as the Climate Change Crop Performance Index (CCCPI) and doesn't exist in the literature. This index might help in focussing on monitoring or keeping a watch on the qualified indicators and formulating suitable adaptation and mitigation strategies, develop suitable crop and land management technologies, frame relevant climate change policies to overcome or deal with the negative or positive consequences of climate change on the cereal crop production.

II. MATERIALS AND METHODS

2.1. Study Area

This study adopted fourteen heterogenous locations across the world representing various climatic regions. The criteria for selection of these countries include: locations having agriculture especially cereal production as one of the main livelihoods, their vulnerability to climate change variability and extremes, and their contribution towards GDP. The countries selected for the study were: Canada and Mexico from North America; Argentina and Cuba from Latin America and Caribbean; France, and Portugal from Europe; Iran, and Israel from Middle East; Liberia and Somalia from Africa; and Mongolia, Nepal, Myanmar, and Philippines from Asia.

2.2. Climatological and socio-economic Indicators

The variables used in the present study as indicators include the climatological indicators, which are, temperature and precipitation, and the socio-economic indicators, which are, Carbon dioxide emissions(CO_2e), cereal yield (CY), and land area under cereal crop production (LACP) (ha). The data for these indicators has been obtained from the World Bank data repository for a period from 1961 to 2013[18]. The observation records include the monthly temperature and precipitation for all the above-mentioned locations. CO_2emissions data, measured in kilo tons (kt), includes the emissions from burning of the fossil fuels and the manufacture of cement, consumption of solid and gas fuels and gas flaring. The cereal yield data, measured in kilograms per hectare (kg ha^{-1}), includes the dry grain yields for wheat, rice, maize, barley, oats, rye, millet, sorghum, buckwheat, and mixed grains and excludes the crops harvested for fodder, harvested for hay and food, feed, grazing, and silage purpose [18].

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2.3. Methodology for identification of key indicators and computation of Climate Change Crop Performance Index (CCCPI)

The prime objective of this study is to identify the key agri-climatic indicators and compute the crop performance indices under varied climate conditions termed as "climate change crop performance index" (CCCPI). The methodology to calculate this index has been adopted with a few modifications from Andrews et al., Doran and Parkin [19], [20], followed by Sharma et al. [21, 22]. Although similar approach in computing the CCCPI has been employed in the present study, the current study will use the agriculture related climate change indicators for the first time to assess the impacts under climate change conditions. Principal component analysis and linear scoring techniques are used in calculating the index.

The initial step includes testing the indicators for their levels of significance using Mann-Kendall test and eliminate the non-significant indicators from the assessment process [23]. In the present study, as the number of indicators is low, all the indicators, irrespective of their levels of significance, have been included in the study. This is followed by identification of minimum data set (MDS) which is the smallest set of the indicators, that best represents the specific region and can be used to assess the impacts of climate change. All the indicators which have been retained in the minimum data set need not to be considered as the key indicators, but only the most appropriate ones selected based on further statistical analysis. It is only these key indicators which are included in computation of CCCPI. This is done by using principal component analysis (PCA) where the data is subjected to reducing the dimensionality (number) of the variables and retains only the original variability. Hence the principal components which received the Eigen values ≥ 1 and which explained at least 5 % of the variation in the data set [24] and which had high factor loadings are considered as the best representatives of the system attributes. Within each PC, only the highly weighted factors are retained for the MDS. In cases, where more than one variable was qualified, correlation analysis was performed to determine if any of the variables could be considered redundant and dropped from the MDS. Among the well correlated (r > 0.70) variables, only one variable with higher correlation sum is considered for the MDS. However, flexibility criteria were followed depending upon the importance of the variables for some of the locations. In cases, when the highly weighted variables are not well correlated, they were retained in the minimum data set. The variables thus qualified under these series of steps were termed as the key indicators. These key indicators were considered for computing the CCCPIs of various locations under the study.

Once the key indicators were identified, all the observations for these indicators were transformed using linear scoring technique [20]. To assign these scores, first the indicators were identified if a higher or the lower values were considered 'good' or 'bad'. In case of indicators with "higher values are better", each observation was divided with the highest value such that the observation with the highest value gets a score of one. In the case of "lower values are better" indicators, the lowest value was divided with each observation such that the lowest value gets a score of one. Once the observations are transformed using linear scoring, each observation of the MDS indicators were multiplied with the weighted factor using the PCA results. The weighted factor was obtained by dividing the % of variation explained by each PC with the total percentage of variation explained by all the PCs with only eigen value >1. Once these weighted scores were obtained for the MDS indicators, they are summed up for each observation to arrive at the CCCPIs using the following relation:

$$\text{CCCPI} = \sum_{i=1}^{n}(W_i S_i)$$

where, W_i is the weighted factor obtained from the PCA and S_i is the variable score. The underlying assumption is, higher the CCCPI value, greater is the crop performance under the prevailing climatic changes. Thus, the relative crop performance during various years of the study period under various locations has been assessed and the key climate change indicators which are responsible for causing any change in the crop performance can be monitored.

III. RESULTS

The results of identifying the minimum dataset, selection of key indicators and computation of the CCCPIs are presented here and the importance of the key indicators identified are also discussed briefly.

3.1. Identification of key agri-climate change indicators

To identify the key indicators of climate change which influence the cereal crop production, all the indicators i.e. temperature (Temp), rainfall (RF), CO_2 emissions (CO_2e), cereal yield (CY), and land area under cereal production (LACP) were considered. In the primary step, out of the five indicators selected for the study, rainfall showed statistical significance only for few countries out of all the locations (Table1). However, this climate variable has been retained in the data set for further processing since it is one of the most

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climatologically important indicators. The results of the PC analysis (Table 2) reveal that only two PCs i.e. PC1 and PC2 with eigen value >1 are qualified for all the locations except for Philippines and Myanmar. These PCs explained a cumulative variability ranging from 61.5 to 89.0% across the locations.

For both Canada and Mexico, PC1 included similar highly weighted variables i.e. temperature, CO_2e and the crop yields. But in PC2, Canada had LACP while Mexico had RF as the highly weighted variables. For PC1 of Canada, temperature did not show any correlation with other indicators while CO_2e and the CY showed a significant correlation (r = 0.78) due to which only CO_2e indicator has been retained for CCCPI calculation. While for Mexico, as all the three indicators were significantly correlated, correlation sums were calculated for all the three indicators. CO_2e and CY showed similar and higher correlation sum of (r = 2.70), and hence only CO_2e has been selected. Though temperature showed a lower correlation sum, it was also retained in the MDS based on its significant role in the climate change. Hence, for Canada, the indicators retained as the key indicators in the MDS included temperature, CO_2e and LACP while for Mexico, the key indicators retained were Temperature, CO_2e and RF.

Both Argentina and Cuba showed only two PCs with eigen values >1. Out of these in PC1, both the locations had same highly weighted variables i.e. CO_2e and CY while in PC2 both the locations showed RF as the highly weighted variable with a cumulative variance of 71.6 and 65.2% respectively. Significant correlation existed between CO_2e and CY of PC1 for Argentina (r = *0.81*) and Cuba (r = 0.70) due to which only the CO_2e has been retained for the MDS. Hence, for both Argentina and Cuba, the highly weighted variables which were retained as key indicators in the MDS were CO_2e and RF.

Similarly, for France and Portugal, only two PCs were qualified. Under PC1, in case of France, three variables i.e. temperature, CO_2e and CY were highly weighted variables while for Portugal LACP is also qualified along with the above three variables. But in PC2, both the locations showed RF as the highly weighted variable in the data set. Among the variables in PC1 of France, CO_2e did not show any significant correlations with other variables while temperature was well correlated with the CY and CO_2e. Though, CY showed highest correlation sum, it has not been retained in the data set due to its correlation with temperature. Hence, temperature and CO_2e were retained as the variables for the MDS in PC1. In case of Portugal, among the variables of PC1, temperature did not show any significant correlation with other variables while CO_2e, CY and LACP were well correlated between each other. Between CO_2e and CY variables, CO_2e had the highest correlation sum (r = 3.33) compared to CY (r = 3.26) and has been retained. But compared to CO_2e, LACP had slightly higher correlation sum (r = 3.39) and is retained as an indicator in the MDS. Hence, for the European countries, the variables which are retained as key indicators in the MDS include: Temperature, CO_2e and RF for France; and Temperature, CO_2e, LACP, and RF for Portugal.

In the case of Iran and Israel, two PCs were qualified and explained a cumulative variability of 83.4% and 82.3% respectively. Under PC1, for Iran, temperature, CO_2e and CY were highly weighted and for Israel, temperature, CO_2e and LACP were found to be highly weighted variables. In PC2, for both the locations, RF was the only variable which was found to be highly weighted. In case of Iran, under PC1, though all the variables were found to be highly correlated, all the three variables were retained for the MDS irrespective of their correlation sums considering the importance of these indicators. In the case of Israel, among the three variables, both CO_2e with the highest correlation sum (r =2.64) and temperature with correlation sum of r =2.42 were retained in the MDS due to their primary role in these areas. However, the LACP with the lowest correlation sum was dropped out from the MDS. Hence, the variables which are retained as the key indicators in the MDS for Iran include: Temperature, CO_2e, CY and RF; while for Israel: temperature, CO_2e and RF.

For both Liberia and Somalia, the PCA analysis showed two qualified PCs explaining a less percent cumulative variability of 58.8% and 62.7% respectively. In PC1, for Liberia, CO_2e and CY were found to be the highly weighted variables while, for Somalia, temperature, and CO_2e were the highly weighted variables. In the case of PC2, for Liberia, only temperature has been found to be the highly weighted variable while for Somalia, both RF and LACP were qualified as highly weighted variables. It was quite interesting to note that none of the variables either in PC1 or in PC2 for both the locations showed any significant correlation and each were independent. Hence all these variables were inevitably retained in the MDS as indicators. Overall, the variables which are qualified and retained as the key indicators in the MDS were:CO_2e, CY and temperature for Liberia; and temperature, CO_2e, RF and LACP for Somalia.

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Table.1: Tests of significance for the indicators used in the computation of climate change crop performance index

	Temperature (°C)	Rainfall (mm)	CO_2 emissions (kt)	Cereal Yield (kg ha^{-1})	Land area (ha)
North America					
Canada	**	**	**	**	**
Mexico	**	NS	**	**	**
South America					
Argentina	**	NS	**	**	**
Cuba	**	NS	**	**	**
Europe					
France	**	NS	**	**	NS
Portugal	**	NS	**	**	**
Middle East					
Iran	**	NS	**	**	**
Israel	**	NS	**	**	**
Africa					
Liberia	**	NS	**	**	NS
Somalia	**	NS	**	**	NS
Asia					
Mongolia	**	NS	**	*	*
Nepal	**	NS	**	**	**
Myanmar	**	**	**	**	**
Philippines	**	NS	**	**	**

*Note: * = $p<0.05$ and ** = $p<0.001$*

Among the Asian countries, Mongolia and Nepal showed two qualified PCs with eigen values greater than 1 and which explained a cumulative percent variability of 67.5 % and 86.4 % respectively. However, in PC1, for Mongolia, Temperature and CO_2e were found to be highly weighted variables while for Nepal, temperature, CO_2e and CY were found to be highly weighted variables. In the case of PC2, CY and RF were found to be highly weighted variables for Mongolia and Nepal respectively. However, the variables qualified under PC1 for Mongolia were not significantly correlated with each other and hence were retained in the MDS. But in the case of Nepal, all the three variables were found to be significantly correlated with each other and hence the correlations sums were worked out. Among the three variables of PC1 of Nepal, CO_2e and CY showed similar correlation sums of r=2.63 and r=2.62 respectively while temperature had the lowest (r=2.47). Hence, CO_2e and CY were retained to be qualified as indicators in the MDS. While in case of Myanmar and Philippines, only one PC with eigen value >1 was qualified for both the locations explaining 88.9 % and 84.3 % cumulative variation respectively. PC1 of Myanmar showed all the variables i.e. temperature, RF, CO_2e, CY and LACP to be highly weighted while Philippines also showed similar variables except RF. For both the locations, correlation analysis was performed which showed significant correlations between the variables. For Myanmar, temperature did not show much significant correlation with any of the parameters except CY while the rest of the indicators CO_2e, CY and LACP were found to be well correlated. Among these three variables, CO_2e had the highest correlation sum followed by CY, LACP and RF. Hence, among these variables, temperature has been retained due to its non-significant correlation with other variables except CY. But CO_2e was retained due to its high correlation sum. But in the case of Philippines, all the variables viz., temperature, CO_2e and CY were well correlated while LACP did not show any significant correlation with any of the variables and had the lowest correlation sum. Hence for Philippines, temperature and CO_2e were retained as indicators in the MDS.

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Table.2: Principal component analysis (PCA) – Factors, correlations sums, percent variance and variables selected for all the countries under study

	Components and variables qualified		Correlation sums
	PC1	PC2	
North America			
Canada	Temp, CO_2e, CY	LACP	2.08, 2.32, 2.32
Mexico	Temp, CO_2e, CY	RF	2.44, 2.70, 2.70
Latin America			
Argentina	CO_2e, CY	RF	1.81, 1.81
Cuba	CO_2e, CY	RF	1.70, 1.70
Europe			
France	Temp, CO_2e, CY	RF	1.76, 1.71, 2.01
Portugal	Temp, CO_2e, CY, LACP	RF	2.72, 3.33, 3.26, 3.39
Middle East			
Iran	Temp, CO_2e, CY	RF	2.40, 2.55, 2.57
Israel	Temp, CO_2e, LACP	RF	2.42, 2.64, 2.16
Africa			
Liberia	CO_2e, CY	Temp	1.39, 1.39
Somalia	Temp, CO_2e	RF, LACP	1.39, 1.39
Asia			
Mongolia	Temp, CO_2e	CY	1.26, 1.26
Nepal	Temp, CO_2e, CY	RF	2.47, 2.63, 2.62
Myanmar	Temp, RF, CO_2e, CY, LACP	-	3.51, 3.82, 4.18, 4.07, 4.08
Philippines	Temp, CO_2e, CY, LACP	-	2.97, 3.27, 3.31, 2.81

Note: PC = Principal component; Temp = temperature; RF = rainfall; CO_2e = CO_2 emissions; CY = Crop Yield; LACP = Land area under cereal production

In this case, LACP was also required to be included but as it was not found as a limiting factor in the study and hence, it was not considered. However, based on various assumptions and decisions, the variables chosen to be retained as key indicators in the MDS were: temperature, CO_2e and CY for Mongolia; CO_2e, CY and RF for Nepal; Temperature and CO_2e for Myanmar; and temperature and $CO_2e$for Philippines.

3.2. Climate Change Crop Performance Indices (CCCPI)

After identifying the key indicators, linear scoring was performed for all the selected variables in dataset. Temperature which was identified as the key indicator for most of the locations, was considered "more is better" for countries like Canada, Cuba, France, Portugal, and Mongolia while for rest of the countries, it was considered "less is better". Rainfall has been assigned "more is better" status for most of the locations where it has been qualified as an indicator except for Nepal. CO_2ewasconsidered "less is better" indicator for all the locations due to its deleterious role in climate change and its impact on agricultural sector. The CY and LACP were considered as "more is better" indicators for all the locations for which they were qualified. Each observation of the indicators was assigned the linear scores, multiplied by the weighted factors of the qualified PCs (Table 4) and the values were summed up to arrive at CCCPI values. These CCCPI values were computed for all the locations for all the years and would help to assess the relative performance of the cereal crops between the years as influenced by the climate change.

The graphical representations of the CCCPI indices against all the indicators (Fig 1 to 4) elucidates the relative trend in response to the indicators and correlations were worked out (Table 4) to make a comparative analysis.

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Table.3: Weighted factors obtained from the qualified principal components of the PCA

	% variance		Cum. variance
	PC1	PC2	
North America			
Canada	54.51 (0.73)	20.19 (0.27)	74.69
Mexico	57.15 (0.69)	25.41 (0.31)	82.56
South America			
Argentina	45.40 (0.63)	26.20 (0.37)	71.60
Cuba	44.74 (0.69)	20.52 (0.31)	65.25
Europe			
France	46.25 (0.69)	20.68 (0.31)	66.92
Portugal	64.07 (0.76)	20.22 (0.24)	84.29
Middle East			
Iran	58.85 (0.71)	24.54 (0.29)	83.39
Israel	61.52 (0.75)	20.81 (0.25	82.33
Africa			
Liberia	32.14 (0.55)	26.80 (0.46)	58.84
Somalia	36.59 (0.58)	26.09 (0.42)	62.68
Asia			
Mongolia	36.07 (0.54)	31.38 (0.47)	67.45
Nepal	66.29 (0.77)	20.11 (0.23)	86.39
Myanmar	79.03 (0.89)	9.83 (0.11)	88.86
Philippines	64.55 (0.77)	19.73 (0.23)	84.28

Note: Figures in parenthesis are the weighted factors for each PC from the PCA

For the North and Latin American countries, CCCPI indices were slightly higher in the early years and thereafter tended to decline slowly up to 1970s and remained steady with some variabilities in between. Canada's CCCPI curve, declined slowly in the beginning and remained nearly constant thereafter throughout the years with rise in the climatic and socio-economic indicators. For Mexico and Argentina, the CCCPI showed a decline with rise in all the indicators expect the land area in case of Argentina. Cuba showed a lot of variability in the crop performance as influenced by climate change.

Of the European countries (Fig 2), the CCCPI indices for France tended to slightly rise over the years irrespective of the increase or decrease in the climate change variables. The CCCPIs for Portugal showed a slight decline during the years 1961 to 1975 and then remained steady until 2001 and thereafter showed an increase. In the case of the Middle East countries, Iran showed a slight decline in the CCCPI up to 1966 and thereafter remained constant. While for Israel, the CCCPI tended to decrease and was observed to decrease gradually over the years. In case of African countries, the CCCPI did not show any consistent rise or decline and displayed inconsistencies between the years. However, Liberia depicts an increase in the CCCPIs during the initial years. For Asian countries, a varied performance in the CCCPI has been observed across the study locations. Mongolia showed a lot of variations while in case of Nepal, Myanmar, and Philippines, the CCCPIs presented an initial steep decline and thereafter a gradual decline except for Nepal which showed a slight rise.

CCCPI showed a positive correlation with temperatures for Canada, Portugal, France, Iran, and Liberia while the rest of the countries showed a negative correlation. However, the correlations were significant and positive for France and significantly negative for Myanmar and Philippines. CCCPI showed a non-significant negative correlation with rainfall for Canada, Mexico, Liberia, and Philippines while the rest of the countries exhibited a positive correlation, of which

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Table.4: Correlations between CCCPI Indices and the climatological and socio-economic indicators

	Temperature (°C)	Rainfall (mm)	Cereal Yield (kg ha^{-1})	CO_2 emissions (kt)	Land area under cereal production (ha)
Canada	0.12	-0.34	-0.52	-0.73	0.17
Argentina	-0.29	0.08	-0.81	-0.91	0.32
Mexico	-0.57	-0.02	-0.86	-0.90	-0.51
Cuba	-0.32	0.22	-0.69	-0.92	-0.32
Portugal	0.31	0.38	0.38	-0.01	-0.03
France	0.84	0.13	0.91	-0.59	-0.44
Iran	0.11	0.15	0.20	0.00	-0.37
Israel	-0.59	0.41	-0.63	-0.87	0.85
Liberia	0.21	-0.23	0.33	-0.51	-0.41
Somalia	-0.40	1.00	-0.05	-0.11	0.21
Mongolia	-0.20	0.18	0.14	-0.13	-0.14
Nepal	-0.15	0.002	0.12	-0.17	-0.50
Myanmar	-0.62	0.66	-0.87	-0.89	-0.76
Philippines	-0.72	-0.15	-0.75	-0.81	-0.78

only Somalia and Myanmar were significant. CCCPI exhibited a significant negative correlation with CO_2e for all the locations and the correlations were non-significant for Portugal, Iran, Somalia, Mongolia, and Nepal. Increase in the CO_2 emissions showed a decline in the CCCPI performance. France is the only country which showed a decrease in CO_2 emissions and hence the rise in CCCPIs while Liberia, Somalia, and Mongolia, did not show any conspicuous response irrespective of the huge variability. CCCPI revealed a positive correlation with cereal yields for Portugal, France, Iran, Liberia, Mongolia, Nepal. However, the correlation was significant only for France. For the rest of the locations, CCCPI had significant negative correlations except for Somalia. In the case of land area, the CCCPIs showed a positive correlation for Canada, Argentina, Israel, and Somalia of which Israel showed high positive significance. For rest of the locations, CCCPI showed a negative correlation of which Myanmar and Philippines showed a high significance while Portugal showed the lowest. However, it is beyond the scope of this study to discuss and present the underlying reasons for all the trends observed.

IV. DISCUSSION

The key agri-climate change indicators which have been identified for each country were most relevant to the climatic pattern of each location. For most of the locations, temperature and CO_2e were qualified to be the major indicators in PC1 except for Argentina, Cuba, and Nepal. Temperature affects the crop yields to a greater extent [27] by playing a significant role at all stages of plant development influencing the key physiological processes of flowering and grain filling in cereal crops [25, 26]. Additionally, the significant alterations in global climate due to rapid increases in CO_2 emissions is also an influential climate factor [28, 29] causing declines, stagnations, collapses and increased year to year variations in crop yields [7, 30, 31]. Thus, both temperature and CO_2 emissions have interconnected influence on agricultural production. Doubling of CO_2 might raise the temperature and absolute humidity indirectly influencing the soil and plant evaporation, soil and ground moisture storage, length of growing season, heating degree days, and crop heat units. High temperatures during growing season, might speed up crop maturity, but temperature induced water stress might contribute largely to yield decreases in crops such as barley, wheat, oats, sunflower, and canola. Conversely, the projected warm temperatures and the long growing seasons, might be beneficial to crops such as corns, soybeans, potatoes, beans, and sorghum and less affected by plant water stress. Indirect effects of temperature changes would be, the incidence of pests and pathogens and the dependence of existing varieties more on irrigation for sustenance [32].

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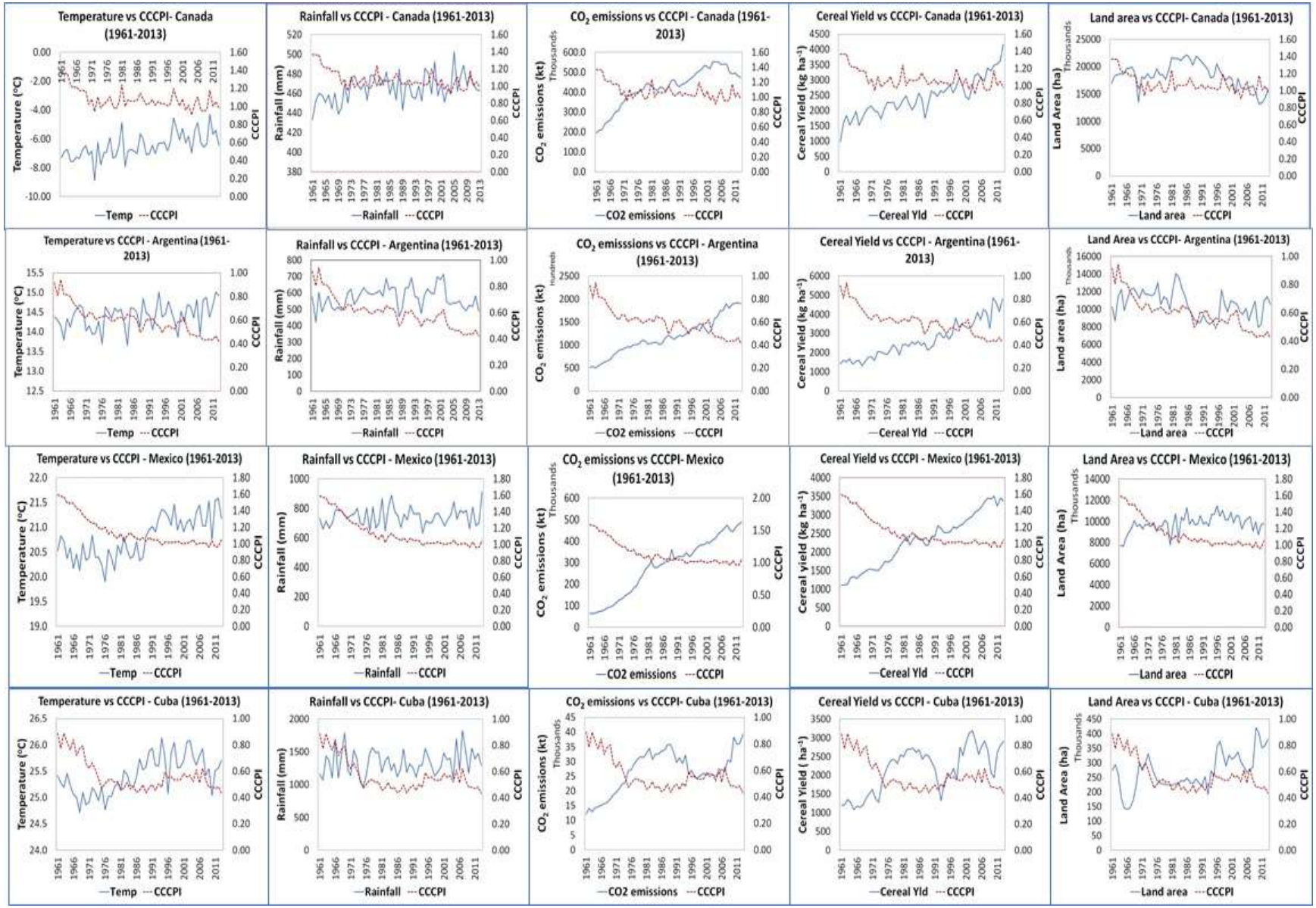

Fig.1: Climate Change Crop Production Indices (CCCPIs) plotted against each indicator for North and Latin American countries for the period 1961-2013

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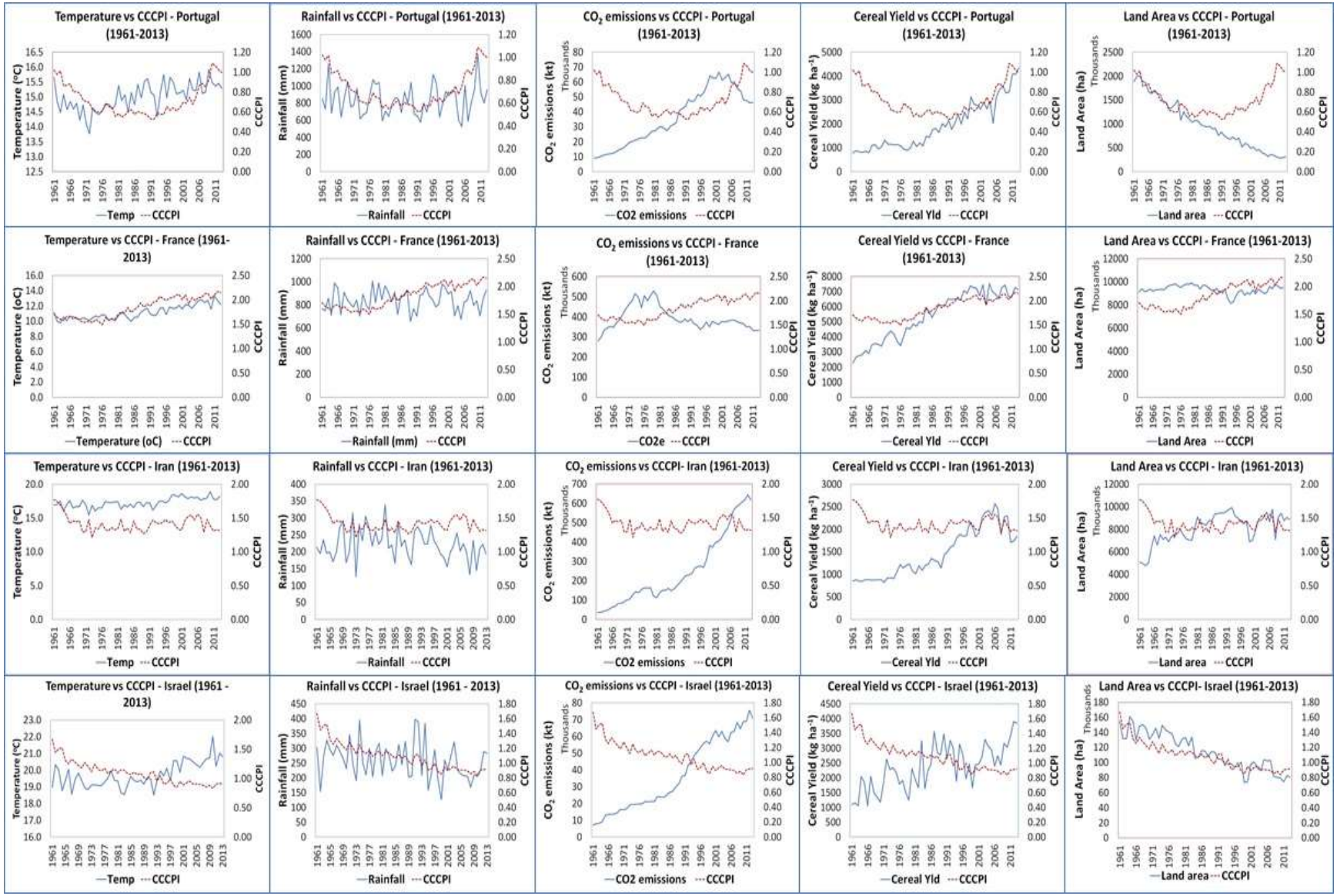

Fig.2: Climate Change Crop Production Indices (CCCPIs) plotted against each indicator for European and Middle East countries for the period 1961-2013

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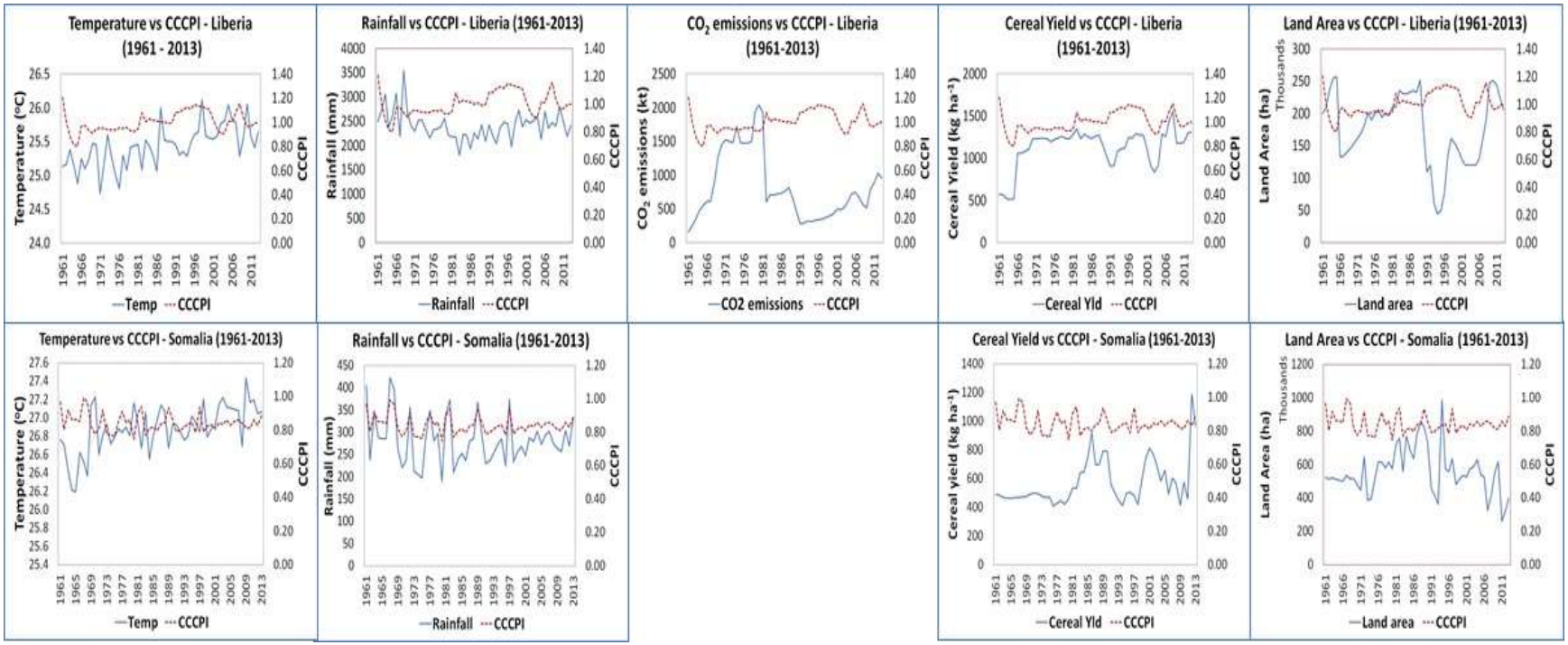

Fig.3: Climate Change Crop Production Indices (CCCPIs) plotted against each indicator for African countries for the period 1961-2013

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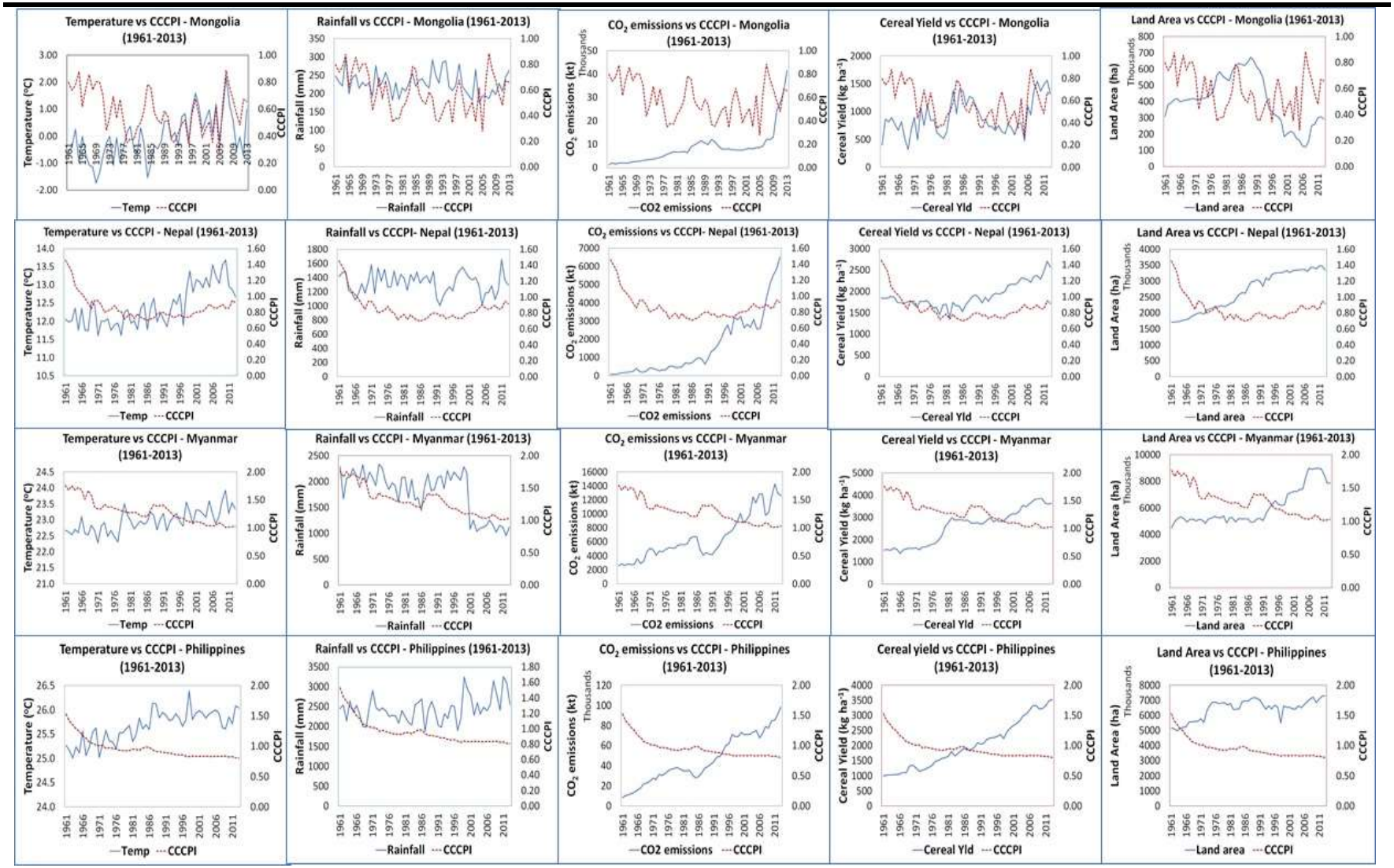

Fig.4: Climate Change Crop Production Indices (CCCPIs) plotted against each indicator for Asian countries for the period 1961-2013

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Rainfall has emerged as one of the key indicators in PC2 for most of the countries, except Canada, Liberia, Mongolia, Myanmar, and Philippines. During the period of study, almost all the countries, with a few exceptions like Nepal and Portugal showed a decrease in rainfall over the successive decades with some variations in between (Fig 1 to 4). The African and the Asian countries with higher temperatures showed a decrease in rainfall, while the American and European countries showed very slight increase. Though rainfall might not be a limiting factor for countries receiving abundant rainfall per annum, but the change in its magnitude and timing is one of the most significant consequences of climate change [33].Increased rainfall events might reduce the pesticide efficacy requiring heavier and more frequent applications thus leading to increased costs and externalities [34]. For countries like Somalia, Israel, Iran, which receive very less amount of rainfall, it is an important indicator. In the case of Mongolia and Canada, though they receive less amount of rainfall, they also experience long and cold winter with precipitation as snow. Hence, rainfall has not emerged as an indicator for these countries. For high rainfall areas, rainfall distribution remains to be prime determinant of mitigation effects of adopting specific sustainable land management practices [35, 36]. In general, due to the uncertainties in the rainfall, shifts in seasonality, and the extent of rainfall, the concern and adaptation strategies for potential climate change are mostly focussed on agriculture sector [37].

Land area under cereal production (LACP) emerged as a key indicator only for some of the locations such as Canada, Portugal, Somalia, and Philippines and has been retained in the MDS. Canada, Portugal, Somalia, and Israel showed a strong decreasing trend in LACP over the decades. In Canada, the agricultural landscapes are impacted differently by temperature and precipitation by way of increase in spring precipitation and runoff, and high intensity storms, reduced sea ice cover, reduced summer rainfall, increasing drought frequency, and increasing demands for water. These factors challenge the water management causing decline in land suitability for some of the small grain crops [38]. The increase in droughts in Southern Prairies of Canada, thus shifting the production areas northward, could be one of the factors in reduction in land use [39]. In the case of Portugal, there is a drastic reduction in the land area under cereal production from 1961-2013 but the cereal yields showed an increase with high variability. This reduction might be due to the industries and service sectors increasing at a faster pace than agriculture [40] during 60s and 80s, urbanization and agricultural land abandonment in coastal areas during 1990 to 2000 [41], and permanent grassland and meadows gaining importance resulting in a decrease in the arable land during the 2000s [42]. There was agricultural intensification during this period thus improving the cereal yields [41].

Crop yield (CY) emerged as an indicator for most of the locations but has been retained only for Iran, Liberia, Mongolia, and Nepal. For Iran, the crop yields increased gradually up to the years 2005 and thereafter started declining which requires further insight. Liberia, and Mongolia showed huge fluctuations in cereal yields. Nepal showed increase in cereal yields over the years which might be due to increase in the land area under cereal production. It is necessary to understand the complex interactions of the effects of insects, weeds, and diseases on agricultural production thus necessitating the crop diversification as a measure to fight the climate change effects [34]. Despite the advancement in the crop production technologies including intensive research and breeding techniques, the climate and weather have a strong hold on the agricultural production in many ways [43]. Environmental stresses such as droughts and high temperatures will also become the key stress factors having a major impact on crop yields [44].

A clear perception of the possible impacts of climate change on crop production over time across the locations is needed to facilitate more informed climate change mitigation and adaptation strategies and policies [45] without which the long-term mean crop yields are likely to decline [25]. However, it would be difficult to isolate the compounding impacts of climate change, the technological advancements and the socioeconomic conditions on crop production. Hence, this indicator selection approach is an excellent option to investigate the effects of individual output by keeping all other factors constant [46]. These key indicators identified for each country can be centred to frame the adaptation and mitigation strategies.

The key indicators identified for Canada include temperature, CO_2e and LACP and the CCCPIs had significant negative correlation with the CO_2e. The temperatures of Canada are increasing gradually, CO_2e are already in rise while LACP is decreasing (Fig 1). These are the indicators which need to be given a careful consideration. Already a declining trend in CCCPI has been observed and future changes in any of these indicators might alter the CCCPI. Despite the predictable risks of extreme temperatures and droughts, the cereal yields have been

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managed and improved over the years with the adoption of processes like minimum tillage [38], conservation tillage, reduction in summer fallow, adoption of precision agriculture, enhanced crop rotation, improved cultivars, increased nitrogen use efficiency and improvements in technology. These processes also facilitated the increased removal of $CO_2$from the atmosphere and sequestration in the soil [47, 48, 49]. Although, there is expected possibility of increase in yields with increase in temperatures for some crops, the negative consequences from the introduction of new diseases and pests can negate the yield increases [50, 51]. Studies predict increased length of growing season, less cool periods, more hot periods, increase in crop heat units and growing degree days by 2040-2069 due to temperature rises; lower water stress for crops due to increased water use efficiency of crops under elevated CO_2 and increased crop yields [51]. However, predictions also exist for decreased snow accumulation, reducing spring runoff and increasing desertification thus calling for improved land and water management strategies [34].

The key agri-climatic indicators identified for Mexico were Temperature, CO_2e and RF and the CCCPI showed a negative correlation with all these indicators (Table 4 and Fig 1). The factors like low and intense rainfall, high CO_2 emissions and risk of frequent droughts and floods, hurricanes, climate change variability, long and hotter periods, and land use transformations constrain the food production in Mexico. But it can be observed that there is an increase in crop yields over the decades which might be due to the adaptation measures undertaken like formulating irrigation development policies, increased use of chemical inputs, land development and transformation, infrastructural and technological development, etc., to fight against the climatic change and the crop production risks [52, 53].

The key indicators qualified for Argentina and Cuba include CO_2e and RF. The CCCPI exhibited a positive correlation with rainfall and negative with CO_2 emissions (Table 4 and Fig 1). In Argentina, weather remains to be one of the most uncontrollable factors affecting agriculture mainly due to its inter-annual variability [46]. There was an increase in the temperature during the last decade and a slight decrease in the total annual rainfall and continuous increase in CO_2 emissions over the years of study period. Over the years, land area varied and increased crop yields during 1970s [54] while in the later years, it showed a decrease due to urban expansion [55]. Despite these variabilities, significant increases in productivity have been observed due to the agricultural transformation during the past 50 to 70 years owing to the adoption of no-till farming practices along with improved technologies, judicious fertilizer use, and less aggressive pesticides and use of improved varieties and hybrids [56, 57]. Though the cereal yields are increasing over the years, the declining trend in CCCPI from the present study, and the future predictions of increasing temperatures and decreasing crop yields calls for an attention to frame or strengthen the crop production strategies.

Similar to Argentina, Cuba also had RF and CO_2e as the key indicators which showed a positive and negative correlation respectively with CCCPI. Cuba is highly vulnerable in terms of water resources availability and water distribution. As the country lacks fresh water from rivers, and rainwater being the only available source for irrigation, makes RF as an important indicator. Cuban soils also exhibit poor soil structural and fertility properties. Added threats to agriculture from climate change in Cuba include seawater intrusions, rising median temperatures, shorter rainy seasons, suffocating summer temperatures, heavy rains, extended periods of drought and modifications in pest behaviour. Intense hurricane of 2008 resulted in decline in agricultural production [58]. Cuba has implemented sustainable organic farming as against the industrial farming like in Canada to mitigate the environmental impact on climate [59]. The use of most competent meteorological and extreme weather warning and response system, and the renewable energy sources has become a part of adaptation and mitigation programs [60].

For Portugal, the key indicators qualified were: temperature, CO_2e and LACP. The correlation effects of these indicators with CCCPIs were positive for temperature and negative for CO_2e and LACP but not significant. Agriculture mostly relies on wheat, corn, and rice. The overall agricultural performance in Portugal was unfavourable due to various factors. These include low level of agricultural investment, very low usage of machinery and fertilizer quantities, small and fragmented farms in the north not more than 5 ha maximum (mostly <1ha), incapability of modernizing the farms, poor productivity associated with low education levels of the farmers and finally, inadequate distribution channels and economic infrastructure [61]. In terms of climate change, Portugal has adapted conservation agriculture which reduced CO_2 emissions by decreasing number of farm machinery and by increasing soil carbon sequestration during the later years [62].

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For France, temperature, and CO_2e were qualified as the key indicators and showed significant positive and negative correlation with CCCPIs respectively. However, the CCCPI indices for France has tended to slightly increase over the years irrespective of the increase or decrease in the climate change variables. The average temperature of France showed an increase of 0.5ºC per decade which might increase the warmer summers rather than cooler summers. The CO_2 emissions here, especially the industrial sources are substantial and are the most worrisome due to their long residency in the atmosphere as well as the societal reliance on energy and industrial processes emitting them. Hence, France has adapted the mitigation technology of deploying the CO_2 emissions only from the industrial sources by way of CO_2 capturing, transportation, and storage within the country, to address the CO_2 emissions across the sectors [63]. Mitigation measures are being taken to bring the CO_2 emission levels down. Negative responses were observed in wheat and barley yields due to spring and summer temperatures in France [64]. In terms of crop performance, France shows a positive trend and might continue if the temperatures do not increase further. With the prevailing temperatures, France was not exempted to experience the severe heat waves during 2003 and 2006 as well as severe flooding in 2010. Hence, there is every possibility that, increase in temperatures might cause an increase in the water stress for which adaptive measures need to be taken [65].

For the Middle East countries, i.e. Iran and Israel, the key indicators qualified for both the countries were temperature, CO_2e and RF and showed non-significant correlations with CCCPI. For Iran, the temperature changed by 0.2ºC per decade while rainfall showed less variations. The CO_2 emissions showed a continuous increase over the study period. The land area under cereal production and the crop yields were also increasing with a drop only during the last decade. The CCCPI also showed a slight decline up to the years 1966 and thereafter remained steady throughout the years. Under these prevailing circumstances, Iran is just planning to take some proactive steps to reduce its carbon foot prints [66]. However, it needs to develop adaptive and mitigation measures to reduce the water crisis and CO_2 emissions apart from other direct and indirect climate change effects before the situation gets to any worse. Switching to low water requirement and temperature tolerant varieties might be a good adaptation measure, but lack of technology and knowledge of the process and consumption are important factors to be considered [67].

For Israel, the key indicators qualified were temperature, CO_2e and RF. Despite being small, its climate shows remarkable variability and changes over minor distances [68]. During the study period, the temperatures showed a slight increase after 2000 and the rainfall remained to be similar with a very slight decrease during 2000-2010 due to the severe drought [69]. The CO_2 emission levels showed an increase of approximately 1.5 times per decade since 1961. It was quite interesting to note that the cereal yields increased over the decades while the land area under cereal production showed a continuous decrease. CCCPIs tended to decrease gradually over the years and showed a significant negative correlation with temperature and CO_2e and a significant positive correlation with RF. Specially in drylands, agricultural production mainly depends upon the temperature and rainfall and the crop performance becomes vulnerable with the vulnerability in these indicators mainly when associated with the accumulation of greenhouse gases like CO_2 [2]. Israelis agriculture depends more on water, and to cope up with the temperature and rainfall anomalies, the government already issues incentives to farmers to use the water efficiently. Another interesting aspect of Israel is to have a unique system of investing its capital to substitute water for land by adopting drip irrigation and cover technologies thus shaping their agriculture to take advantage of the heat rather than becoming a victim [70]. Hence, it can be observed in the present study, that according to the prevailing climate, the CCCPI is decreasing but, despite the decrease in land area under cereals, the crops yields tended to increase because of the adaptive measures taken. However, the observed changes from the study suggest that Israel's climate is entering a new period of uncertainty where it is likely to influence the water resources and agriculture [69].

The key indicators qualified for Liberia were CO_2e, CY, and temperature. Rainfall did not emerge as an indicator because Liberia is blessed with abundant rainfall [71]. Liberia showed a variability in temperatures and the CO_2 emissions were low showing a great variability especially up to 1980 and increased during the last decade. The cereal yields showed a drastic increase over the decade 1961-70 and continued to maintain the same yields [72]. Irrespective of the wide variability in the indicators, the CCCPIs showed a non-significant positive correlation with temperature and negative correlation with CO_2e and CY and was not affected much by the variability of the indicator patterns over the years. Agricultural production in Liberia is based on rainfed farming with rice as the staple crop. Heavy reliance on

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rainfall exposes these farmers to vagaries of weather and rice would be negatively affected by the higher temperatures even though the precipitation is adequate [72]. Liberia, however, is vulnerable to climate variability thus presenting challenges to socio-economic development of the country. The civil war of 1989-2003 has played a detrimental effect in making the country low in its adaptive capacity to respond to climate change. Agriculture itself supports 75 per cent of its population and contributes to approximately 76.9 per cent of GDP as of 2016. Given the dependency on agriculture and lower adaptive capacity, it might become sensitive to future rainfall shocks with extreme rainfall to become more common and the future is likely to experience more social conflicts due to climate change. Hence, the country should focus on improvements on water storage capacity and irrigation systems, improved crop varieties, measures to prevent flood damage and transparency in the government functions [73].

For Somalia, the variables qualified as indicators include temperature, CO_2e, RF and LACP. The climate is predominantly desert with a year-round hot climate, with a high average annual temperature of 26.9ºC and less total annual rainfall of 270.1 mm. Temperature and rainfall showed slight increases over the decades with high variability (Fig 3). The CO_2 emissions are very meagre and are similar over the decades from 1961. The cereal yields showed an increase over the decades and the land area under cereals increased up to 1980 and thereafter decreased. However, during 1991-92, the country experienced severe famine, thus decreasing the crop yields. The CCCPIs did not show any decline or rise throughout the years but remained at the same level with variability between the years. However, CCCPIs showed a significant positive correlation with RF and non-significant correlation with CO_2e and LCAP. Somalia has typically low and highly variable rainfall throughout the country with an annual rainfall ranging from 158 mm to 423 mm. The major cereal crops cultivated include sorghum and maize which are grown both under irrigated and rainfed conditions. At present, this country is worst hit by the drought, thus worsening the food security, escalating food prices, and increased malnutrition. Though early warnings for the 2011 droughts were issued, immediate action plans and risk management initiatives were not triggered due to major constraints of funding, access, and responsible mechanisms [74, 75, 76]. Water scarcity is a major problem as it is receiving less amounts of rainfall since the 1990s. Rehabilitation of malfunctioning boreholes, water trucking to exhausted areas would be a great asset [77]. Somalian agriculture contributes to 60.2 per cent towards GDP and remains to be an important economic sector. Though Somalia is a food deficit country, potential exists for the country to reduce its dependence on food imports and where irrigation is possible along the rivers of Shabelle and Juba [78]. It cannot rely upon the present agricultural production for its future food demands. Despite the 1.6 per cent land put to agricultural use, more amount of land can be brought under cultivation. Impudent strategies need to be evolved to engage in modern, intensive, and sustainable agriculture. Once grain sufficient in 1970s and 80s, can again be made grain sufficient with good farming system instead of remaining as food imports dependent [79]. The coping strategies available in other regions to cope with shocks and to mitigate long term stresses might be unavailable or inappropriate [80].

The indicators retained for Mongolia include temperature, CO_2e and CY. It is a land-locked developing country in the northern latitudes and is a place for the occurrence of highest global warming [81]. Due to its high altitude, it is generally colder than other countries at same latitude. It also has a harsh continental climate, with high annual and diurnal temperature fluctuations, and low rainfall. During the study period, from the year 1961, there is an increase in temperature each decade while during the last decade a decrease was observed. The CO_2 emissions increased each successive decade and was more pronounced during the last decade especially. The cereal yields also increased every successive decade and showed drastic changes recording high yields during 1981-90 and 2011-15. However, the land area under cereal production fluctuated with alternate increase and decrease. The CCCPIs also showed a great variability throughout the years and showed non-significant correlations with temperature, CO_2e and CY. Heavy rains, snowfall, strong winds, sandstorms, snowstorms hail and flooding are the major natural disasters affecting the socio-economic situations of the country. It is necessary to understand the complex interactions of the effects of insects, weeds, and diseases on agricultural production. Crop diversification is an essential measure to fight the climate change effects for a country like Mongolia [34].

For Nepal, the key indicators to determine the CCCPI include CO_2e, CY, and RF. During the study period, the CO_2 emissions drastically increased from the decade 1991-2000, while the cereal yields and rainfall did not show much variation between the decades except during the last decade, where it showed a sudden increase. The CCCPIs showed a

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decline during 1961-70 and remained at the same level for the rest of the period and then slightly increased at the end. CCCPIs showed a no significant positive correlation with rainfall and cereal yield and negative correlation with CO_2e. Precipitation is a major factor affecting the crop yields and the present study also reveals a decrease in rainfall. In 2006, the west Nepal experienced flash floods while the Eastern Nepal experienced extreme drought thus leading to a decrease in crop yields. Late or erratic monsoons might result in crop damages and subsequent food insecurity. A decline in agricultural productivity by 17.3% has been predicted if no adaptation or carbon fertilization strategies are to be implemented with the current technological growth [82]. Nepal, one of the least developed countries, categorises the climatic impacts to be severe due to its static adaptation capacity and high vulnerability. Hence, initiation of government supported large-scale planned strategies like controlling excess water flows arising from flash floods, and seasonal landslides are very important to protect the crops [82].

For Myanmar temperature and CO_2e were retained as the key indicators to determine the CCCPIs. Both these indicators showed a significant negative correlation with the CCCPIs. During the study period, temperature showed a variation of 0.1°C per decade while the CO_2 emissions showed a continuous and drastic increase from 1961 to 2015. Rainfall showed a greater variation and was observed to decrease. By 2100, there was a temperature prediction of 0.5^{o}C to 5.5^{o}C rise. Being a least developed nation, Myanmar is highly vulnerable to negative effect of changing climate. With its extensive coastline, it is inherently prone to extreme weather events like flooding, cyclones, tsunamis, droughts, heavy monsoon rains, and storm surges. The changing climate might increase the frequency of these extreme weather events posing new threats. Sea level rises might decrease the coastal rice producing shore level [83, 84]. Development of climate resilient cultivars by exploiting the genetic variability and yield potentials would be essential to sustain the crop productivity [85].

For Philippines, the key indicators retained to determine the CCCPI include temperature, CO_2e, and LACP. Temperatures and CO_2e showed a significant negative correlation with CCCPIs while LACP showed a significant positive correlation. Considering the temperatures, during the study period, Philippines had an average annual temperature of 25.5^{o}C with an increase of 0.1^{o}C over the years. The CO_2 emissions increased drastically over the past few decades and the land area also increased over the decades. According to a study, climate change is expected to impact agriculture by PHP145 billion dollars through 2050 [86]. Our study revealed a declining trend in the CCCPIs up to 1967 and thereafter remained steady for few years and then ultimately decreased slightly over the years, but the cereal yields showed an increasing trend which could be attributed to increase in land area. Philippines is usually highly vulnerable to adverse impacts of climate change especially the floods, droughts, heat waves, and typhoons which alter the agricultural output and productivity. Consequently, it has already experienced crop losses. Adaptation strategies like improved stress tolerant varieties and farm management techniques might have contributed to the improved yields added to the increase in land area [87]. In the recent past, measures like improving disaster risk management and reduced dependence on agriculture have been taken up to reduce its extreme vulnerability to climate change.

V. CONCLUSION

The climate change crop performance index calculated from this study gives an idea of the performance of the crops under varied climatic conditions over the years and comparison can be made to assess the impacts of range of climate variability. Just for the limitation of data availability, there would have been a broader scope of choosing additional indicators of agricultural related climatic and socio-economic variables, which are more relevant to the study location. Amidst the body of extensive literature of earths warming, crop production remains to be inherently sensitive to climate change and variability. Various adaptation and mitigation measures have already been adopted by some countries while some of the countries like Somalia are still at the initial stages of development of these measures.

In general, the study reveals a gradual increase in the temperatures, constant rise in CO_2 emissions (except for France), gradual or no change in rainfall, and rise or decline in land area under cereal crops. In most of these cases, the CCCPIs showed only a declining or no change trend. The negative correlations of the CCCPIs with most of the key indicators suggest every possibility of altering the cereal crop performance even with slight changes in any of the indicator. Contrary to the existing climatic situations, if future IPCC predictions of temperature increases, and rainfall reductions tend to set in, might result in direct or indirect influence on other indicators causing detrimental

effects on cereal production. This demands a constant vigil and monitoring especially on the key indicators apart from other indicators. Suitable adaptation and mitigation strategies centered around these key indicators are very much essential for these countries. If appropriate adaptation and mitigation strategies have already been taken, a general appraisal of these strategies and policies should be made for developing new strategies. Despite the vast adaptation and mitigation practices, there still exist room for further adoption of climate proof and climate friendly practices.

The index could not be standardized as this is the first study of its kind. Detailed year-wise comparison in evaluating or assessing the crop performance for each location could not be taken up as it is beyond the scope of the paper. These indices can be further strengthened, and some critical points can be identified based on which the crop performance can be monitored regularly. The methods used to monitor these indicators should be fully defined to make easy comparisons. Thus, the findings of the present study might be found useful for the future researchers, land mangers, policy makers or any other stakeholder engaged in developing measures to sustain the cereal yields thus contributing to country's economy and its food security.

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Marketing of Crop Residues in Niamey city: Socio-organizational Aspects.

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***Abstract*—** *A sample of 93 stakeholders was surveyed and monitored to analyze the socio-economic and organizational aspects of the marketing of crop residues used in animal feed in the city of Niamey. It emerges from this study that almost all the actors are male. More than 45% of actors are between 40 and 60 years old. The Hausa ethnic group is the majority of wholesalers / semi-wholesalers (54.5%). Those of the Zarma ethnic group are more numerous at the level of sellers / resellers (92.7%), while those of the Fulani ethnic group represent the majority of street vendors (57.9%). Inheritance and profitability were the main reasons that led to the activity of wholesalers / semi-wholesalers (30.3% and 24.2%) and street vendors (26.3% and 31%). 6%). Among sellers / resellers, it is rather survival (24.4%), passion (22%) and constraint (22%). Most wholesalers / semi-wholesalers (60.6%) and sellers / resellers (68.3%) are supplied and delivered locally at their own point of sale. cash settlement is the most common (79% for wholesalers / semi-wholesalers, 61% for sellers / resellers and 74% for street vendors). The daily gross margin of a wholesaler / semi-wholesaler of crop residues is 14753.03 ± 25724.24FCFA. It is higher than that of street vendors (6946.05 ± 2204.23FCFA), which is also higher than that of sellers/resellers (5855.54 ± 5065.49FCFA). In short, the marketing of crop residues is a very profitable activity for the actors, especially in the cold dry season. However, the recurrent displacements of actors for lack of fixed places and the harassment of the collection agents from the municipalities represent a brake on the development of this activity. It is therefore imperative to regulate their implementation while facilitating administrative procedures leading to the granting of authorization for installation.*

Keywords*— *Cultivated residues, breeding, sale, Niamey, Niger.

I. INTRODUCTION

Livestock farming in tropical countries occupies a very important place in household activities (Zoffoun et al., 2013). It is a source of considerable income for the population and plays an important socio-cultural role (Steinfeld et al., 2010). In Niger, drought cycles have led in recent years, the descent of farmers in agricultural areas and their establishment almost permanently. This has resulted in a change in dietary practices, including the switch from extensive livestock farming using large areas to a semi-intensive or intensive system in urban centers (Faye and Alary, 2001). In addition, the low forage productivity despite the large area of primary pasture production (620 000 km²), makes animal feed one of the main constraints of livestock farming in Niger (Chaibou et al., 2012; Rhissa, 2010; Maidadji, 2003). In order to alleviate this dietary constraint of booming urban and peri-urban farms, fodder food markets have developed (Sanou et al., 2011), in which speculation has taken place with several types of actors around crop residues. Thus, the marketing of crop residues is a profitable economic activity that is essential for the prosperity of urban and peri-urban livestock farming in Niamey. The objective of this study is to analyze the socio-economic and organizational aspects of the marketing of crop residues in the city of Niamey.

II. MATERIALS AND METHODS

2.1. Study areas

The study was conducted in the urban area corresponding to the large metropolitan area of Niamey which is subdivided into five communes (Niger, 2016). It is located in the western part of the country, between 2 ° 10 'and 2 ° 14' east longitude and 13 ° 33 'and 13 ° 36' north latitude (Beidari 1999, Niger 2015a). It covers an area of about 55227 ha with a tropical climate of the Sudano-Sahelian type characterized by an average temperature of 30.25 ° C; a high of 42.1 ° C in April and a low of 17.8 ° C in December. The average rainfall is 563.3 mm / year (Niger, 2015a). The vegetation is generally shrubby, sparse with seasonal-season herbaceous plants. Agriculture is practiced in a traditional way and occupies a good part of the population. Livestock occupies a prominent place in the activity of the population, and the herd is 105,212 TLU in 2015 (Niger, 2015b).

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2.2. Data collection

It was identified in the first months by an identification of the actors involved in the marketing of crop residues.

These actors are generally settled around livestock markets and on the main boulevards of the city of Niamey.

Thus, 296 actors identified during the period from November to December 2016 served as sampling frame. Then, 1/4 of the 296 identified actors, or 93 actors were randomly drawn. Among these 93 actors, there are 33 wholesalers / semi-wholesalers and 41 sellers / resellers. To these 93 actors drawn at random, 19 street vendors met during the same phase of identification were added. This gave a sample of 122 actors. The latter were selected for seasonal monitoring to see the effect on the activity. These include the hot dry season (March-June, 2017), the rainy season (July-October, 2017) and the cold dry season (November 2017-February2018). The information sought focused on the socio-economic characteristics of the actors, the different types of crop residues sold, the types of packaging, the price and weight of the local unit, and the stock available.

2.3. Statistical analysis

The collected data were coded and entered into a model built on the SPSS version 19 software. A descriptive statistic (Khi2 test, Fisher's exact test at the 5% threshold) and an ANOVA (multivariate analysis, followed by the Levene error variances test) through the general linear model were performed between the variables. The averages were compared according to Duncan's test at the 5% threshold. Some data has been exported to the Excel spreadsheet for the production of tables and figures.

III. RESULTS

3.1. Socio-economic characteristics of actors

The majority of the actors surveyed (more than 45%) are relatively old (age between 40 and 60 years old). This study also shows a very low proportion of female actors (6.1% wholesalers / wholesalers and 2.4% sellers / resellers), and a large proportion of single actors (52.6%). at street vendors. We notice more actors of Hausa ethnic group at the level of wholesalers / semi-wholesalers; actors of ethnic Zarma at the level of sellers / resellers and actors of Fulani ethnicity at the level of street vendors. Also, the actors do not have much experience (less than 5 years) in the activity of selling fodder (51.5% wholesalers / wholesalers, 34.1% sellers /resellers and 63.2% street vendors) and their majority activity is the sale of livestock feed (60.6%) for wholesalers / semi-wholesalers; agriculture (53.7%) for sellers / resellers and the sale of fodder (68.4%) for street vendors (Table 1).

Table.1: Socio-economic characteristics of the respondents

Variable	Modality	Wholesaler / semi wholesaler		Seller/ reseller		Street vendor		P value
		N	%	N	%	N	%	
Age	> 20 ≤ 40 years	13	39.4	4	9.8	6	31.6	*
	> 40 ≤ 60 years	15	45.5	9	63.4	50	47.4	
	> 60 years	5	15.2	11	26.8	4	21.1	
	Total	33	100	41	100	19	100	
Sex	Male	31	93.9	40	97.6	19	100	Ns
	Female	2	6.1	1	2.4	0	0	
	Total	33	100	41	100	19	100	
Marital status	Married	31	93.9	39	95.1	10	52.6	***
	Single	2	6.1	1	2.4	9	47.4	
	Widower/widow	0	0	1	2.4	0	0	
	Total	33	100	43	100	19	100	
Ethnic group	Haussa	18	54.5	3	7.3	0	0	***
	Zarma	11	33.3	38	92.7	8	42.1	
	Fulani	2	6.1	0	0	11	57.9	
	Kanuri	2	6.1	0	0	0	0	
	Total	33	100	41	100	19	100	
Core business	Agriculture	12	36.4	22	53.7	6	31.6	***
	Fodder sale	0	0	5	12.2	13	68.4	
	Sale of livestock feed	20	60.6	12	29.3	0	0	
	Sale wood + Secko	1	3	2	4.9	0	0	
	Total	33	100	41	100	19	100	
Number of year of exercise	≤ 5 years	17	51.5	14	34.1	12	63.2	Ns
	> 5 ≤ 10 years	6	18.2	9	22	6	31.6	
	> 10 ≤ 15 years	1	3	3	7.3	1	5.3	

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> 15 ≤ 20 years	4	12.1	5	12.2	0	0
> 20 years	5	15.2	10	24.4	0	0
Total	33	100	41	100	19	100

Ns = not significant; * = p<0.05; *** = p<0.001; N = effective; % = Percent

3.2. Reasons for the practice of the commercialization of crop residues

The main reasons that led to the marketing practice of crop residues are among other reasons, inheritance and profitability respectively for wholesalers / semi-wholesalers (30.3% and 24.2%) and street vendors (26.3% and 31.6%). As for sellers / resellers, it is rather survival (24.4%), passion (22%) and constraint (22%) (Figure1).

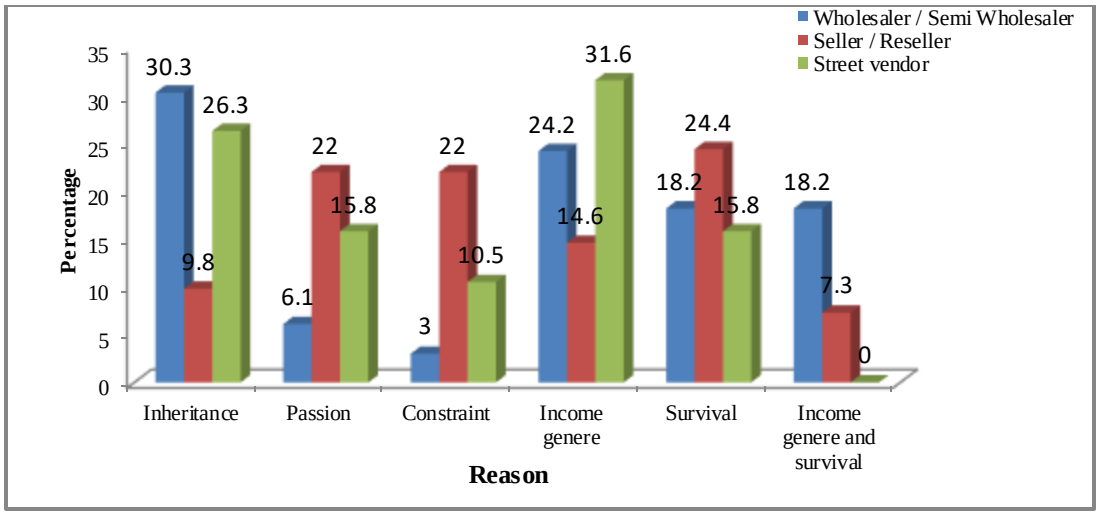

Fig. 1: Reasons for the practice of marketing crop residues

3.3. Trade Organization

3.3.1. Type of crop residues sold

The different types of crop residues encountered in the marketing system as livestock feed are cowpea haulm, groundnut haulm and rice straw. These last are object of specific sale, or of combined sale at the level of the various actors. Figure 2 shows that all street vendors surveyed sell cowpea haulm and 36.84% of them sell extra rice straw. Wholesalers / semi-wholesalers mainly combine cowpea and groundnuts haulm (57.6%). The largest proportion of sellers / resellers sells specifically cowpea haulm (43.9%), followed by the sale of cowpea haulm to groundnut haulm (31.7%).

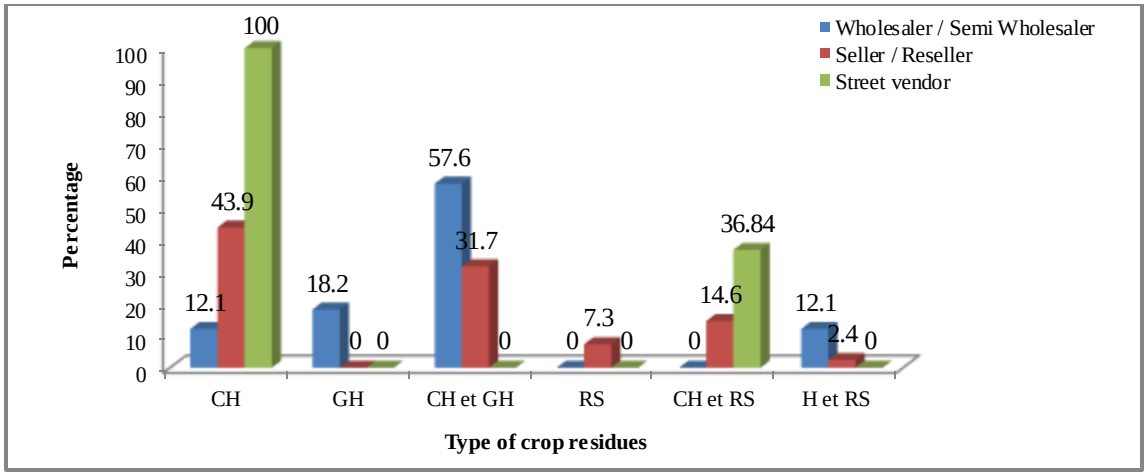

Fig. 2: The different types of crop residues sold

3.3.2. Supply and delivery

All street vendors obtain supplies from the producers. Most wholesalers / semi-wholesalers (60.6%) and sellers / resellers (68.3%) are supplied and delivered locally at their own point of sale (Figure 3).

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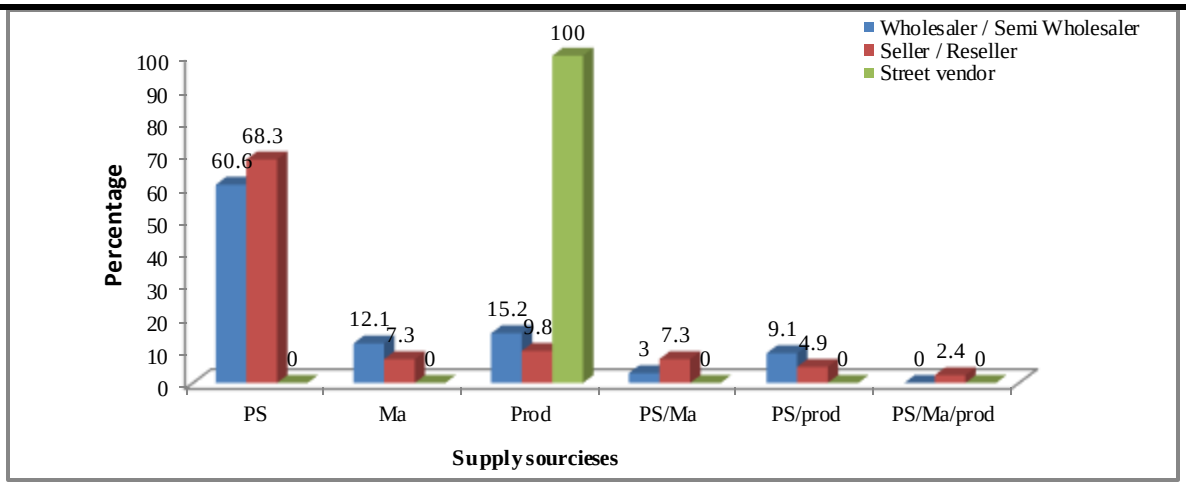

Fig. 3: Supply and delivery

3.3.3. Quantity of crop residues per supply

Street vendors stock up on boot of cowpea and rice straw. The quantities per supply are mainly between 50 and 100 boots of cowpea haulm (78.9%) and 100 and 200 boots of rice straw (100%). Wholesalers / semi-wholesalers supply mainly groundnut and rice straw. The quantity per supply is generally greater than 100 boots of groundnut haulm (41.4%) and more than 200 boots of rice straw (66.66%). The vast majority of sellers / resellers are delivered relatively small quantities. A proportion of 57.1% is delivered 10 to 25 boots of groundnut haulms; 41.5% are fed a quantity of cowpea grass less than 25 boots and 49.68% are supplied with a quantity of rice straw of between 100 and 200 boots (Figure 4).

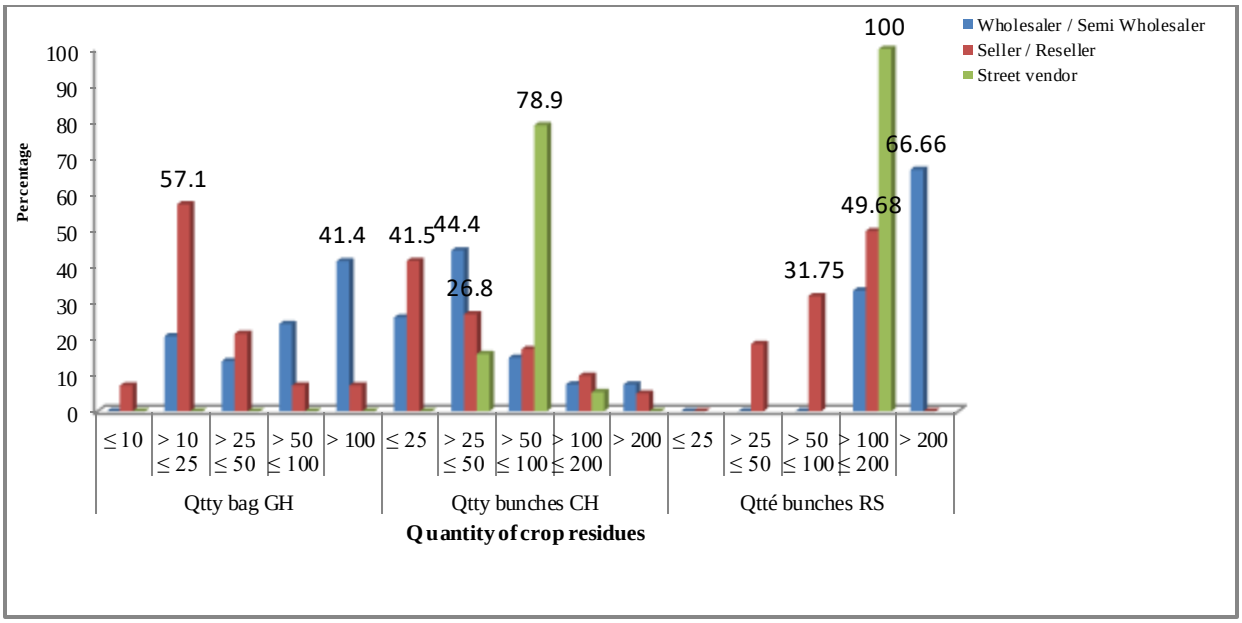

Fig. 4: Quantity of crop residues per supply

3.3.4. Payment method of supply

Overall, there are two modes of supply settlement: cash payment or cash and credit combination. This latter formula is the most widely practiced (79% for wholesalers / semi-wholesalers, 61% for sellers / resellers and 74% for street vendors) (Figure 5).

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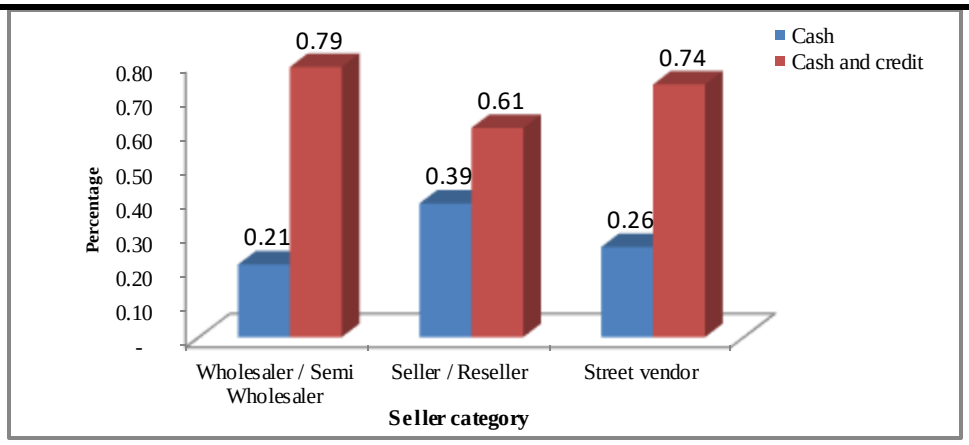

Fig. 5: Supply payments mode

3.3.5. Storage

The most common method is to store crop residues on bricks and / or tires and cover them with plastic (72.7% wholesalers / semi-wholesalers and 87.5% resellers) (Figure 6).

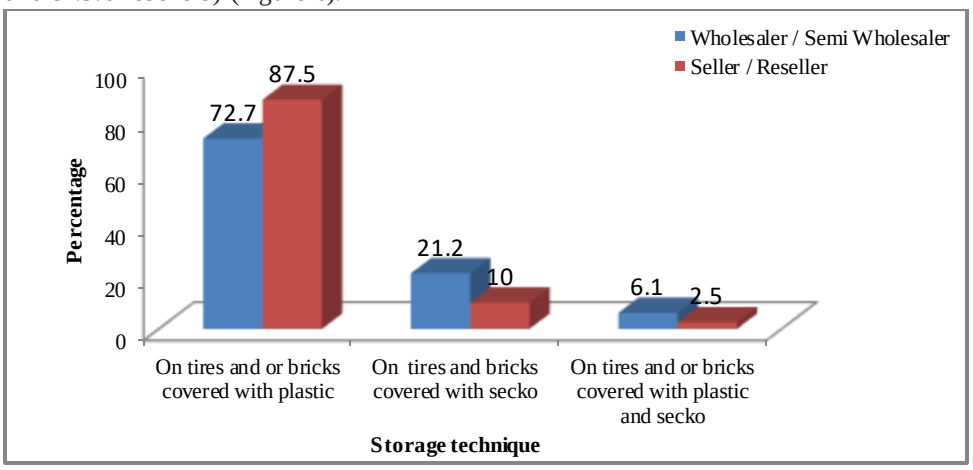

Fig. 6: Storage

Labor is used only by wholesalers / semi-wholesalers and sellers / resellers. Thus, 72.7% of wholesalers / semi-wholesalers and 46.3% of sellers / resellers claim to use labor. It is mainly family-based (91.7% for wholesalers / semi-wholesalers and 100% for sellers / resellers). In most cases this labor is used during all the periods of the year. The majority of wholesalers / semi-wholesalers (87.9%) and sellers / resellers (68.3%) pay a tax for the exercise of the crop residue marketing activity. It is mainly the installation tax for the town hall or the synthetic patent. It should be noted that street vendors are exempt from these (Table 3).

3.3.6. Daily recipe

Daily revenues are higher at wholesalers / semi-wholesalers, mainly in the cold dry season (59803.03 ± 60381.979FCFA). They are generally lower among sellers / resellers, especially during the rainy season (Table 4).

Table.2: Use of labor

Variable	Modality	Wholesaler / semi wholesaler		Seller/ reseller		Street vendor		P value
		N	%	N	%	N	%	
Use of labor	Yes	24	72.7	19	46.3	-	-	***
	No	9	27.3	22	53.7	19	100	
	Total	33	100	41	100	19	100	
Period of use of the labor	Cold dry season	1	4.2	-	-	-	-	Ns
	Hot dry season	-	-	1	5.3	-	-	
	All the dry season	9	37.5	6	31.6	-	-	
	Dry and rainy season	14	58.3	12	63.2	-	-	

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	Total	24	100	19	100	-	-	
Type of labor used	domestic	22	91.7	19	100	-	-	Ns
	wage	2	8.3	-	-	-	-	
	Total	24	100	19	100	-	-	

Ns = not significant; *** = p<0.001; N = effective; % = Percent

Table.3: Tax Payment

Variable	Modality	Wholesaler / semi wholesaler		Seller/ reseller		Street vendor		P value
		N	%	N	%	N	%	
Tax payment	Yes	29	87.9	28	68.3	0	0	***
	No	4	12.1	13	31.7	19	100	
	Total	33	100	41	100	10	100	
Type of tax	Town hall	14	48.3	20	71.4	0	0	Ns
	patent	1	3.4	2	7.1	0	0	
	Mayor and patent	14	48.3	6	21.4	0	0	
	Total	29	100	28	100	0	0	

Ns = not significant; *** = p<0.001; N = effective; % = Percent

Table 4: Daily recipe according to the seasons

Actor category	Daily recipe						P value
	Rainy Season		Cold Dry Season		Hot Dry Season		
	N	Mean	N	Mean	N	Mean	
Wholesaler / Semi wholesaler	28	36767.86±35341.06	33	59803.03±60381.98	32	53421.88±64048.16	***
Seller / Reseller	21	13433.33±16510.1	41	15136.59±19767.21	26	19119.23±23664.35	
Street vendor	9	28888.89±9279.61	19	21171.05±9410.42	13	21173.08±9323.68	
Total	58	27096.55±28540.99	93	32218.82±43399.99	71	34955.63±48123.73	

*** = p<0.001; N = effective

3.4. Determinants of margins by type of actors

The expenses related to the marketing of crop residues are mainly the costs related to refueling, packaging and storage; to labor and to the payment of tax. According to this study, in Niamey, the daily gross margin of a wholesaler / semi-wholesaler of crop residues is 14753.03 ± 25724.24FCFA. It is higher than that of street vendors (6946.05 ± 2204.23FCFA), which is also higher than that of resale sellers (5855.54 ± 5065.49FCFA) (Table 5).

Table 5: Daily gross margin by types of actors

Topics	Categories of actors		
	Wholesaler / semi wholesaler	Seller/ reseller	Street vendor
	Mean	Mean	Mean
Loads (FCFA)			
Feeding in fodder	34802.31±29391.62	9175.96±14633.72	15332.08±7112.04
Conditioning and storage	571.97±286.27	676.83±328.05	587.72±358.94
Labor	357.14±303.05	-	-
Tax	187.59±47.37	198.49±173.6	-
Total expenses	35919.01±30028.3	10051.28±15135.37	15919.8±7470.98
Products (FCFA)			
Daily recipe	50672.04±55752.55	15906.82±20200.86	22865.85±9675.21
Gross margin	14753.03±25724.24	5855.54±5065.49	6946.05±2204.23

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IV. DISCUSSION

4.1. Socio-economic characteristics of the actors

The marketing of crop residues is more an activity of male actors, whose age is between 40 and 60 years (Oumarou, 2016). This is apparent from this study, or only about 40% of wholesalers / semi-wholesalers are under 40 years of age. Compared with sellers / resellers (with almost 10%) and street vendors (with nearly 30%) of young people under 40, this proportion represents the category of actors with the youngest people. The sellers / resellers were former street vendors of bush hay. They are of Zarma ethnicity and after getting older, settle down in strategic fixed places in search of potential customers (Lawal, 2014). The street vendors, for their part, are rural, single, of Fulani ethnicity for the most part. This is justified on the one hand, by the fact that the surrounding villages of the city of Niamey are mainly made up of Fulani, on the other hand, by the precariousness which reigns in the rural world making the conditions of life difficult in these environments. In wholesalers / semi-wholesalers, it is rather actors of Hausa ethnic group, with a relatively young fringe. This situation can be explained by the fact that it is a profitable activity on the one hand (Maimouna, 2012), and on the other hand, by its ethno-linguistic character (Ouseseini et al, 2017) through the marketing of groundnut haulm. As a result, a large proportion of these (60.6%) make the marketing of livestock feeds, their main activity. Although the marketing of fodder is a profitable activity for the actors (Aboh, 1999, Kiema et al, 2012, Sanou et al, 2011, Sanou et al, 2016), several reasons lead to the practice of commercialization of crop residues. This is inheritance and profitability, respectively for wholesalers / semi-wholesalers (30.3% and 24.2%) and street vendors (26.3% and 31.6%). Among sellers / resellers, it is rather the survival (24.4%), the passion (22%) and the constraint (22%) which constitutes the fundamental reasons for the practice of the activity. Usually, the sale of fodder is an activity of actors of ethnic Zarma (Ouseseini et al, 2017) from the Zarmaganda. Young people are engaged in street vending on carts and camels, and older sellers, usually over 40, settle into fixed places and are handed over by young people. Those with significant capital maintain relationships that allow them to be delivered in large quantities and become wholesalers / semi-wholesalers. The others who do not have substantial means remain sellers / resellers by constraint because their survival depends on it. Nevertheless, a large group of wholesalers / wholesalers and street vendors have engaged in this fodder business because it generates a lot of revenue (Maimouna 2012, Dan Gomma et al, 2017). Sellers / resellers are usually sourced locally at the point of sale, usually by street vendors or a few wholesalers / semi-wholesalers. Although this situation exempts shipping and handling costs from the products, it significantly reduces the margin of the sellers / resellers' customer base, because of having products with a relatively high price.

4.2. Marketing and supply management

The different types of crop residues used as livestock feed, encountered in the marketing channel, are cowpea, groundnut and rice straw. In Bobo Djioulasso in Burkina Faso, it is rather stalks of millet, sorghum and corn instead of rice straw (Sanou, 2016). These last are object of specific sale, or of combined sale at the level of the various actors. In fact, all the street vendors surveyed sell cowpea hay and 36.84% of them sell the rice straw and more. This situation is explained by the fact that the production of groundnuts in the urban community of Niamey and its surroundings is almost non-existent. The banks of the Niger River are valued mainly by growing rice through two productions per year (Siddo 2010, Gergely 2014, Dan Gomma et al 2017). Cowpea is generally associated with cereals (millet and sorghum). Cereal stalks are not found in the marketing market at the level of the different actors because they are either used by the producer himself for feeding his animals and other uses such as fences, or sold directly to breeders (Dan Gomma et al, 2017). The quantity and nature of crop residues by supply depends on the category of actor (Sanou et al, 2011). Street vendors are more interested in cowpea haulm and rice straw boots. Supply quantities are in most cases between 50 and 100 boots of cowpea hay (78.9%) and 100 and 200 boots of rice straw (100%). These quantities generally represent the carrying capacity of the carts. The amount of cowpea haulm is relatively inferior to that of rice straw because the packing of cowpea haulm does not allow them to be compacted in order to better preserve the leaves, while the rice straw can. It should also be noted that rice straw has a lower nutritional value, with a price that is relatively low. Therefore, to make the trip profitable, you need a relatively large load. As for wholesalers / semi-wholesalers, the importance of supply is mainly in groundnut and rice straw fodder, with quantities per supply generally greater than 100 boots of groundnut fane (41.4%) and 200 boots of rice straw (66.66%). Groundnut leaves come from the interior of the country mainly from the Maradi and Tahoua region (Niger, 2015c) and rice straw from the riverbanks at the producer level (Dan Gomma et al, 2017). Wholesalers / semi-wholesalers acquire it per production plot and carry it on carts. Sometimes street vendors serve as carriers for this task. These quantities of groundnuts and rice straw are most often used to supply sellers / retailers who obtain small quantities because they have small means, but also to feed the herdsmen through a small part used to feeding the stand of the wholesaler / semi-wholesaler in person. The method of payment of the cash supply is very rare. Cash

and credit are the most common methods of payment (79% for wholesalers / wholesalers, 61% for sellers / resellers and 74% for street vendors). This allows those who cannot afford cash to have the products on credit for a small advance. The rest of the money will be paid in full at the next delivery. This observation is similar to that made by Oumarou (2016).

The subsequent storage and storage of crop residues is done on old tires and / or bricks. They are then covered by tarpaulins or polystyrene plastic (Lawal, 2014, Oumarou, 2016, Ousseini et al, 2017) or secko. Tarpaulins are used for their thickness, hardness and weather ability whereas polystyrene plastics are used for their easy handling and relatively low cost. The seckos, on the other hand, are those resulting from slump, which are at the beginning of degradation or completely degraded. In this way, the cost of storage and storage is reduced.

In this crop residue marketing activity, a significant number of wholesalers / semi-wholesalers (72.7%) and sellers / resellers (46.3%) use labor. This workforce, mainly family consists of members of the family of the actor. Sometimes they are schoolchildren who serve as labor during the off-class periods. This workforce is unpaid because it is an integral part of the family. However, in case of marriage or baptism and or religious festival, the expenses of the latter are the responsibility of the actor. Through this study, (87.9%) wholesalers / semi-wholesalers and (68.3%) sellers / resellers claim to pay taxes for the town hall.

It is mainly the installation tax and the synthetic patent. It should be noted that these settle without prior authorization from the municipality. For this purpose, they are subject to recurrent displacement for lack of permanent place. Thus, for the maintenance and preservation of their place, they are confronted with a system of racketing by the collection agents of the municipality. This is one of the main constraints linked to the marketing of fodder in general (Lawal 2014, Soulé 2014).

4.3. Determinants of margins by type of actors

The proper conduct of the crop residue marketing activity requires costs related to refueling, packaging, storage, labor and the payment of taxes and duties. Daily revenues are higher at wholesalers / semi-wholesalers, mainly in the cold dry season (59803.03 ± 60381.979FCFA). It is lower among sellers / resellers during the rainy season. In fact, the variations and fluctuations in the price of fodder for general stoves depend on the season, the type of fodder and the type of packaging (Manzo 2009, Soulé 2014, Oumarou 2016, Dan Gomma et al 2017). According to Sadoud (2010), feed prices depend mainly on the periods and behavior of the storers. This activity generates a daily gross margin of 14753.03 ± 25724.24FCFA at a wholesaler / semi-wholesaler. It is higher than that of street vendors (6946.05 ± 2204.23FCFA), which is also higher than that of resale sellers (5855.54 ± 5065.49FCFA). The quantity of the product and its availability play a very important role in the realization of profit.

V. CONCLUSION

It follows from this study that the marketing of crop residues in the urban community of Niamey is an activity reserved mainly for male actors, whose age is between 40 and 60 years. The main reasons that lead to this activity are legacy and profitability, survival, passion and coercion. The quantity and nature of crop residues by supply is a function of the category of actor and the method of payment of cash supply is very rare. The storage of products is done on old tires and or bricks, covered by tarpaulins or polystyrene plastics. The labor used is mainly family and consists of members of the actor's family. It also shows that the marketing of crop residues is a very profitable activity for the actors, especially in the cold dry season. However, the recurrent displacements of the actors for lack of fixed place coupled with the harassment of the agents of recovery of the municipalities represent a brake with the development of this activity. It would therefore be imperative to regulate their implementation while facilitating the procedures leading to the granting of installation authorizations.

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Studies on Physicochemical Analysis of Water from Different Sources

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Abstract— Introduction: *Water is one of the important natural sources for all living organisms. It is one of the ecological systems. It's the essential source for human health, food production and economic development. The quality of water is important is an important parameter to be noted. The quality of water is affected by various contaminants. The consumption of contaminated water may cause serious health problems due to the activity of microorganism present in it .Due to the activity of microorganism the quality of water becomes very poor and also causes harmful diseases. Thus in this study we are aimed to test the quality of water from different sources by means of physicochemical studies.*

Objectives: *The objective of the present studies is to provide information on the physicochemical characteristics & detailed ecological studies of Portable water and Lake water (Habitat) in order to discuss it's suitability for human consumption. Physicochemical aspects of the water have been investigated to assess the quality of water.*

Result: *The variations of physicochemical properties and comparative analysis of water different sources were analyzed.*

Keywords— Biochemical aspects, Ecological system, habitat, physicochemical studies.

I. INTRODUCTION

Water is one of the important natural sources for all living organisms. It is one of the ecological systems. It's the essential source for human health, food production and economic development (1). The quality of water is important is an important parameter to be noted. The quality of water is affected by various contaminants (2, 4). The consumption of contaminated water may cause serious health problems due to the activity of microorganism present in it(2,4) .Due to the activity of microorganism the quality of water becomes very poor and also causes harmful diseases. Thus in this study we are aimed to test the quality of water from different sources by means of physicochemical studies (6, 10, 14).

Sample Collection:

Portable water sample was collected from five different areas of Kanchipuram.

Habited water sample was collected from four different lakes of Kanchipuram, Mathuranthagamn Lake, Kolavai Lake, Karunguzhi Lake and Vedanthangal Lake.

II. Material and Methods

The water sample was collected from four different lakes for the project work. The sample was taken in a closed bottle was dipped into the lake and opened the cap inside the lake and was closed again to bring it out at the surface (9). From the time of sample collection, biological and chemical reactions may change the quality of water sample. To minimize the growth of microorganism, we have to preserve soon after the collection of water sample (13, 9). Once the water sample is collected, the odour, taste and TDS should be analyzed and preserved by adding chemical preservations and lowering its temperature (13). The water analysis process was carried out for a period of four months. The collected water samples were brought to the laboratory and analysis were performed. .pH was determined using pH meter, and similarly turbidity is measured by turbiditymeter (Verma Pradeep et al, 2012).

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Table.1: Portable Water Samples

Sr. No.	***Test***	***Processed Water Sample***	***Chengalpet Municipal Water Sample***	***Madhuranthagam Municipal Water Sample***	***Vedanthangal municipal Water Sample***	***Kolavai Municipal Water Sample***
1	Temperature (ºC)	28	28	28	28	28
2	Colour (Unit)	<1	<1	<1	<1	<1
3	Odour	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable
4	Taste	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable
5	pH	6.9	7.0	6.9	6.8	7.0
6	Turbidity (NTU)	0.38	0.29	0.30	0.28	0.30
7	TDS (ppm)	141	140	144	140	146
8	Dissolved oxygen (ppm)	6.1	6.2	6.3	6.1	6.3
9	Dissolved carbon dioxide(ppm)	38	36	40	38	37
10	Alkalinity (ppm)	8	8	9	8	10
11	Chloride (ppm)	58	30	41	33	35
12	Calcium (ppm)	6.2	7	6	8	7
13	Barium (ppm)	Nil	Nil	Nil	Nil	Nil
14	Magnesium (ppm)	2.2	3	3	3.5	2.5
15	Total Hardness (ppm)	4	4.5	4.5	5	4.9
16	Copper (ppm)	0	0	0	0	0
17	Sulphate (ppm)	8	10	14	16	11

Table 2: Lake Water Samples

S. No.	**Test**	**Chengalpet Lake Sample**	**Mathuranthagam Lake Sample**	**Vedanthangal Lake sample**	**Kolavai Lake Sample**
1	Temperature	28	27	29	28
2	Colour (Unit)	< 2.3	<3	<2.5	<3
3	Odour	Disagreeable	Disagreeable	Disagreeable	Disagreeable
4	Taste	Disagreeable	Disagreeable	Disagreeable	Disagreeable
5	pH	8.3	8.6	8.7	8.8
6	Turbidity (NTU)	8	9	11	10
7	TDS (ppm)	900	946	987	735
8	Dissolved oxygen (ppm)	5.7	5.9	4.4	5.0
9	Dissolved carbon-di-oxide(ppm)	7.0	6.9	6.6	6.1
10	Alkalinity (ppm)	150	168	164	170
11	Chloride (ppm)	84	83	60	74
12	Calcium (ppm)	72	73	68.6	67
13	Barium (ppm)	41	32	31	36
14	Magnesium (ppm)	32	15.2	7.8	8.2
15	Total Hardness (ppm)	280	279	343	321
16	Copper (ppm)	19.76	15.27	17.43	15.89
17	Sulphate (ppm)	74	62	61	71

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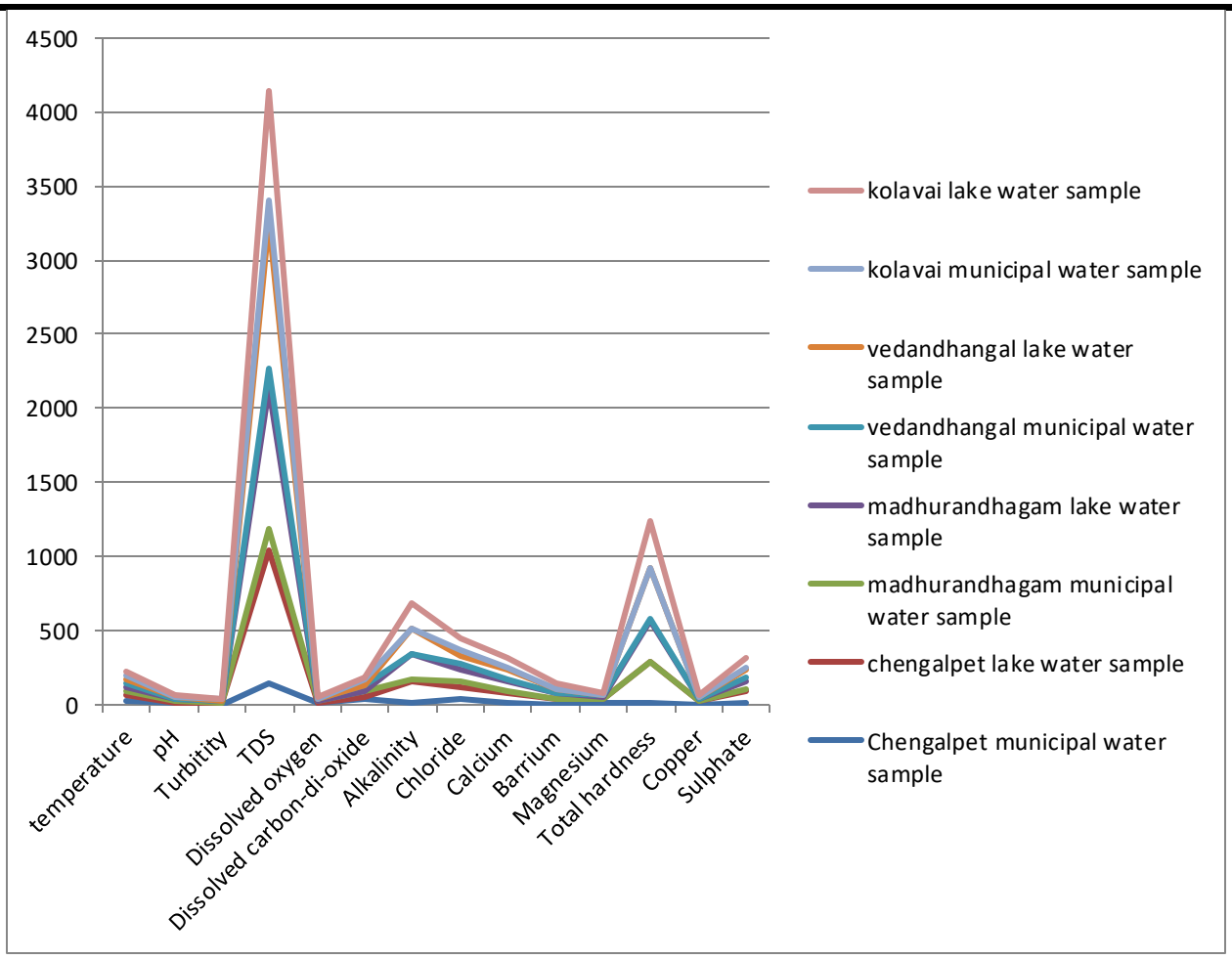

Fig.:1: Comparative graph for municipal water sample and lake water sample

III. DISCUSSION

Physical parameters like Odour & Taste was agreeable in portable water. The general ISI standard for Drinking water's Turbidity is 5 NTU is considered unhealthy (2). In Different areas of portable water the Turbidity ranging from 0.2 NTU to 0.4 NTU (13). In Municipal water, observed higher Turbidity than other area. The normal pH range of drinking water is between 6.5 to 7.0. The pH municipal water was observed between 6.5to7.0. So that the criteria of pH range was acceptable. For Portable water, Dissolved carbon dioxide & Dissolved oxygen were found to be 6.2 and 38 (Average value of five different areas) respectively (8). TDS of water sample showed range below 1000 ppm & it complied with the given criteria of Indian standard. Minerals like Calcium, Magnesium, Chloride, Sulphate, Barium, and Copper are essential for human body. Tests of these minerals were performed on portable water sample (9). The results complied with the given range to be Test for Minerals. Alkalinity & Total Hardness of potable water samples should less than or equal to 10 and 300 ppm respectively (13). Results were complied with the given limits of both tests. Turbidity of lake water sample ranges from 4 NTU to 11 NTU. The Total dissolved solid recorded ranges from 668 ppm to 942 ppm.

IV. CONCLUSION

The result obtained during study was compared with ISI standards. Portable water is water safe enough to be consumed by the humans and the habited water is generally used by animals & birds & aquatic life. After physicochemical analysis we found that the sample of Portable water and habited water are free from pollution & ecologically balanced.

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Lipase Producing Potential of Different Bacteria Species Isolated from Cooking Oil Contaminated Soils

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***Abstract*—** *In this study, we investigated the lipolytic activity of different bacteria strains isolated from different cooking oil contaminated soils.This was with a view to finding the isolate with the highest lipase-producing potential.A total of 70 bacteria strainswere isolated from the soil samples and the morphological examination ofeach isolate was done and characterized. 20 bacterial isolates were screened for lipase activity using chromogenic composed medium and the lipase producing microorganisms produced clear zones on this media. The isolate showing maximum activity was identified as Pseudomonas sp using Berger's manual. The result showed that Pseudomonas sp. has the highest lipase activity with the largest diameter of 20.4 mm, 20.4 mm and 20.9 mm at 24, 48 and 72 hours of incubation while Escherichia coli, Klebsiella sp., Alcaligens sp., Citrobacter sp., Streptococcus sp., and Serratia sp. showed no lipase activity with 0.00 mm within the hours of incubation.The prevalence of lipase activity of each suspected bacterial isolate was investigated. Pseudomonas sp. has the highest prevalence of lipase activity with a value of 32.3% and the bacterial isolates with the lowest prevalence rate include Klebsiellasp, Escherichia coli, Serratia sp., Citrobacter sp., Streptococcus sp., and Alcaligens sp. with 4.2% each.. Staphylococcus sp. has a prevalence rate of 21.2%, Bacillus sp. with 6.1% prevalence rate while Bifidobacterium sp. and Corynbacterium sp. has 7.6% each.*

Keywords*— *Lipase, Isolate, Pseudomonas, cooking oil, contaminated soil.

I INTRODUCTION

Lipases are the enzymes that play a principal role in the hydrolysis and synthesis of esters formed from long-chain fatty acids and glycerol (Sharma *et al*., 2001; Svendsen*et al*., 2000). Several studies have reported the ability of some microorganisms to produce specializeenzyme (Itakorode*et al*., 2019; Oyedeji*et al*., 2013). Large quantities of useful metabolites are mostly obtained from microorganisms with extracellular lipases producing potential. About 98% of the world's microorganisms have not been explored as enzyme sources. Soil, a region where biology, geology and nature meet provides a home to animal, plants and microbial life. The soil is also a region on the earth's crust that teems with both microscopic and macroscopic life. The different kinds of microorganisms present in soil depend on many environmental factors such asthe number of nutrients available, the degree of aeration, pH, temperature and available moisture (Akpor*et al*., 2006). Soil bacteria and fungi play prominent roles in various biochemical cycles most especially in recycling of organic compounds. It also influencesecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility (O'Donnell *et al*., 2001). Lipase (EC 3.1.1.3), an ester hydrolase catalyzes the hydrolysis of its substrate (triacylglycerol) to glycerol and fatty acids (Sharma *et al*., 2001). Lipases are ubiquitous and are produced by many microorganisms and higher eukaryotes (Kamimura*et al*., 2001; Elibol and Ozer, 2000). Several attempts have been made to isolate an organism with lipase producing potential since this enzyme is becoming popular in numerous biotechnological processes most especially wastes management industries(Sharma *et al*., 2001). The use of microorganism for lipase production have varieties of advantages over higher eukaryotes that are lipase producers. Microorganisms have avariety of enzymatic activities, high production within a short period of time and the ease with which they can be genetically manipulated. Microorganisms have been found to produce high yields of lipases compare to the animal and plants. This high yield might be because of their ease of production and genetic manipulation (Akoh*et al*., 2007). The oily environment (oil mill effluent) may provide a good

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environment for the isolation of lipase producing microorganisms. Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physicochemical factors, such as temperature, pH, nitrogen and carbon sources, inorganic salts, agitation and dissolved oxygen concentration (Gupta *et al*., 2004).This study, therefore, aims at isolating lipase producing bacteria from differentsoil samples that can be of use in the treatment of oil-contaminated soil.

II MATERIAL AND METHOD

Study Area

The study was carried out on different oil contaminated soil samples collected from different locations in Ile-Ife, Ipetumodu, Moro, and Asipa towns, Osun state.Ile-Ife is an ancient Yoruba city in south-western Nigeria. Ile-Ife city lies on the geographical coordinates of Latitude $7^0$28ˈN and Longitude $4^0$34ˈE.Moro, Ipetumodu and Asipa are towns located in Osun state, in the southwestern part of Nigeria with geographical coordinates of latitude $7^0$22'N and longitudes $4^0$30'E. Ipetumodu is also a university town of Oduduwa University. It shares a common boundary with Ile-Ife, the spiritual headquarters of the Yoruba Kingdom.

Sample Collection

Oil spilled soil samples were collected from different sites located at Ile-Ife, Ipetumodu, Moro and Asipa by adapting the aseptic technique from a depth of 5-10 cm. The samples were collected aseptically in sterilized polyethylene bags, transferred to the laboratory immediately and processed for microbiological analysis within one hour of collection.

Media

The media used included: Nutrient Agar (NA), Buffered Peptone water, Simmons Citrate Agar (SIM), Agar, Methyl-red-VogesProskuer (MR/VP) broth

Preparation of Culture Media

The medium used was prepared according to the manufacturer's specification (28g of Nutrient agar was measured into sterile conical flasks; 1000 ml of distilled water was dispensed into the conical flasks). The conical flasks were shaken very well to dissolve the medium, and then boiled on the hot-plate to homogenize. 9 ml of distilled water was dispensed into different sterile test tubes using a syringe, the test tubes were covered with cotton wool, aluminum foil and paper tape. The medium and the test tubes were sterilized in the autoclave at 121^0C for 15minutes.

Isolation of Bacteria

1g of each sample was dispensed into their respective test tubes containing 9 ml of the sterile water each, the test tubes were shaken very well to dissolve so as to prevent tiny particles from settling at the bottom. The samples were then serially diluted up to 10^{-3} (Olutiola*et al*., 2000). A volume of 1 ml of each diluent was dispensed into labelled Petri dishes and the sterilized medium was dispensed into the Petri dishes and left to solidify. The Nutrient agar plates were incubated together with one sterile Nutrient agar plate to serve as control at 37^0C for 18-24 hours to check for growth of bacteria. After 24hrs, the growth of bacteria appeared in the form of colonies and the numbers of colonies was counted using the colony counter.4 distinct colonies from each growth plate were sub-cultured on another sterile Nutrient agar plates each and incubated for 24 hours at 37^0C.

Characterization of Bacterial Isolates

The pure bacterial isolates were further identified by microscopic and several biochemical examinations. Bergey's Manual of Determinative Bacteriological (Holt *et al*., 1994) was used as a reference for the identification based on the result of various biochemical tests.

Detection of lipase activity

A chromogenic substrate medium composed of0.5 g Congo red, 10 g Peptone, 5 g Sodium chloride (NaCl), 0.1g Calcium Chloride ($CaCl_2$), 6.36 g Agar and 1 ml immersion oil in 1 litre of distilled water. The composition was dispensed into a beaker, boiled on hot-plate to homogenized and dispensed into two sterile conical flasks and autoclaved at 121^0C for 15 minutes. The sterile medium was dispensed into Petri dishes and left to solidify. The organisms were inoculated on the sterile medium plate and incubated at 37^0C for 24, 48 and 72hours. This was carried out using the modified method of Gupta *et al*. (2004). The clearance zones of inhibition and diameters of colonies were measured after 24, 48 and 72hours of incubation.

III RESULTS

A total of 11 soil samples contaminated with cooking oil were collected from different locations in Ile-Ife, Moro, Ipetumodu and Asipa. The soil samples were processed and serially diluted and pour plated using Nutrient agar medium, the growth colonies on each plate were counted and recorded as shown in Table 1. The result shows that sample A has the lowest bacterial count with 4.80×10^2 CFU/g while sample H has the highest bacterial count with 6.40×10^4 CFU/g. The bacteria strains isolated are presented in Table 2.The frequency distribution of the suspected bacteria strains as shown in Table 3 shows that *Staphylococcus sp*. has the highest occurrence in the soil sample collected while *Escherichia coli*, *Alcaligens sp*., *Citrobacter sp*., and *Serratia sp*. has the lowest occurrence. Out of the 70

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isolates, 20 bacterial isolates were screened for lipase activity by culturing them on a chromogenic composed medium. Table 4 shows the zone of inhibitions for the suspected bacterial isolates in diameter after 24, 48 and 72 hours of incubation; 12 bacterial isolates showed lipase activity while 8 bacterial isolates showed no lipase activity within the hours of incubation. The result shows that *Pseudomonas sp*. has the highest lipase activity with the largest diameter of 20.4 mm, 20.4 mm and 20.9 mm at 24, 48 and 72 hours of incubation while *Escherichia coli, Klebsiella sp., Alcaligens sp., Citrobacter sp., Streptococcus sp., and Serratia sp*. showed no lipase activity with 0.00 mm within the hours of incubation. This shows that lipase-producing bacteria have been isolated from the soil samples collected.

Figure 1 shows the prevalence of lipase activity of each suspected bacterial isolate. This graph shows that *Pseudomonas sp*. has the highest prevalence of lipase activity with a value of 32.3% and the suspected bacterial isolates with the lowest prevalence rate include *Klebsiella sp., Escherichia coli, Serratia sp., Citrobacter sp., Streptococcus sp*., and *Alcaligens sp*. with 4.2% each.. *Staphylococcus sp*. has a prevalence rate of 21.2%, *Bacillus sp*. with 6.1% prevalence rate while*Bifidobacterium sp*. and *Corynbacterium sp*. has 7.6% each.

Table.1: Total Number of Heterotrophic Bacterial Count in Cooking Oil Contaminated Soil Samples

SAMPLE CODE	TOTAL HETEROTROPHIC BACTERIAL COUNT CFU/g
A	4.80×10^2
B	1.42×10^4
C	1.76×10^4
D	1.96×10^4
E	1.80×10^4
F	4.95×10^4
G	5.90×10^4
H	6.40×10^4
I	2.34×10^3
J	2.63×10^4
K	2.10×10^3

CFU/ml (Colony Forming Unit per gram)

Table.2: The biochemical characteristics and probable identification of bacteria isolated from soil samples contaminated with cooking oil

Isolate code	Cat	Coa	I	Cit	Oxi	MR	VP	Probable Identity
A01	NA	NA	_	NA	_	_	+	*Serratia sp.*
A02	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
A06	NA	NA	+	_	_	+	_	*Escherichia coli*
A13	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
A14	+	NA	_	+	_	_	+	*Bacillus sp.*
A15	+	NA	_	+	_	_	+	*Bacillus sp.*
B01	+	NA	_	+	_	_	+	*Bacillus sp.*
B02	+	NA	_	+	+	_	+	*Bacillus sp.*
BO3	+	NA	_	+	+	_	+	*Bacillus sp.*
B04	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
B12	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
B13	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
B14	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
C01	_	_	NA	NA	_	NA	NA	*Streptococcus sp.*
C02	_	_	NA	NA	_	NA	NA	*Streptococcus sp.*
C03	_	_	NA	NA	_	NA	NA	*Streptococcus sp.*
C04	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
C05	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
C06	+	NA	_	+	_	_	+	*Bacillus sp.*
C07	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
C08	NA	NA	_	+	_	_	+	*Klebsiella sp.*
C12	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
D06	+	NA	_	+	_	_	+	*Bacillus sp.*
E01	_	NA	NA	+	NA	NA	NA	*Bifidobacterium sp.*

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E05	+	+	NA	NA	_	NA	NA	*Staphylococcus sp.*
E07	NA	NA	_	+	+	+	_	*Citrobacter sp.*
F01	_	NA	NA	+	NA	NA	NA	*Bifidobacterium sp.*
F03	+	NA	_	+	_	_	+	*Bacillus sp.*
F04	+	NA	_	+	_	_	+	*Bacillus sp.*
F05	_	NA	NA	+	NA	NA	NA	*Bifidobacterium sp.*
F08	+	NA	_	+	_	_	+	*Bacillus sp.*
F12	+	NA	_	+	_	_	+	*Bacillus sp.*
F13	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
F14	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
F15	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
G01	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
G02	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
G03	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
G04	+	_	NA	NA	_	NA	NA	*Staphylococcus* sp.
G12	NA	NA	_	+	_	_	+	*Klebsiella sp.*
G13	NA	NA	_	+	_	_	_	*Pseudomonas sp.*
G14	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
G15	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
H02	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
H03	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
H04	NA		_	+	+	_	_	*Pseudomonas sp.*
H05	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
H06	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
H13	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
H14	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
H15	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
I01	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
I02	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
I03	+	NA	_	+		_	+	*Bacillus sp.*
I04	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
I05	+	NA	_	+		_	+	*Bacillus sp.*
I06	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
I08	+	NA	NA	_	NA	NA	NA	*Corynebacterium sp.*
J01	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
J02	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
J05	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
J06	+	+	NA	NA	_	NA	NA	*Staphylococcus sp.*
J07	NA	NA	_	_	+	_	_	*Alcaligens sp.*
J08	+	NA	NA	_	NA	NA	NA	*Corynebacterium sp.*
J12	+	NA	_	+	+	_	+	*Bacillus sp.*
J13	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
J15	+	NA	_	+	+	_	+	*Bacillus sp.*
K01	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
K02	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
K13	NA	NA	_	+	+	_	_	*Pseudomonas sp.*

Keywords: + (Positive), _ (Negative), NA (Not Applicable), MR (Methy-red test), VP (VogesProskauer test), Cat (Catalase test), Coa (Coagualse test), I (Indole test), Oxi (Oxidase test), Cit (Citrate test)

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Table.3: Frequency distribution of the suspected bacteria isolates from soil samples contaminated with cooking oil

Suspected Bacterial Isolate	Frequency	Percentages
Serratia sp.	1	1.4%
Escherichia coli	1	1.4%
Bacillus sp.	15	21.4%
Pseudomonas sp.	11	15.7%
Staphylococcus sp.	30	42.9%
Bifidobacterium sp.	3	4.3%
Klebsiella sp.	2	2.9%
Corynebacterium sp.	2	2.9%
Streptococcus sp.	3	4.3%
Citrobacter sp.	1	1.4%
Alcaligens sp.	1	1.4%
Total	**70**	**100**

Table.4: Screening of the bacterial strains for lipase production on solid agar

		Lipase activity in diameters(mm)		
		Incubation time (hours)		
Isolates code	**Probable Identity**	**24**	**48**	**72**
A01	*Serratia sp.*	0.00	0.00	0.00
A06	*Escherichia coli*	0.00	0.00	0.00
B01	*Bacillus sp.*	0.00	10.2	20.0
B13	*Pseudomonas sp.*	20.1	20.1	20.1
B14	*Pseudomonas sp.*	20.3	20.4	20.9
C05	*Pseudomonas sp.*	10.8	20.1	20.1
E01	*Bifidobacterium sp.*	0.00	0.00	0.00
F01	*Bifidobacterium sp.*	0.00	10.9	20.5
F13	*Staphylococcus sp.*	0.00	20.1	20.1
G12	*Klebsiella sp.*	0.00	0.00	0.00
H02	*Staphylococcus aureus*	0.00	0.00	0.00
H04	*Pseudomonas sp.*	10.6	10.8	20.3
I04	*Staphylococcus aureus*	0.00	10.7	20.5
I08	*Corynebacterium sp.*	0.00	10.9	20.5
J05	*Staphylococcus sp.*	10.8	10.8	20.0
J07	*Alcaligens sp.*	0.00	0.00	0.00
J13	*Pseudomonas sp.*	0.00	10.5	10.9
K01	*Pseudomonas sp.*	20.2	20.2	20.2
E07	*Citrobacter sp.*	0.00	0.00	0.00
C03	*Streptococcus sp.*	0.00	0.00	0.00

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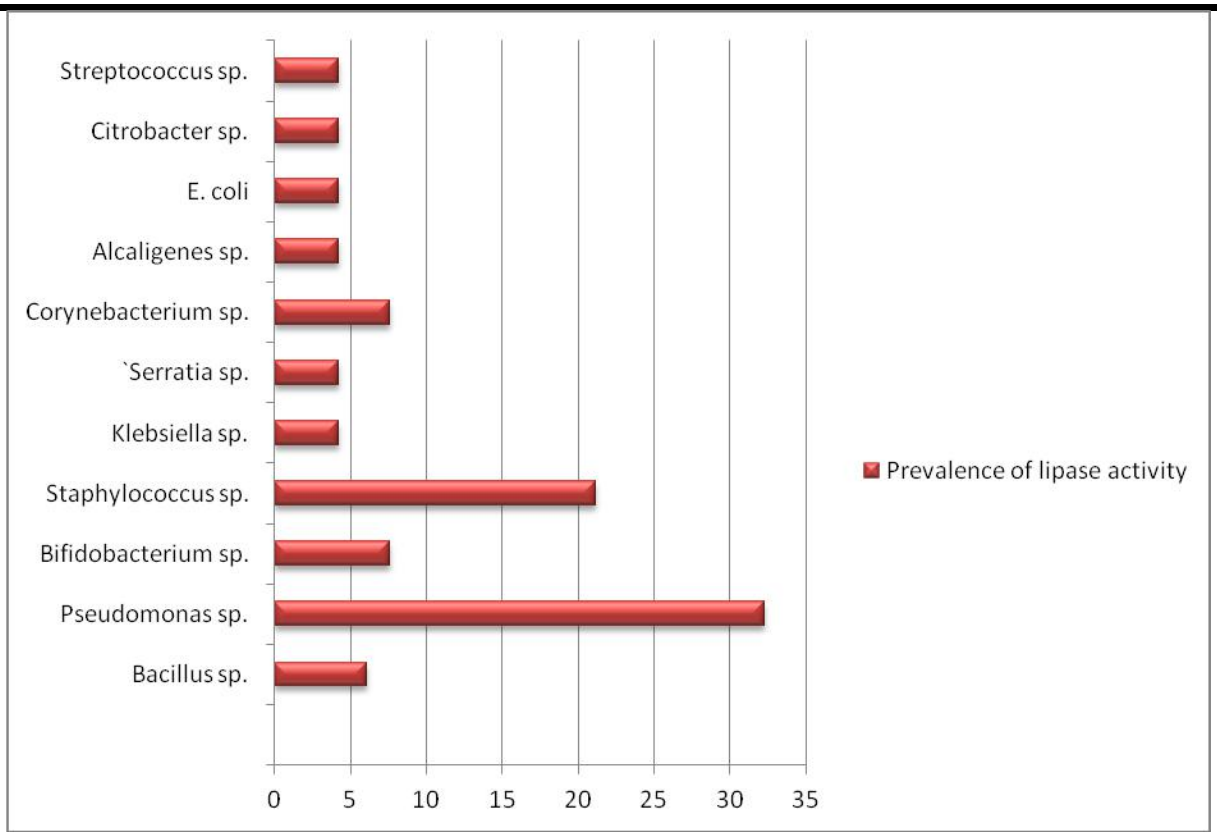

Fig.1: Prevalence of the lipase activity of suspected bacteria isolates in percentage

IV

DISCUSSION

Lipases are serine hydrolases that catalyze both the hydrolysis and synthesis of long-chain triacylglycerol. Extracellular lipases are produced by microorganisms, fungi and bacteria and the use of bacteria lipases in a variety of biotechnological applications is becoming popular. Pseudomonas lipases have proved to be the most important ones which have a significant potential in detergent industry and organic chemistry.Bacteria isolated from the environmental soil samples contaminated with cooking oil were screened for their lipase producing ability on solid agar. A total of 11 bacteria genus (*Pseudomonas sp., Bacillus sp., Corynebcaterium sp., Alcaligens sp., Staphylococcus sp., Streptococcus sp., Bifidobacterium sp., Klebsiella sp., Citrobacter sp., Serratia sp. and Escherichia coli*) were isolated from different soil samples contaminated with cooking oil collected from different locations in Ile-Ife, Asipa, Moro and Ipetumodu towns. The total heterotrophic bacteria count per gram of each soil sample ranged from 6.40×10^4 CFU/g of sample H and 4.80×10^2 CFU/g of sample A. Table 3 shows the percentage frequency of occurrence of the different bacteria isolated from the samples. *Staphylococcus sp.* had the highest frequency of occurrence (42.9%), followed in order by *Bacillus sp.* (21.4%), *Pseudomonas sp.* (15.7%), *Streptococcus sp.* (4.3%) and *Bifidobacterium sp.* (4.3%), *Klebsiella sp.* (2.9%) and *Corynebacterium sp.* (2.9%), *Escherichia coli, Serratia sp., Alcaligens sp., Citrobactersp* (1.4%). Similar results have been reported by Riaz*et al.* (2010) and Pandey *et al.* (1999). The screening of bacterial isolates for lipase production on solid agar is shown in Table 4. The lipolytic activity ranged within 10.6-20.3 mm, 10.2-20.4 mm, and 10.9-20.9 mm at 24, 48 and 72 hours of incubation respectively. *Pseudomonas sp.* (B14) had the highest activity at different hours of incubation followed by *Staphylococcus sp.*The rest of the isolate showed little lipolytic activity (Figure 1).Sirisha,*et al.* 2010 also reported the lipase producing potential of *Staphylococcus sp.*

V. CONCLUSION

Lipase producing microbes have been found in diverse habitats such as industrial wastes, vegetables, oil contaminated soil, oilseeds, and decaying food. Lipases are important enzymes in the chemical industry and the production of wine chemicals.In this study, lipase producing bacteria were isolated from the soil contaminated with

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cooking oil and the bacteria were identified as *Pseudomonas sp.*,*Staphylococcus sp., Bacillus sp.*, *Bifidobacterium sp.*, and *Corynebacterium sp*. The high lipase producing potential of *Pseudomonas sp*. suggests that the organism might be of use in waste management processes and in chemical industries.

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Road Transport and their Impacts on Health

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Abstract**—** *Traffic contributes to a range of gaseous air pollutants and to suspended particulate matter (PM) of different sizes and composition. The effects on health of transport related air pollution are among the leading concerns. Research in recent decades consistently indicates the adverse effects of outdoor air pollution on human health.*

Keywords**— Road Transport, PM, IARC.**

In the coming decades, road transport is likely to remain a significant contributor to air pollution in cities. Traffic contributes to a range of gaseous air pollutants and to suspended particulate matter (PM) of different sizes and composition. The effects on health of transport related air pollution are among the leading concerns of transport. Research in recent decades consistently indicates the adverse effects of outdoor air pollution on human health. The evidence points to air pollution stemming from transport are an important contributor to these effects.

In this view, a systematic reporting of diseases related to air pollution as well evaluation of health risks posed due to such pollution are documented. The discussion of the adverse effects on health aims to consider both epidemiological studies and toxicological assessments and studies of biological mechanisms are of great concern for further research work.

A major challenge facing India is to attain a proper balance between economic growth and environmental quality, of which air pollution is an important aspect. Particulate are mainly produced from coal combustion, diesel engines, construction and industrial activity.

Presence of toxic elements in the atmospheric is of great concern due to their adverse affect on human health and ecosystem. *Air pollution is a form of environmental degradation which has become widespread regarding economic and population growth. Such environmental degradation leads to public health consequence, thereby causing diseases impairing community welfare* ***(1,3)*** *a relationship between PM10, exposure and negative effects on health leading to respiratory and cardiovascular morbidity and mortality has already been established* ***(4,2)***. Air toxics can be defined as having three characteristics a. they have the potential to cause serious adverse health effects in the general population or to organisms in the environment as a result of air borne exposures b. they are released from anthropogenic sources c. they include 189 hazardous air pollutants listed in section 112.b.1 of the clean air act of 1990. WHO estimates that some 80% of outdoor air pollution related premature deaths were due to ischemic heart disease and strokes while 14% of deaths were due to chronic obstructive pulmonary disease or acute lowered respiratory infections, and 6% of deaths were due to lung cancer. A 2013 assessment by WHO's International Agency for Research on Cancer (IARC) concluded that outdoor air pollution is carcinogenic to humans, with the particulate matter component of air pollution most closely associated with increased cancer incidence, especially cancer of the lung. An association also has been observed between outdoor air pollution and increase in cancer of the urinary tract / bladder.

Ambient (outdoor air pollution) in both cities and rural areas was estimated to cause 3.7 million premature deaths worldwide per year in 2012; this mortality is due to exposure to small particulate matter of 10 micro or less in diameter (PM_{10}), which cause cardiovascular and respiratory disease and cancers.

Accurate estimates of human exposure to inhaled air pollutants are necessary for a realistic appraisal of the risks these pollutants pose and for the design and implementation of strategies to control and limit those risks. Except in occupational settings such estimates are usually based on measurements of pollutant concentration in outside (ambient) air, recorded with outdoor fixed site monitors. Whether a person is exposed once a week or several times a day can be an important determinant of air pollution injury. Individual exposure versus population exposure is as follows. The pollutant concentrations experienced by an individual during normal daily activities are referred to as personal or individual exposures. A personal exposure depends on the air pollutants concentration that are present in the locations the person moves through, as well as on the time spent in each location. Measuring any one person's exposure is a relatively straight forward procedure, but from a public health perspective it is important to determine the population exposure. The aggregate exposure for a specified group of people such as community or an occupational cohort is rarely necessary to measure the exposure of each member of the group. But some

measures of the distribution of individual exposure is needed. This typically includes at least a measure of the central tendency (ex: mean exposure and of its variability (ex :variance). An accurate and statistically valid characterization of even these simple descriptors of population exposure may require many personal exposure measurements.

However, even if one established that a particular specified hazard can be responsible for some unwanted health effect (asthma and air pollutants), this level of information on its own is of limited practical use, what is really needed is a quantitative measure of the likelihood of an adverse health effects i.e. the risk for a given level of exposure.

Another necessity is to determine the exposure response relationships for mixed exposures and the interaction between these and other risk factors. Having established causation, and exposure response relationships (no mean task) it may be important to translate these into health economic terms.

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Assessment of Wheat Genotypes for Yield and its Components Developed through Gama Rays

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***Abstract*—** *An experiment was conducted to assess the advance spring wheat mutant lines for some quantitative traits in ten genotypes of wheat (Triticumaestivum L.) evolved through mutation breeding techniques along with two check varieties (NIA- Sunahri and Kiran-95). The experimental trial was conducted in the field of Plant Genetics Division, Nuclear Institute of Agriculture (NIA), Tandojam, during Rabi season 2011-2012. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The experimental material was examined for mean performance, correlation coefficient and regression coefficient of important yield components towards grain yield plant^{-1}. The mean squares (ANOVA) and mean performances of ten wheat genotypes revealed highly significant differences at ($p<0.01$) level fordays to 75% heading, days to 75% maturity, spikelets spike^{-1}, grains spike^{-1}and grain yield of main spike, while harvest index is significant at ($p<0.05$) level. The trait grain yield plant^{-1} was positively and highly significantly associated with days to 75% heading, spikelets spike^{-1}and grains spike^{-1} while significantly and positively correlated with grain yield of main spike (g), however, it was non-significantly associated with days to 75% maturity and harvest index (%).Regression analysis showed that an increase in the days to 75% heading, days to 75% maturity, spikelets spike^{-1}, grains spike^{-1}, grain yield of main spike and harvest index (%) will increase grain yield plant^{-1} by 0.261, 0.195, 1.120, 0.376, 10.432 0.398 grams, respectively. It was, therefore, suggested that days to heading, spikelets spike^{-1}and grains spike^{-1} should be given emphasis for future wheat yield improvement programs.*

***Keywords*— Wheat, correlation, mutation breeding, agronomic traits.**

I. INTRODUCTION

Bread Wheat (*Triticumaestivum*L.) has got a very unique position among all the cereals being cultivated throughout the world. It occupies the central position in formulating agricultural policies (Farooq, 2011). Creative human studies have produced tangible results in the evolution of new wheat varieties from the crosses of old and new varieties in view of an enhanced consumption pressure of growing population. The reduction in production of wheat stresses the need to develop genotypes with consistent performance over a wider range of environments.

Crop improvement using induced mutagenesis is now well Standardized. A large number of new promising varieties in wheat crop have successfully been developed world wide using both physical and chemical mutagens. Recent advances in technology combined with classic crop improvement using induced mutagenesis is now well being, mutation breeding offers new and exciting challenges for development of new varieties. (Datta, 2005).

Correlation is the simultaneous variation of two variables. It is often desirable to observe and measure the relationship between two series because in case of relationship, the fluctuation in one result into a proportionate variation in the other. Hence, the breeding work gets simplified and facilitated. (Bhutto et al., 2005).

II. MATERIALS AND METHODS

To evaluate ten wheat genotypes viz., Mutant-9, Mutant-12, Mutant-17, Mutant-22, Mutant-28, Mutant-29, Mutant-32, Mutant-34, evolved through mutation breeding techniques along with two check varieties (NIA- Sunahri and Kiran-95), the experiment was conducted in Randomized Complete Block Design (RCBD) during Rabi season 2011 with three replications at

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experimental area of Nuclear Institute of Agriculture (NIA), Tandojam. Fourrows/entrywere kept, with 30cm row to row distance and 2 meter row length. The data was recorded on days to 75 % heading, days to 75 % maturity, spikelets spike^{-1}, grains spike^{-1}, grain yield of main spike (g) and harvest index (%). The data collected was subjected to statistical analysis according to the Gomez and Gomez (1984) andSnedecor and Cochran (1987). Correlation coefficient and regression coefficient were calculated for following character combinations.

1. Days to 75% heading V/S Grain yield plant^{-1}
2. Days to 75% maturity V/S Grain yield plant^{-1}
3. Spikelets spike^{-1} V/S Grain yield plant^{-1}
4. Grains spike^{-1} V/S Grain yield plant^{-1}
5. Grain yield of main spike plant^{-1} V/S Grain yield
6. Harvest index V/S Grain yield plant^{-1}

III. RESULTS

The mean squares (ANOVA) and the mean performances of ten wheat genotypes are presented in Table- 1 and Table- 2 respectively. Table- 1 showed that genotypes are highly significant at 0.01 level for days to 75% heading, days to 75% maturity, spikelets spike^{-1}, grains spike^{-1}, grain yield of main spike (g), while harvest index is significant at ($p<0.05$).

Table.1: Analysis of variance (mean squares) of ten genotypes for twelve important agronomic characters of bread wheat

Characters	Replication mean square(DF.2)	Genotypes mean square (DF.9)	Error mean square (DF.18)	F-value
Days to 75% heading	0.1333	34.7444	2.4667	14.09**
Days to 75% maturity	0.10000	8.16296	0.84074	9.71**
Spikelets spike^{-1}	7.50533	5.09704	0.60904	8.37**
Grains spike^{-1}	29.457	250.199	34.560	7.24**
Grain yield of main spike (g)	0.10192	0.31122	0.04475	6.95**
Harvest index (%)	71.4885	30.1492	11.5059	2.62*

Table.2: Mean performance of yield and yield contributing characters of wheat genotypes.

Genotypes	Days to 75% heading	Days to 75% maturity	Spikelets spike^{-1}	Grains spike^{-1}	Grain yield of main spike (g)	Harve-st index (%)
Mutant-9	88	116.67	20.4	60.133	2.177	40.523
Mutant-12	88.667	116.67	21.267	64.067	2.277	38.365
Mutant-17	84	115.33	19.667	54.133	2.03	34.373
Mutant-22	82.333	115.00	19.533	55.067	2.203	35.123
Mutant-28	88.667	117.33	22.133	68.267	2.243	37.84
Mutant-29	90	117.00	22	64.467	2.247	40.72
Mutant-32	82.333	114.67	18.2	47.4	1.703	37.386
Mutant-34	89.333	118.33	20.2	51.533	1.553	36.914
NIA-Sunahri	81.667	113.33	18.867	52	2.23	37.277
Kiran- 95	89.333	113.67	19.4	77.2	2.717	29.84
LSD @ 5%	2.6941	1.5729	1.3387	10.084	0.3629	5.8187

Days to 75% heading

The analysis of variance for days to heading of different wheat genotypes is given in Table-1. The F-value of 14.09 showed highly significant difference among the genotypes. The mean performance for days to heading is presented in Table-2.The mean values revealed that the genotypes differed significantly from each other. The check variety NIA-Sunahri took minimum days to heading (81.667) followed by genotype M-32 and M-22, the genotypes M-29, M-34 and Check variety Kiran-95 took maximum days to heading as compared to all other genotypes. While the genotypes M-9, M-12, M-17, M-28 took more or less equal days to heading.

Days to 75% maturity

The analysis of variance for the character days to maturity is given in Table-1. The analysis of variance showing F-value of 9.71 indicated highly

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significantly difference among the genotypes. The mean performance for days to maturity is presented in Table-2. The mean values of genotypes were differed significantly from each other. These values were ranged from 113.333 to 118.333. In the present study check varieties NIA-Sunahri, Kiran-95 and genotype M-32 took minimum days to maturity. The genotypes M-28, M-29 and M-34 took maximum days to maturity.

Spikelets spike $^{-1}$

In Table-1, F-value (8.37) indicating highly significant difference among all genotypes for the character of spikelets spike $^{-1}$. In Table-2, the mean values of the genotypes are ranging between 18.2 to 22.133. The genotype M-28 produced maximum number of spikelets spike^{-1} (22.133) as compared to all other genotypes. The minimum number of spikelets^{-1}spike were produced by genotype M-32 (18.2). The mean performance of check varieties NIA-Sunahri and Kiran-95 statistically at par to rest of the genotypes.

Grains spike $^{-1}$

The analysis of variance for grains spike $^{-1}$of different genotypes and check varieties are presented in Table-1. The F-value of 7.24 indicated highly significant differences among all the genotypes. The mean performance of all the genotypes for grains spike^{-1} is depicted in Table-2, which reveals that the genotype differed significantly from each other. Their values are ranging from 47.4 to 77.2. The check variety Kiran-95 produced maximum number of grains spike^{-1} (77.2) followed by genotype M-28 (68.267), M-29 (64.467) and M-12 (64.067). However, the genotype M-32 reflected minimum number of grains spike^{-1} (47.4) followed by genotype M-34 (51.533) and check variety NIA-Sunahri (52.000).

Grain yield of main spike (g)

The data regarding analysis of variance for the character grain yield of main spike is summarized in Table-1. The F-value 6.95 indicating highly significant difference among the genotypes.The mean performance of M_5 genotypes and check varieties for the traits grain yield of main spike, is shown in Table-2, which revealed great differences among the genotypes. The mean values of all the genotypes are ranging between 1.553 to 2.717. The check variety Kiran-95 (2.717) and genotype M-12 (2.277) produced maximum grain yield of main spike followed by genotype M-28 (2.243) and check variety NIA-Sunahri (2.23). The genotype M-34 produced the lowest grain yield of main spike (1.553). While other genotypes remains at par to check variety NIA-Sunahri.

Harvest index (%)

The mean square for harvest index is presented in Table-1, the F-value 2.62 indicating significant differences among all the genotypes. The mean performance of genotypes for harvest index is depicted in Table-2, reveals that the genotypes differed from each other and the values are ranging b/w 29.84 to 40.72. The genotype M- 29 (40.72) showing maximum harvest index % followed by genotype M-9 (40.523). However, the check variety Kiran-95 (29.84) showing minimum harvest index %, while other genotypes remain at par to check variety NIA-Sunahri.

Table.3: The value of correlation coefficient (r), coefficient of determination (r^2), regression coefficient (b) and their significance for main agro-economical traits and grain yield plant^{-1} combinations in wheat.

S.No.	Characters	Correlation 'r'	Coefficient of determination $(r)^2$	Regression coefficient'b'	Remarks
1	Days to 75% heading v/s Grain yield plant^{-1} (g)	0.617	0.38024	0.261	**
2	Days to 75% maturity v/s Grain yield plant^{-1} (g)	0.351	0.12337	0.195	N.S
3	Spikelets spike^{-1} v/s Grain yield plant^{-1} (g)	0.790	0.62489	1.120	**
4	Grains spike^{-1} v/s Grain yield plant^{-1} (g)	0.674	0.45476	0.377	**
5	Grain yield of main spike (g) v/s Grain yield plant^{-1} (g)	0.543	0.29550	10.432	*

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Correlation coefficients and regression analysis

Correlation coefficient coefficients (r), coefficients of determination (r^2), regression coefficients (b) and their test of significance (t) between grain yield and other quantitative traits were conducted in ten genotypes for twelve characters viz., days to 75% heading, days to 75% maturity, spikelets spike^{-1}, grains spike^{-1}, grain yield of main spike (g) and harvest index (%) are presented in table-3. The results are described hereunder

Days to 75% heading v/s grain yield plant^{-1} (g)

Highly significant correlation coefficient (r =0.617)** had been obtained between days to 75% heading and grain yield plant^{-1}, (Table-3). Coefficient of determination revealed that 38.02% of the total variability in grain yield plant^{-1} was due to its association with days to 75% heading. The regression coefficient determined an increase of 0.261 grams in grain yield plant^{-1} with increase a day in days to 75% heading.

Days to 75% maturity v/s grain yield plant^{-1} (g)

The Table-3 reveals that the character days to 75% maturity showed non-significant association (r= 0.351)$^{N.S}$ with grain yield plant^{-1} (g), which indicating that 12.34 per cent variation in grain yield plant^{-1} was caused due to the variation in days to 75% maturity. Regression coefficient indicated that with delay of one day in maturity, the grain yield increased by 0.195 grams.

Spikelets spike^{-1} v/s grain yield plant^{-1} (g)

The Table-3 shows a highly significant positive correlation between spikelets spike^{-1} and grain yield plant^{-1} (r= 0.790). The coefficient of determination indicated 62.48 per cent of the total variability in grain yield plant^{-1}to be accounted for its association with spikelets spike^{-1}. The value of regression coefficient indicated that because of a unit increase in spikelets spike^{-1} will result in simultaneous increase of 1.120 grams in grain yield plant^{-1}.

Grains spike^{-1} v/s grain yield plant^{-1} (g)

The Table-3 depicts a highly significant positive correlation (r= 0.674) between grains spike^{-1} and grain yield plant^{-1}. The coefficient of determination indicated that 45.48 per cent of the total variability in grain yield plant^{-1} can be accounted for its association with grains spike^{-1}. Regression coefficient indicated that with a unit increase in grains spike^{-1}, the grain yield plant^{-1} increased by 0.376 grams.

Grain yield of main spike (g) v/s grain yield plant^{-1}

A significant positive correlation (r= 0.543) has been computed between grain yield of main spike and grain yield plant^{-1} (Table-3). The coefficient of determination indicated that 29.55 per cent variation in grain yield plant^{-1} could be accounted for its association with grain yield of main spike. The coefficient of regression displayed that an increase of a yield in main spike will results in a corresponding increase of 10.432 grams in grain yield plant^{-1}.

Harvest index % v/s grain yield plant^{-1} (g)

The Table-3 reveals non-significant and positive correlation (r= 0.329) between harvest index and grain yield plant^{-1}. the coefficient of determination indicated that 10.83 per cent of the total variability in grain yield plant^{-1} was due to its relationship with harvest index (%). The regression coefficient indicated that with a unit increase in harvest index, the grain yield plant^{-1} increased by 0.398 grams.

IV. DISCUSSION

1) Mean performance of genotypes

The mean squares revealed highly significant differences among the genotypes for the characters viz., days to 75% heading, days to 75% maturity, plant height, tillers plant^{-1}, spike length, spikelets spike^{-1}, grains spike^{-1}, grain yield of main spike, grain yield plant^{-1} and biological yield. Whereas significant for harvest index and non-significant for 1000- grains weight. It reflects great genetic variability among the material studied.

The results regarding to mean performance of the genotypes for twelve important traits reveals that check variety NIA-Sunahri, genotype M-22 and M-32 were early in days to heading, whereas the genotypes M-29, M-34 and check variety Kiran-95 were late in days to heading. As for as days to maturity is concerned, the check varieties NIA-Sunahri and Kiran-95 were early in maturity and late maturing genotypes were M-28 and M-34.For the character spikelets spike^{-1}, genotypes M-28 and M-29 produced maximum spikelets spike^{-1}. The maximum grains spike^{-1} produced by check variety Kiran-95 followed by the genotype M-28. As far as the trait grain yield of main spike^{-1} is concerned, the check variety Kiran-95 produced maximum grain yield of main spike followed by the genotypes M-12, M- 28 and M-29. The maximum harvest index (%) value were attained by the genotypes M-9 and M-29, The present findings revealed great difference among the genotypes, which

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reflecting utilization of such variability for the improvement of crop as well as for the trait concerned. Several researchers like Memon*et al*. (2007), Mohammad *et al*. (2008), Anwar *et al*. (2009), Haq*et al*. (2010)and Mohibullah*et al*. (2012) indicated the observed variability among the genotypes in wheat in various morphological characters.

2) Correlation coefficients and regression analysis

Correlation coefficient and regression studies between grain yield and other quantitative traits were conducted in ten genotypes for the characters viz., days to 75% heading, daysto 75% maturity, plant height (cm), tillers plant^{-1}, spike length (cm), spikelets spike^{-1}, grains spike^{-1}, grain yield of main spike (g), 1000-grains weight (g), biological yield plant^{-1} (g) and harvest index (%) combinations with grain yield plant^{-1}. The results obtained for correlation coefficients (r), coefficients of determination (r^2), regression coefficients (b) and their test of significance (t) are presented in table-3. The results are described hereunder.

The present findings revealed that there existed a highly significant positive correlation between these two characters. These results are in agreement with the findings of Akhter*et al*. (2011), who also reported significant and positive correlation between these characters. The coefficient of determination indicated that variability of 38.02 per cent in grain yield plant^{-1} was due to its association with days to 75% heading. The regression coefficient determined an increase of 0.261 grams in grain yield plant^{-1} with a unit increase in days to 75% heading. Therefore early heading genotypes would be important selection criteria for improved grain yield plant^{-1} in the breeding material studied. A non-significant positive correlation has been obtained between days to 75% maturity and grain yield plant^{-1}. Coefficient of determination indicated that 12.34 per cent variation in grain yield plant^{-1} (g) was caused by its association with days to 75% maturity. Regression coefficient indicated that with a delay of one day in maturity, the grain yield decreased by 0.194 grams. Present results are supported by the findings of Anwar *et al*. (2009) and Haq*et al*. (2010). From these results it is evident that while selecting for increased grain yield plant^{-1}, early maturing plants should be selected.

In the present study, these two characters displayed almost positive relationship which exhibited that 62.48 per cent of total variation in grain yield plant^{-1} was induced by spikelets spike^{-1}. Regression coefficient also determined that, a unit increase in spikelers spike^{-1} produced a corresponding increase of 1.120 grams in grain yield plant^{-1}. present findings are also in agreement with those of Khan and Dar (2010), Haq*et al*. (2010) and Akhter*et al*. (2011) who also found similar results for these two characters. The very high positive correlation between spikelets spike^{-1} and grain yield plant^{-1}can be successfully exploited while selecting for high grain yield in any breeding programme.Highly significant and positive correlation has been recorded between these two characters. Present results are in agreement with those obtained byMajumder*at al*. (2008) and Akram*at al*. (2008) who also found similar type of relationship between grains spike^{-1} and grain yield plant^{-1}. Coefficient of determination indicated that 45.48 per cent of the total variability in grain yield plant^{-1} was due to its association with grains spike^{-1}. regression coefficient indicated that with a unit increase in grains spike^{-1}, the grain yield plant^{-1} increased by 0.376 grams. This high positive and significant correlation between these two characters shows that this yield component is considerably important in breeding programme for high yielding wheat varieties.

The present findings revealed that there existed a significant positive correlation between these two characters. These results are in agreement with the findings of Akhter*et al*. (2011) who also reported significant positive correlation between these two characters. The coefficient of determination indicated that the variability of 29.55 per cent in grain yield plant^{-1} was due to the corresponding variation in grain yield of main spike^{-1}. The regression coefficient revealed that a unit increase in grain yield yield of main spike^{-1} cause a linear increase of 10.432 grams in grain yield plant^{-1}. Therefore for breeding and selection programmes for high yielding varieties of wheat, emphasis should be placed on high yielding spikes genotypes.In the present study, these two characters displayed non-significant and positive relationship which exhibited that 10.83 per cent of the total variation in grain yield plant^{-1} was due to its relationship with harvest index (%). The regression coefficient also determined that a unit increase in harvest index, the grain yield plant^{-1} increased by 0.398 grams. Present findings are also in agreement with those of Mohammad *et al*. (2008).

V. CONCLUSIONS

It is therefore concluded that while formulating any strategy in a breeding programme for higher grain yield of wheat, emphasis should be placed on the selection for more number of tillers plant^{-1}, longer spikes, more number of spikelets spike^{-1}, more number of grains spike^{-1} and biological yield plant^{-1} which had highly

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significant direct and indirect influences on the grain yield of wheat.

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Effects of Nickel Toxicity on Seedling Growth, Photosynthetic Pigments, Carotenoids and Phenols Contents of Cowpea *Vigna unguiculata* (L.)

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***Abstract*—** *Nickle pollution is worldwide problem due to industrial and anthropogenic activities. Seedlings of cowpea (Vigna unguiculata) were treated to different ($NiSO_4.6H_2O$) concentrations as 0.0 mM, 0.6 mM, 1.2 mM, 1.8mM and 2.4 mM salt into distilled water. The seedlings of V. unguiculta became less tolerable to nickel at higher concentration 2.4 mM as compared to control treatment. Nickel in trace amount is required for plant growth. In present study, the high level of nickel concentration 1.8 and 2.4 mM significantly ($p<0.05$) affected seedling growth performance and physiological, biochemical parameters of V. unguiculata. The nickel treatments at 1.2 mM concentrations significantly ($p<0.05$) affected the number of leaves, shoot, seedling length, fresh weight and dry weight of seedling and root / shoot ratio of V. unguiculata. The treatment of Ni^{+2} at 1.20 mM also significantly affected shoot root and seedling fresh and dry weight of cowpea. The leaf area, leaf weight ratio and root fresh and dry weight of V. unguiculata showed less affect at 0.6 to 2.4 mM treatment of nickel. Nickel treatment at 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM concentrations showed considerable effects on relative water content, chlorophyll 'a', chlorophyll 'b' total chlorophyll, carotenoids and phenols of Vigna unguiculata.*

***Keywords*—Cowpea, nickel, photosynthesis pigments, root, seedling growth.**

I INTRODUCTION

$Ni^{+2.}$ is a solid silver white hard transition element [23] and used in manufacturing of stainless steel, coin, jewelry, nickel plating, nickel refining and dental care products. The micronutrients are responsible for plant growth and exposure at higher level considered toxic for human health risk and plant growth [13, 17 and 26]. Literature is available on the effects of nickel toxicity in plants. Inhibition in rate of seed germination percentage, photosynthesis, seedling growth, metabolism, growth and development of plants [4, 5, 14, 15, 19, 20, 21, 25 and 33]. In an investigation, the decline in chlorophyll content of the leaves of maize and *Vigna mungo* were recorded [12 and 28]. The nickel stress at 40 mg L^{-1} showed decrease in the photosynthetic pigments of *Vigna radiata* [1]. Nickel content in food may vary considerably from place to place due to the difference in nickel content of the soil while, certain foods are routinely high in nickel content [9]. Studies have shown toxic effects of nickel on seed germination and seedling growth of plants. The concentration of nickel normally considered in soil 5-500µg/gram, plant tissue 0.5-5µg/gram, and animal tissue 0.1-5µg/g and fresh water 5-100 µg/liter [2]. Nickel approximately 0.008% available in Earth′s crust, and in soil contains 40 ppm on average basis [8]. The concentration of nickel in soil vary due to soil types, use of synthetic fertilizers, pesticides, nickel smelters, industrial effluents and urban wastes [7]. Nickel is an essential element for healthy plant life, and trace amounts naturally found in most vegetables, fruits, nuts and in slightly greater amounts in chocolate and wine [22].

Nickel is tough silvery hard metal with atomic number 28 and found in living organism and mainly in plants. It is generally believe that nickel at higher concentrations in environment produce toxic effects on plant growth. The researchers are working on the impact of heavy metals on plant growth since last few years. This

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paper gives information about the effect of nickel toxicity on seedling growth and some physiological and biochemical parameters of an important legume bean crop cowpea (*Vigna unguiculata* L.) cultivating in agricultural areas of Pakistan.

II MATERIALS AND METHODS

The study was conducted in the green house of the Bio saline Research Laboratory, located at the Department of Botany, University of Karachi, Pakistan, during July to August. The sand was collected from Sands pit sand dunes, Karachi. The sand was passed through 2.00 mm sieve to remove gravels and other material. The sand was washed 5 to 6 times with running tap water and later with distilled water in order to make it free from all nutrients and minerals. The washed sand was filled up to 2/3 in plastic pots measuring 7.3 cm in diameter and 9.6 cm in height. At the bottom of pot, holes were made for the purpose of absorption of nutrients and water. The filter paper was also placed at the bottom of pots before adding sand. Four pots were place in each plastic tray, which contains irrigation medium.

A modified Hoagland solution was prepared according to Epstein [11]. The composition of Hoagland solutions given in Table 1. Different $NiSO_4.6H_2O$ solutions (0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) were prepared in Hoagland solutions. The 0.0 mM nickel solutions considered as control.

Table 1. Composition of modified Hoagland nutrient solution.

Compounds		Concentration of stock solution (mM)	Volume of stock solution per liter of final solution (ml)
Macronutrients	KNO_3	1000	6
	$Ca(NO_3)_2.4H_2O$	1000	4
	KH_2PO_4	1000	2
	$MgSO_4.7H_2O$	1000	1
Micronutrients	KCL	25	2
	$H3BO_3$	12.5	
	$MnSO_4.H_2O$	1.0	
	$ZnSO_4.7H_2O$	1.0	
	$CuSO_4.5H_2O$	0.25	
	MoO_3	0.25	
Fe Na EDTA		64	1

The average pH and E.C. (Electrical conductivity) of irrigation medium was determined by pH meter (AD 1000 p H/ mv and temperature meter) and conductivity reading meter, respectively.

Table 2. The average EC ($dS.m^{-1}$) and pH of the irrigation medium

$NiSO_4.6H_2O$ solution (mM)	EC ($dS.m^{-1}$)	pH
0	2.375	8.16
0.6	2.415	8.185
1.2	2.50	8.36
1.8	2.56	8.445
2.4	2.615	8.515

The healthy seeds of cowpea (*Vigna unguiculata* L.) were bought from the local market and were surface sterilized with 0.2% solution of sodium hypochlorite (NaOCl) for one minute to avoid any fungal contamination. Then the seeds were imbibed for 30 minutes in distilled water. The seeds were germinated in sterilized Petri plates, moistened with distilled water. Four seedlings of the same size were selected and transplanted into pots at nearly equal distance. The seedlings were initially irrigated with ¼ strength Hoagland solutions for one week. The solution was replaced after two days interval. Then the seedlings were irrigated with ½ strength Hoagland solution two times at the interval of two days. The irrigation medium was changed to full strength Hoagland solution and the seedlings were established and were subjected to the respective desired Ni^{+2} treatment. The treatment was given twice a week. During the experiment, the range of minimum and maximum temperature and relative humidity were in between 28 to 33 ºC and 65 to 74%, respectively The experiment was completely randomize block design

consisted of eight replicates. Four replicate were used for growth analysis while four were used for relative water content and biochemical analysis.

After five weeks, the seedlings were harvested and different growth parameters were determined including number of leaves per plant, leaf area, length of root and shoot, fresh and dry weight of leaves, root and shoot. For dry weights, the root and shoots were dried at 80º C for 48 hours in oven. The root, shoot ratio, leaf weight ratio, specific leaf area and leaf area ratio were determined by the following formula, respectively.

Root/ shoot ratio = root dry weight / shoot dry weight
Leaf weight ratio = leaf dry weight / total plant dry weight
Specific leaf area (cm^2 g^{-1}) = Leaf area / leaf dry weight
Leaf area ratio = Leaf area / Total plant dry weight

Relative water content (%)

For the determination of relative water contents, fully expanded leaf was excised from one plant of each pot. The dust particle were removed. The leaf sample was immediately weighted to take the fresh weight (FW) and then immersed in distilled water at 4º C for 10 hours. The saturated leaf sample was removed from water and excess water was removed by tissue paper. The leaf sample was weighted to obtain turgid weight (TW) and the dried in an oven at 70º C for 48 h to record dry weight (DW). The R.W.C. of leaf was determined by the following formula

R.W.C (%) = [FW-DW] / TW-DW] X 100

Physiological and biochemical analysis:

Phenols were determined by using folin reagent method while, soluble sugars were obtained by Anthrone reagent method.

Soluble phenols were determined by the methods of Singleton and Rossi [29]. The dried leaf powder was homogenized in 80% methanol and centrifuged. In 1 ml of diluted extracts, 5 ml of Folin-Ciocalteu reagent (1.9 ratio in distilled water) and 4 ml of 7.5 % Na_2CO_3 were added. The absorbance was recorded at 765 nm after incubation of 30 minutes at 25º C. The soluble phenols concentration in leaf tissues was determined against Gallic acid and calculated from best-fit standard curve. The concentration of total phenols mentioned in $\mu g.g^{-1}$ dry weight of leaves.

Biochemical analysis

Photosynthetic pigments

The leaf samples were excised from the plants and immediately frozen in liquid nitrogen and stored at -20º C until used for photosynthetic pigments. The leaf samples (0.1 g) were grounded in liquid nitrogen and then homogenized in 5 ml 80% cold acetone, centrifuged at 3000 rpm for 5 minutes. The supernatant was separated and the residue was again dissolved in 3 ml of 80% cold acetone and centrifuged. The process were repeated until all the photosynthetic pigments were extracted. All supernatant fractions were pooled and final volume was adjusted. The absorbance of the extract was recorded at 649 and 665 nm for chlorophylls determination while 480 and 510 nm for carotenoids determination, respectively. The absorbance was recorded on spectrophotometer the chlorophyll and carotenoid contents were determined according to the equation described by Strain, *et al*., [30] and Duxbury and Yentsch [10], respectively.

Chlorophyll a (µg/ml) = 11.63 (A_{665}) – 2.39 (A_{649})
Chlorophyll b (µg/ml) = 20.11 (A_{649}) – 5.18 (A_{665})
Total Chlorophyll (µg/ml) = 6.45 (A_{665}) + 17.72 (A_{649})
Carotenoids (µg/ml) = 7.50 (A_{480}) – 2.63 (A_{510})

Statistical analysis

The means as well as standard errors were calculated. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using personal computer software packages SPSS version 14.0 were statistically analyzed the data. Level of significance for these tests was at $P < 0.05$.

III RESULTS AND DISCUSSIONS

In this paper, the treatment of various concentrations of nickel 0.6, 1.2, 1.8 and 2.4 mM affected number of leaves, root, shoot, seedling length, fresh weight of root, shoot and leaves, dry weights of root, shoot and leaves, leaf area and specific leaf area of *V. unguiculata* as compared to control (0.0 mM). Nickel affects significantly ($p<0.05$) the seedling length and shoot length of cowpea at 0.6 mM as compared to control. Nickel after two weeks of treatment started leaves tip chlorosis and then in third week necrotic lesions appeared on leaves followed by the wilting of cowpea seedlings. In another study, barley plants grown in 100 µM Ni showed typical visual symptoms of Ni toxicity such as chlorosis, necrosis of leaves and browning of the root system [24]. Nickel in adequate quantities has a vital role in a large variety of physiological processes, from seed germination to productivity [31] . Root, shoot and seedling growth of green gram showed tolerance at low concentration of nickel. Metals toxicity is a problem for all living organisms [18]. The report confirmed that seedlings

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treated to Nickel metal at higher concentration induced toxicity and showed reduction in seedling growth of green gram and agrees with the findings of Yang and Zhao [32] who reported that Ni^{2+} treatment affected all growth indices of oilseed rape (*Brassica napus* L.) gradually at higher concentration.

Nickel treatment at 1.2 concentration affected root growth of *V. unguiculata*. While, no significance difference was found in root length of *V. unguiculata* at highest concentration of nickel treatment at 2.4 mM. The treatment of nickel at 1.2 mM significantly affected on number of leaves of *V. unguiculata* as compared to control.

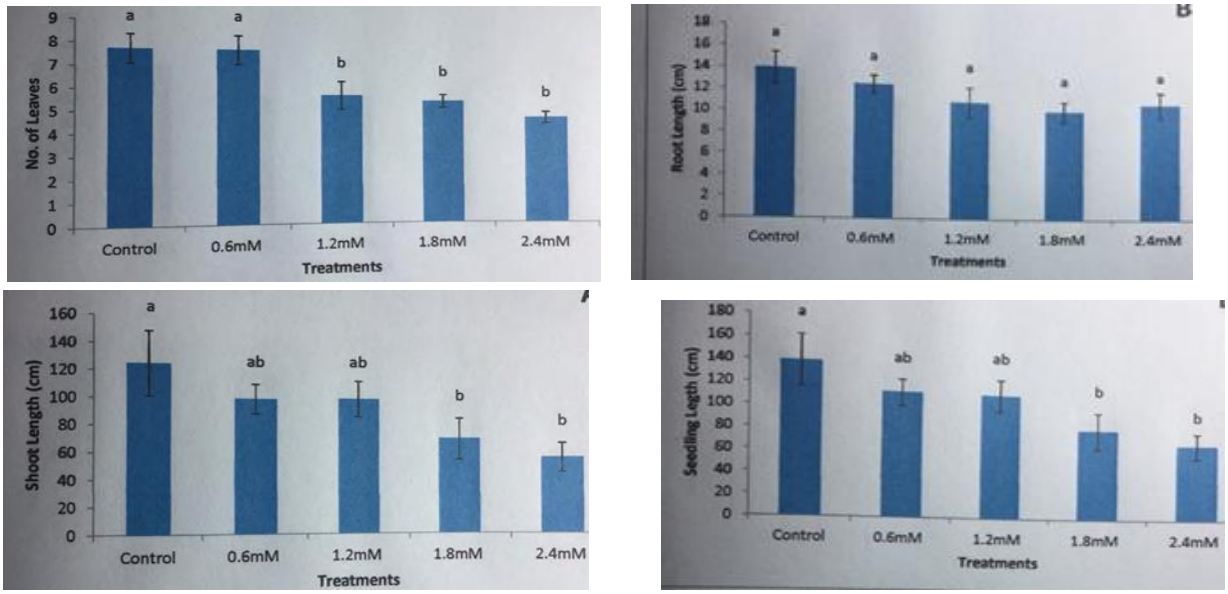

Fig. 1. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on number of leaves, root, shoot and seedling length (cm) of Vigna unguiculata. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different (p<0.05) according to Duncan's multiple range test.

Nickel treatment at all concentration produced toxic effects on fresh weight of root for *V. ungiculata* (Fig. 2). An increase in concentration of Nickel 1.2 mM caused a gradual decline significantly in fresh weight of root for *V. unguiculata*. Nickel treatment at 1.20 mM also significantly affected shoot, leaves and total seedling fresh weight of *V. unguiculata*.

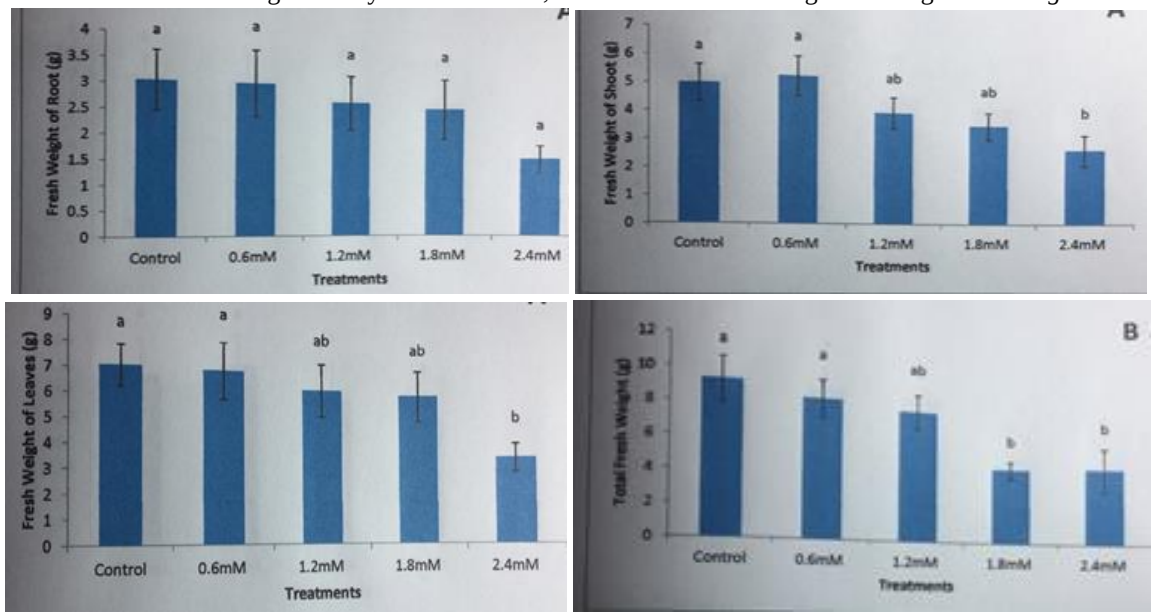

Fig. 2. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on fresh weight of root, shoot, leaves and total fresh weight (g) of Vigna unguiculata. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different (p<0.05) according to Duncan's multiple range test.

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In *V. unguiculata* a constant decline in dry weight of root, shoot, leaves and seedling dry weight was recorded (Fig. 3). The Ni^{+2} treatment at 1.2 mM caused a significant ($p<0.05$) decrease in seedling dry weight of *V. unguiculata*. An increase in concentration of Nickel to 2.4 mM caused further reduction ($p<0.05$) in shoot, leaves and seedling dry weight of *V. unguiculata* as compared to control.

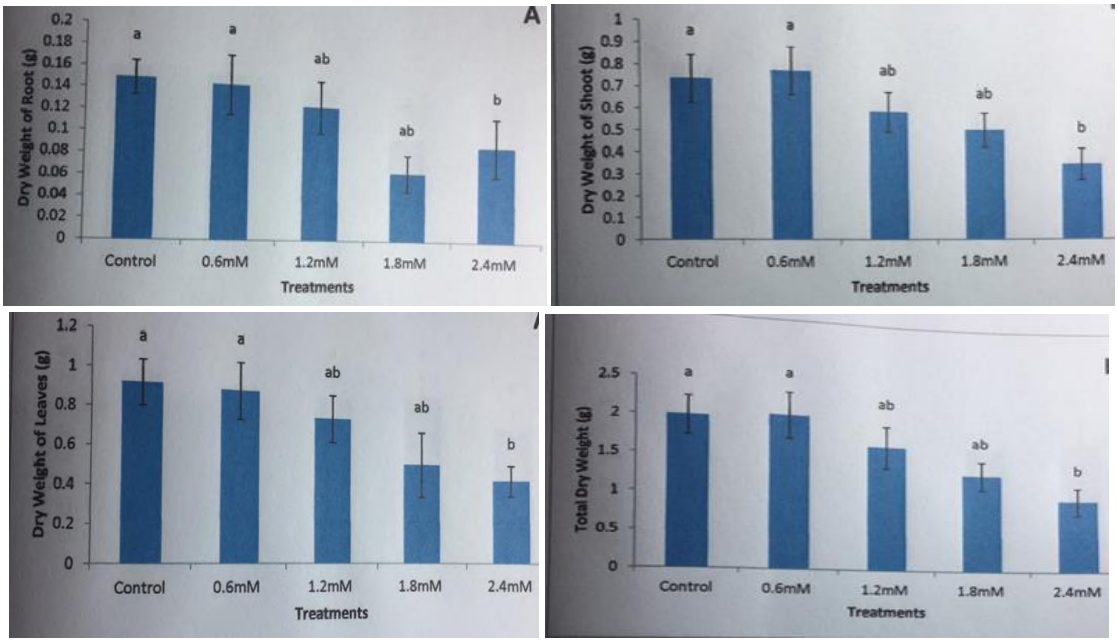

Fig. 3. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on dry weight of roots, shoot, leaves and total dry weight (g) of Vigna unguiculata. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test.

In *V. unguiculata* no significance difference ($p<0.05$) was found in leaf area and root / shoot ratio of *V. unguiculata* with the treatment of nickel at all concentration as compared to control (Fig. 4). An increase in concentration of Nickel 0.6 to 2.4 mM caused a decline in leaf area and root / shoot ratio of *V. unguiculata*. Increase in nickel treatment also decreased the leaf weight ratio, specific leaf area and leaf area ratio of *V. unguiculata*.

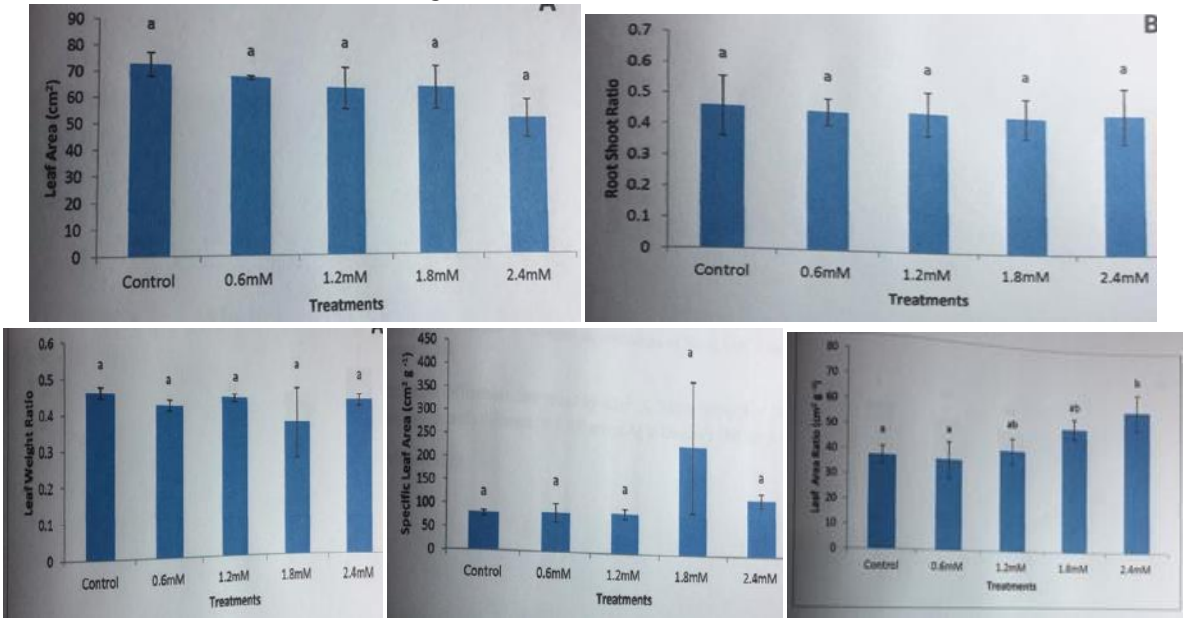

Fig. 4. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on leaf area, root / shoot ratio, leaf weight ratio, specific leaf area, leaf area ratio of V. unguiculata. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test.

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The effects of different concentrations of nickel 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM on relative water content (%), chlorophyll 'a', chlorophyll 'b' total chlorophyll, carotenoids and phenols µg/ml of *Vigna unguiculata* were recorded The effects of nickel on relative water content of *V. unguiculata* was less affected (Fig. 6). In, *V. unguiculata* the decreasing trend was found in relative water content with the increase in concentration of nickel. The variations in photosynthetic pigment content of *V. unguiculata* was found. The chlorophyll 'a' and total chlorophyll content was significantly ($p<0.05$) decreased, but no significant different was found in chlorophyll 'b' content. In a study, Singh and Pandey (2011) []reported the toxic effect of nickel stresses on uptake, pigments and antioxidative responses of water lettuce, *Pistia stratiotes* L. Carotenoid also showed significant decreasing trend from control to higher values. i.e. 1.8 mM Ni and 2.4 mM Ni concentration. The reduction was cleared at 2.4 mM Ni concentration. An increase in phenols contents of *V. unguiculata* at higher concentration i.e. 2.40 mM Ni concentration as compared to control was recorded. Such results are in agreement with the findings of Gopal *et al.*, (2002), Pandey, and Sharma (2002) []. These results suggests that nickel might have oxidative damaged to membranous system of chloroplast (Boaccouch *et al.*, []1998). High levels of this micronutrient can alter various metabolic activities of the plant such as the ratio of mineral nutrients, enzyme inhibition, functioning of the stomata, photosynthetic transport of electrons, and degradation of chlorophyll molecules, consequently reducing the photosynthetic rate, growth and chlorophyll content and biological yield of plants[] (Bybordi and Gheibi, 2009).

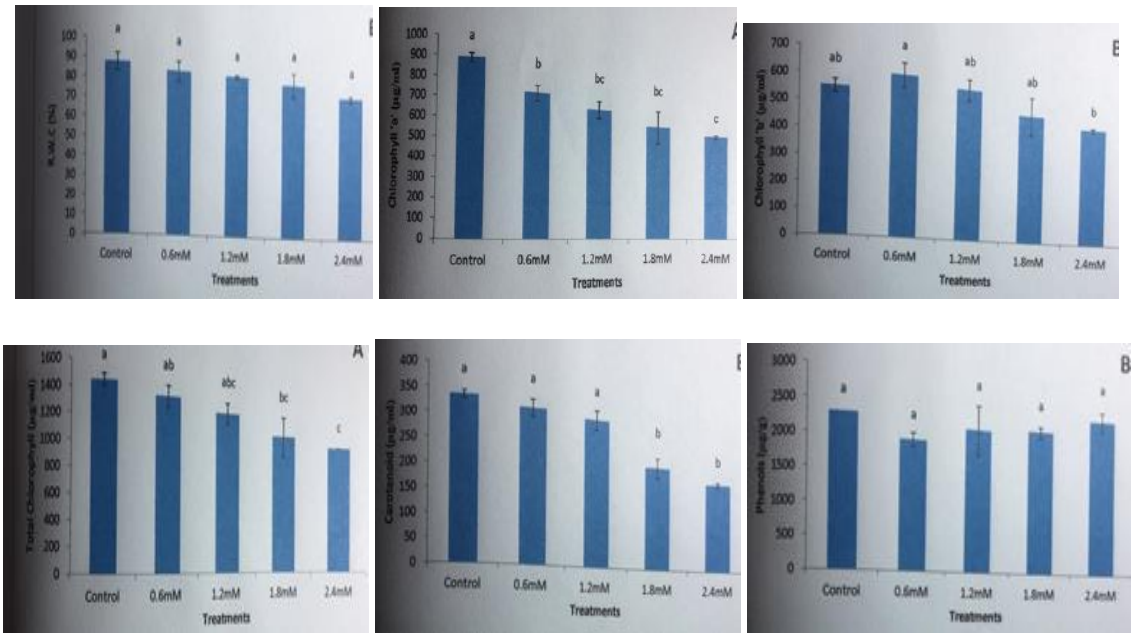

Fig. 6. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on relative water content (%), chlorophyll 'a', chlorophyll 'b' total chlorophyll, carotenoids and phenols µg/ml of Vigna unguiculata. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test.

IV

CONCLUSION

In summary, from the present findings suggest that the nickel treatment at different levels 0.6, 1.2, 1.8 and 2.4 mM) affected seedling growth performance, physical and biochemical parameters of cowpea (*Vigna unguiculata* (L.). Root growth, specific leaf area and leaf area ratio of cowpea seedlings showed tolerance to nickel toxicity at 0.6 mM concentration as compared to control. Seedling fresh and dry weight affected at 1.2 mM Ni concentration. Nickel concentration at 1.2 mM significantly affected number of leaves of cowpea. Shoot growth of cowpea highly affected than root growth. Relative water content of cowpea in response to nickel levels at 0.6 mM were less affected as compared to control. Ni^{+2} treatment at 0.6 mM significantly affected chlorophyll 'b' and carotenoids (µg/ml) content of cowpea. Ni^{+2} treatment significantly increased phenols content of cowpea with the increase in nickel treatment. Overall results suggests that cowpea has a potential of

cultivation in nickel polluted soils having less than 2.4 mM level of nickel and could serve as marker of nickel toxicity.

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Effects of Railway Engine Emission on the Seedling Growth behavior of Woody Plant Species *Acacia nilotica* (Linn.) Delile

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***Abstract*—** *The vegetation growing close to the railway tracks are usually exposed to various types of toxic pollutant discharged from the locomotive diesel engines. In present studies, the seedling growth performance of Acacia nilotica responded differently in soils of Cantonment Station, Drighroad Junction, Malir Station and Landhi Junction as compared to Karachi University Campus (Control) soil. The seedling growth of A. nilotica (Linn.) Delile gradually increased in soil of Drighroad Junction, Malir Station and Landhi Junction. The seedling growth of A. nilotica in Cantonment soil was significantly ($p<0.05$) deceased as compared to the treatment of University Campus soil. The root, shoot, seedling lengths, circumference, root, stem and seedling dry weights of A. nilotica grown in soil of Cantonment Station showed significant ($p<0.05$) reduction as compared to University Campus. The growth parameters such as root, shoot, seedling lengths, number of leaves, leaf area, circumference, root, stem, leaf, seedling dry weights of A. nilotica grown in soil of Malir Station and Landhi Junction were significantly ($p<0.05$) enhanced as compared to University Campus soil.*

***Keywords*— dry weight, kikar, locomotive railway diesel engine, root, seedling growth, tolerance.**

I INTRODUCTION

Pakistan is a developing country and its urban cities are suffering by a series of environmental pollution problems due to increase in automobile and industrial activities [29]. The automobile activities is a major source of different types of pollutants such as suspended particulate matter, carbon monoxide, hydrocarbons (HC), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), ozone (O_3), peroxyacetyl nitrate (PAN) and heavy metals (Pb, Cd, Cr, Ni, Zn) in the environment. The addition of pollutants in soils nearby railway track might produce toxic effects on the biodiversity, trees and crops productivity. The diesel engines showed low concentrations of carbon monoxide and unburned hydrocarbons outlet as compared to spark ignition engines. Currently, the automotive manufacturing industries are facing the serious challenges to meet the future specific requirements for the regulation of both NOx and particulate emissions [19].

Karachi is the 22nd biggest city of the world and is the largest city of Pakistan. It is situated at 64° longitudes and 27º latitude on the shore of Arabian Sea near the Indus River delta with main seaport of the country. The city covers an area of approximately 3,530 square kilometers with more than 18 towns and 6 cantonment boards. The transport system to carry goods and public in the city is comprises on locomotive train, trucks, buses, mini buses, cars, rickshaws and motor cycle which are the primary mode of conveyance. The railway track of Pakistan covers about 11, 755, 00 kilometers including double line track. The Pakistan railway consists of 12 steam locomotives, 16 electric locomotives and 500 diesel electric locomotives for the transport of goods and public Pakistan Railway, 2010-2011, [22]. 115 passenger and most of the cargo trains have been suspended temporarily due to fuel shortage (The Nation, September 29, 2011). According to Rizvi [28], 26 mail and express, 39 intercity, 33 passenger, 24 mixed, 03 international, 03 Karachi shuttle and 02 cargo trains are in operation from Karachi to other parts of the country.

Gasoline and diesel fuels are mixtures of hydrocarbons (made of hydrogen, oxygen carbon atoms). Diesel fuel is a mixture, which may contain approximately 400 distinct hydrocarbons and 20 organic compounds of sulfur and additives [21]. The level of environmental

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pollution with polycyclic aromatic hydrocarbons (PAHs) can be correlate with the region of industrialization and the traffic density. These PAHs accumulate in soil along the roads. PAH, contents found at the depths up to 2 meters. It was concluded that the plants can be absorb and decompose some PAHs which were mostly deposited in the soil, and some of them in the roots [31].

Railway transportation is an important mode of transportation [18] in Pakistan and all over around the world. The railway transportation, leaked cargos, fuel combustion, the use of lubricate oils and sleeper impregnation oils during railway transportation considered the main resources of heavy metals likewise Pb and Cd [5]. The impact of toxic pollutants near the railway track and railway stations on ecological point of view investigated by different researchers [6, 8 and 9]. Polycyclic aromatic hydrocarbons (PACs) and heavy metals assessed in soil and plant samples collected from different areas of the railway junction Ilawa Glowna, Poland. PAH, contamination of soil and plants was reported highest in the platform area and near the railway siding and lowest in loading ramp and cleaning bay areas. Whereas, the heavy metal contamination pattern was different. The soil and plants were very highly contaminated in the cleaning bay and sidetrack areas while the loading ramp and platform areas were less contaminated [17]. The botanists carried out some studies for plants growing along the railway tracks for the last many years [33]. A floristic study in 246 areas along the railway tracks of Poland was carried out [11]. Railway ballast contains little organic material [15]. The exceed concentrations of fine particulate matter ($PM_{2.5}$) levels, carbon dioxide (CO_2) levels and particle number concentrations (PNC) against World Health Organization (WHO) permissible limit (25 µg/m^3) in train carriages on seven routes of the mass transit railway in Hong Kong were recorded [34].

The aim of the present study was to investigate the seedling growth behavior of an important woody tree species, *Acacia nilotica* (Linn.) Delile in the soil collected from near the different railway tracks sites of Karachi, city and compared with the soil of Karachi University Campus.

II MATERIAL AND METHODS

Description of study site

Karachi faces many challenges from the last few decades due to rapid urbanization and industrialization. It is an important hub in economy of Pakistan due to its presence on the coast along the Arabian Sea situated at latitude of 24° 48' N and longitude of 66° 55' E. The study area covers about 20 kilometers from Cantonment Station to Landhi Junction (Fig. 1). The area was disturbed and affected from the activities of rail and road transport. The detail work regarding seedling growth behavior of *Acacia nilotica* in the past years is scanty.

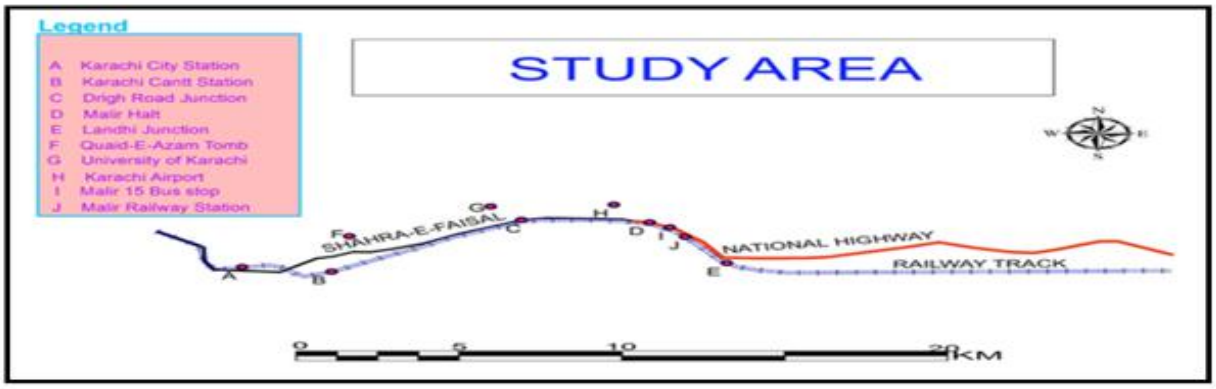

Fig1. Map of the study area

The brief description of the study area is as follows:

A. Karachi University Campus: Karachi University is a public University and away from the city center of Karachi, Pakistan. The University is situated at latitude of 24° 56' N and longitude of 67° 07' E. The Karachi University Campus is considered as pollution free site as compared to other studied sites. The University of Karachi was established by an act of Pakistan parliament in June, 1951. The present campus, to which the University shifted in 1959, is spread over 1279 acres of land, situated 12 Km away from the city center [32] .

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B. Cantonment Station: Karachi Cantonment Railway Station is situated at a latitude of 24° 50' N and longitude of 67° 02' E. Roads with high traffic congestion surround the area around the Cantonment railway station. The main express and local train arrives and depart from here for "up" and "down" side of the country. Most of the passengers use this railway station due to departure and arrival points of many trains. The mechanical diesel workshop at this station is also situated. The service facility is available at this station for cleaning and washing of coaches. Most of the cargo trains and extra coaches are parked at this station (Fig. 2).

Fig.2. Railway locomotive diesel engine workshop at Karachi Cantonment Railway Station.

C. Drigh road Junction: Drigh road railway Junction is an important railway station of Karachi, Pakistan and situated at latitude of 24° 53' N and longitude of 67° 07' E. The up trains have no stoppage at Drigh road Junction while the down trains stop here for 2 to 5 minutes. Only few passengers use this railway station coming from other parts of the country to Karachi. The Drigh road railway station serves for "up" and "down" trains. In the past, this railway junction was used for cargo trains on large scale, but now days this junction is available for parking of the cargo trains (Fig. 3).

Fig.3. Drigh Road Railway Station.

D. Malir Railway Station: Malir railway station is located in Malir 15 and situated at a latitude of 24° 52' N and longitude of 67° 11' E. This railway station was active in the past but now days, the passengers less use this station. The local train runs on this route. The long route trains have no stoppage here and in case of emergency, the trains are used to stop here.

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Fig.4. Malir Railway Station.

E. Landhi Junction: Landhi Railway Station Junction is situated at latitude of 24° 52' N and longitude of 67° 11' E. Although, this junction is a large one. The fast and cargo trains runs on the Landhi railway line. Some of the passengers use this railway station as departure and arrival points for some trains. The station is situated at the east end of the Karachi, city that, make the less disturbance to vegetation and soil as compared to other railway track site (Fig. 5).

Fig.5. Landhi Junction Railway Station.

Species description

Acacia nilotica (Linn.) Delile is a small to medium size tree with more or less rounded umbrella shaped crown and locally known as Kikar (Fig. 1). It belongs to family Fabaceae and sub-family Mimosideae. It is widely distributed in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India [3]. It is widely planted in arid and semi-arid regions of India and Pakistan [24]. *A. nilotica* is also a salt tolerant species and successfully adapted in arid environment [20]. The adult and juvenile plants easily regenerated from the base after removal of top growth and viability of seed remain more than five years [4]. The tap roots of *A. nilotica* rapidly penetrated deeply in soil and access soil moisture for successful growth that ensured the competition to other plants [16] and the seeds remain dormant for long periods due to the presence of hard impermeable seed coats [23]. *A. nilotica* rapidly grow up to 2-3 m in diameter and 15-18 m in height. The pods with 8-12 seeds, increased 7-15 cm in length with a necklace appearance [13]. *A. nilotica* is multipurpose leguminous nitrogen fixing tree which increased the soil fertility [2].

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Fig. 6. *Acacia nilotica* growing in Karachi University Campus.

Seedling growth experiment

The soil samples of polluted sites near the railway tracks (Cant Station, Malir Halt, Malir-15, Landhi Junction) and non polluted site the Karachi University Campus were obtained at 30 cm depth and were brought to the laboratory in polythene bags. The soil samples were air dried and then sieved through 2 mm sieve to remove large size particles, stones and boulders. The healthy seeds of *Acacia nilotcia* (Linn.) Delile were collected randomly from the Karachi University Campus. The experiment was conducted in green house at the Department of Botany, University of Karachi in pots. The top ends of the seeds were slightly cut with a clean scissor to remove any possible dormancy. The seeds were sown in large pots having garden soil at 1 cm depth and watered regularly. After two weeks of their germination, uniform size seedlings was transplanted in pots of 7.0 cm in diameter and 9.8 cm in depth containing the soil of Cant Station, Malir Halt, Malir-15, Landhi Junction and University Campus. There were five replicates for each soil and the experiment was completely randomized. The seedlings were irrigated with tap water after two days intervals. Pots were reshuffled weekly to avoid light/shade or any other environmental effect. After 8 weeks of growth, the seedlings were removed from pots and washed their roots with water. Root, shoot and leaves were separated to dry in an oven at 80ºC for 24 hours. Data on seedling, root, shoot length and leaf area was obtained. Oven dried weights of root, shoot, leaves and total seedling dry weights were taken by electrical balance. Root/shoot ratio, leaf weight ratio, specific leaf area and leaf area ratio were determined as mentioned by Rehman and Iqbal [27].

Statistical analysis

Data of various growth parameters of *A. nilotica* and different variables of soil samples was statistically analyzed by analysis of variance (ANOVA) and Duncan's Multiple Range Test at $p < 0.05$ level on personnel computer using statistical software COSTAT ver. 3.

III RESULTS

The seedling growth performance of *Acacia nilotica* was evaluated in soils of University Campus, Cantonment Station, Drighroad Junction, Malir Station and Landhi Junction. The seedling growth of *A. nilotica* was gradually increased in soil of Drighroad Junction, Malir Station and Landhi Junction, while in Cantonment Station soil, the growth was decreased as compared to University Campus soil. The root, shoot, seedling lengths, circumference, root, stem and seedling dry weights of *A. nilotica* grown in soil of Cantonment Station showed significant ($p<0.05$) reduction as compared to University Campus. The growth parameters such as root, shoot, seedling lengths, number of leaves, leaf area, circumference, root, stem, leaf, seedling dry weights of *A. nilotica* grown in soil of Malir Station and Landhi Junction were significantly ($p<0.05$) enhanced as compared to University Campus soil (Fig. 7a and 7b).

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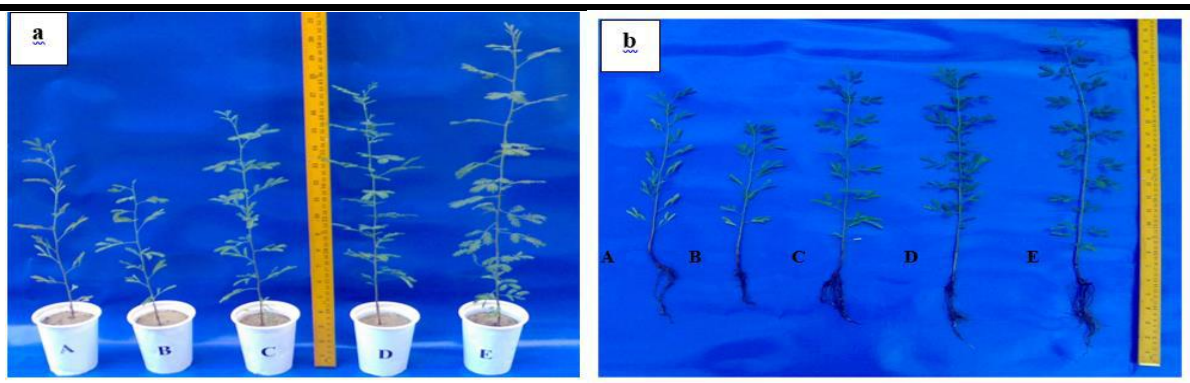

Fig.7: Growth of Acacia nilotica in different soils (a) and after harvest (b)
Symbol used: A = Karachi University Campus; B = Karachi Cantonment Railway Station; C = Drighroad Railway Junction; D = Malir Railway Station; E = Landhi Junction

The seedlings of *Acacia nilotica* showed better growth regarding root, shoot and seedling lengths, number of leaves and circumference in soil of Drighroad Junction, Malir Station and Landhi Junction as compared to University Campus while, the seedlings grown in Cantonment Station soil showed inhibitory effects on root, shoot and seedling lengths, number of leaves and circumference (Table 1). The root length of *A. nilotica* was significantly ($p<0.05$) enhanced in soil of Drighroad Junction (9.40 cm), Malir Station (11.98 cm) and Landhi Junction (12.66 cm) as compared to University Campus (8.20 cm) while, in soil of Cantonment Station a significant ($p<0.05$) reduction was found in root length (7.38 cm). The shoot length of *A. nilotica* was significantly ($p<0.05$) increased, 39.60 cm, 42.80 cm and 46.00 cm in soil of Drighroad Junction, Malir Station and Landhi Junction, respectively as compared to University Campus (33.20 cm). A significant ($p<0.05$) decline was noted in shoot length in the soil of Cantonment Station (26.60 cm) as compared to the University Campus soil. The seedling length of *A. nilotica* seedlings grown in soil of Cantonment Station showed a prominent reduction (33.98 cm), whereas Drighroad Junction, Malir Station and Landhi Junction soil represented a significant ($p<0.05$) increase in seedling length, 49.00 cm, 54.78 cm and 58.66 cm, respectively as compared to the University Campus soil (8.20 cm). The number of leaves of *A. nilotica* was significantly ($p<0.05$) greater, 65.00, 74.00 and 96.20 seedlings grown in soil of Drighroad Junction, Malir Station and Landhi Junction, respectively as compared to University Campus while, the seedlings grown in soil of Cantonment Station showed a significant($p<0.05$) reduction which was recorded as 42.00. The leaf area of *A. nilotica* seedling was significantly ($p<0.05$) increased, 1.29, 1.40, 1.91 and 2.04 cm^2 in soil of Cantonment Station, Drighroad Junction, Malir Station and Landhi Junction, respectively as compared to University Campus (1.07 cm^2). The circumference of *A. nilotica* was significantly ($p<0.05$) reduced to 20.00 cm for the seedlings grown in soil of Cantonment Station as compared to University Campus (29.40 cm). A non significant difference was observed in circumference of the seedlings developed from the soil of Drighroad Junction, Malir Station and Landhi Junction as compared with University Campus soil. The dry weights of root, stem, leaves and cumulative seedlings of *A. nilotica* were recorded high for the seedlings which were grown in soil of Malir Station and Landhi Junction as compared to University Campus (Table 1).

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Table 1. Effects of different soils on growth of *Acacia nilotica* in the field conditions.

Sites	Root length (cm)	Shoot length (cm)	Seedling length (cm)	No. of leaves	Leaf area (cm^2)	Circumference (cm)
A	8.20±0.22b	33.20±0.66b	41.40±0.61b	56.60±0.68b	1.07±0.11a	29.40±0.92b
B	7.38±0.18a	26.60±0.81a	33.98±0.74a	42.00±0.71a	1.29±0.04b	22.00±0.89a
C	9.40±0.14c	39.60±0.81c	49.00±0.76c	65.00±1.67c	1.40±0.03b	30.60±0.92b
D	11.98±0.28d	42.80±0.86d	54.78±0.86d	74.00±1.79d	1.91±0.06c	28.80±1.56b
E	12.66±0.12e	46.00±0.71e	58.66±0.67e	96.20±1.39e	2.04±0.05c	31.00±1.00b
LSD (p<0.05)	**0.58**	**2.28**	**2.20**	**3.93**	**0.19**	**3.18**

Symbol used: A = University Campus; **B** = Cantonment Station; **C** = Drighroad Junctionn; **D** = Malir Station; **E** = Landhi Junction
Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level; ± Standard Error

Table (2) showed a significant (p<0.05) increase in root dry weight of *A. nilotica* grown in soil of Drighroad Junction (0.064 g), Malir Station (0.113 g) and Landhi Junction (0.152 g) in comparison with University Campus (0.072 g). The seedlings of *A. nilotica* developed from the soil of Cantonment Station showed a significant (p<0.05) reduction (0.064 g) in dry weight. The stem and leaf dry weights of *A. nilotica* also showed a significant difference for the seedlings grown in soil of Cantonment Station, Drighroad Junction, Malir Station and Landhi Junction as compared to University Campus (Table 2). The cumulative seedling dry weight of *A. nilotica* was recorded significantly (p<0.05) high for the seedlings grown in Drighroad Junction (0.833 g), Malir Station (0.931 g) and Landhi Junction (1.186 g), whereas the seedlings grown in the soil of Cantonment Station demonstrated a significant (p<0.05) reduction (0.494 g) as compared to University Campus soil (0.782 g). The root/shoot ratio of *A. nilotica* was significantly (p<0.05) different for the seedlings grown in Cantonment Station, Malir Station and Landhi Junction as compared to University Campus soil. Leaf weight ratio of *A. nilotica* showed a significant (p<0.05) difference only for the seedlings developed from the soil of Malir Station and Drighroad Junction as compared to the University Campus soil. The specific leaf area showed significant (p<0.05) difference in seedlings grown in soil of Cantonment Station as compared with University Campus. The leaf area ratio showed significant (p<0.05) increase (2.64 cm^2 g^{-1}) in seedlings grown in Cantonment Station and Malir Station soil as compared to the University Campus.

Table 2. Effects of different soils on dry weights and ratios of different variables of *Acacia nilotica* in the field conditions.

Sites	Root dry weight (g)	Stem dry weight (g)	Leaf dry weight (g)	Seedling dry weight (g)	Root/shoot Ratio	Leaf weight ratio	Specific leaf area (cm^2 g^{-1})	Leaf area ratio (cm^2 g^{-1})
A	0.072±0.002b	0.417±0.01b	0.238±0.04b	0.728±0.01b	0.11±0.003a	0.33±0.002a	4.50±0.41a	1.47±0.15a
B	0.064±0.001a	0.266±0.01a	0.162±0.05a	0.494±0.01a	0.15±0.005c	0.33±0.005a	8.06±0.54b	2.64±0.14c
C	0.095±0.002c	0.424±0.02ac	0.313±0.01c	0.833±0.03c	0.13±0.008ab	0.38±0.006c	4.51±0.22a	1.69±0.05a
D	0.113±0.003d	0.461±0.02c	0.356±0.01d	0.931±0.02d	0.14±0.008bc	0.38±0.011c	5.36±0.10a	2.04±0.04b
E	0.152±0.002e	0.635±0.13d	0.398±0.02e	1.186±0.02e	0.15±0.004bc	0.33±0.012a	5.18±0.35a	1.72±0.07a
LSD (p<0.05)	**0.007**	**0.04**	**0.04**	**0.07**	**0.02**	**0.02**	**1.05**	**1.05**

Symbol used: A = University Campus; **B** = Cantonment Station; **C** = Drighroad Junction; **D** = Malir Station; **E** = Landhi Junction
Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level; ± Standard Error

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Table 3 (a): Physical properties of soil.

Sites	M.W.H.C. (%)	B.D (gcc^{-1})	Porosity (%)	Sand (%)	Silt (%)	Clay (%)	Soil texture class
A	26.59±0.18b	1.36±0.3b	49±1.00a	24.34±0.91a	44.28±0.10a	31.42±1.01a	Clay loam.
B	31.11±0.24c	1.34±0.4b	49±2.00a	41.80±1.00b	29.00±0.50b	29.20±1.00a	Clay loam
C	24.17±0.11a	1.47±0.01c	44±1.00a	60.80±0.94c	31.00±0.50b	08.20±0.44b	Sandy clay loam
D	23.84±0.30a	1.23±0.03a	54±3.00b	64.08±1.00c	13.00±0.50c	22.92±0.55c	Sandy clay loam
E	24.45±0.01a	1.43±0.01bc	46±0.50a	60.08±0.20c	11.00±0.10c	28.92±0.10a	Sandy clay loam

Table 3(b): Chemical properties of soil.

Sites	$CaCO_3$ (%)	Cl (mgL^{-1})	pH	O.M. (%)	T.O.C. (g)	S (μgg^{-1})	EC (dSm^{-1})	TDS (mgL^{-1})	Ex. Na (ppm)	Ex. K (ppm)
A	21.6±0.01a	00.00±0.00a	7.00±0.02a	4.50±0.03c	2.61±0.13b	58.75±0.04a	19.0±0.50a	13.94±0.40a	190.00±6.00b	156.00±3.00a
B	13.0±0.40d	875.0±10.00e	7.25±0.04b	4.39±0.03a	2.55±0.01b	142.50±4.00d	19.6±0.30a	14.1±0.55a	1340.00±25.00d	180.00±7.00a
C	16.7±0.20c	190.0±2.00d	7.40±0.01c	3.35±0.02a	1.94±0.01a	71.25±1.00b	4.4±0.20b	3.3±0.20b	300.00±15.00c	160.00±11.00a
D	14.7±0.10b	100.0±3.00c	8.0±0.04d	3.90±0.01b	2.26±0.01c	150.00±4.00d	1.5±0.40c	1.1±0.20c	120.00±5.00a	80.00±6.00b
E	17.4±0.29c	80.0±5.00b	7.50±0.07c	4.82±0.02d	2.79±0.01d	91.25±3.00c	2.1±0.20c	1.6±0.20c	100.00±2.00a	80.00±3.00b

Symbol used: **A**= University Campus, **B**= Cant Station, **C**=Malir Halt, **D**= Malir15 **E**=Landhi Junction **M.W.H.C.** = Maximum Water Holding Capacity, **B.D.** = Bulk Density, **$CaCO_3$** = Calcium carbonate, **Cl**= Chlorides, **OM** = Organic matter, **T.O.C.** = Total Organic Carbon, **EC**= Electrical Conductivity, **T.D.S.** = Total Dissolved Salts, **Ex. Na^+** = Exchangeable sodium, **Ex. K^+**= Exchangeable potassium. **Numbers followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level.** ± Standard Error. Source: (Farooqi *et al.*, 2016.)

IV. DISCUSSION

The influence of railway exhaust emission on the seedling growth of *Accacia nilotica* was investigated. Railway emission affected seedling growth of *A. nilotica*. A significant ($p<0.05$) reduction in seedling growth of *A. nilotica* raised in soil of Cant railway station observed. The routine works on railway tracks of Karachi is badly affected the soil properties near the railway tracks. The workshops for maintenance of vehicles, train engines and coaches also contaminated the soil of the area. The plants growth directly depends on the soil of the area in which they are growing, because the soil is the medium for plant growth, water and nutrient supplier and give support to plants [10]. In our previous studies the changes in physical and chemical properties of soil near the railway track of Karachi, city were recorded (Table 3a, 3b). The roots of plants penetrate in soil, which act as a water-holding tank and fulfill the needs of water for plants growth. The physical properties of soil such as compactness, structure, and texture and bulk density directly influenced the root penetration, growth performance and production of yield to different crops [12]. The nutrient availability, their absorbance and uptake from water to plants depend on physical attributes of soil [30]. The soil texture, bulk density, porosity and water holding capacity, organic matter, dissolved salts and available sulfates are affecting the plants growth [26].

The physical and chemical characteristics of University Campus, Cantonment Station, Drighroad Junction, Malir Station and Landhi Junction soils were different. The activities of automobiles at the above mentioned study areas are different and the rate of pollutants introduction in environment vary from area to area. The most polluted and disturbed area was Cantonment Railway Station and the less polluted area was Landhi Junction as compared to Karachi University Campus. The growth performance of *Acacia nilotica*, showed a significant ($p<0.05$) variation raised in different soils of the study area due to changes in physical and chemical nature. The height of *A. nilotica* was highly increased in soil of Landhi Junction whereas; in the soil of Cantonment Station height of *A. nilotica* was lowest. The lowest number of leaves and circumference were recorded in soil of Cantonment Station. Rehman [25] carried out similar studies in the polluted soils of Landhi and Korangi industrial areas of Karachi city. The height and number of leaves of *Leucaena leucocephala* showed discontinuous variation after every two weeks in polluted soils of Landhi and Korangi industrial areas [27].

The seedlings of *A. nilotica* grown in soil of Karachi Cantonment Station exhibited significant reduction in root, shoot and seedling lengths, number of leaves and circumference due to most contamination of repairing and servicing activities of train engine and coaches as well as some other activities in that area. The reduction in these parameters of different plants might be due to disposal of waste products and spent engine oil in the vicinity of

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Cantonment railway station. These findings were supported by Akoto *et al*. [1], who described that vegetation in the vicinity of railway servicing workshops in Kumasi city of Ghana was severely affected. The root, stem, leaf and seedling dry weights of *A. nilotica* seedlings grown in soil of Karachi Cantonment Station were significantly reduced as compared to University Campus, Drighroad Junction, Malir Station and Landhi Junction. The same results were coated by Iqbal and Shafiq [14], who described that the reduction in leaf number, plant height, circumference, root, shoot and total plant dry weights of *Prosopis juliflora* and *Blepharis sindica* were suppressed due to low contents of calcium carbonate, high electrical conductivity and high contents of sodium and potassium salts.

V CONCLUSION

The present study showed that the seedling growth of *A. nilotica* raised in the soil of different areas of railway tracks of Karachi responded differently due to anthropogenic activities, grazing, and construction of railway tracks and release of emission from railway engines. The soil of Cantonment Station was highly polluted due to repairing activities, release of exhaust from train engines, chemical used for rolling stock, exploitation (machine grease, fuel oils, and sleeper impregnation oil) and toxic substances processed by railway transport. If the exposure of pollutant on soil near railway tracks goes on then there would likely to be more damage to the vegetation changes in future.

VI ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Physico-Chemical Analysis of Water from Hand-Dug Wells in Wadata Area of Makurdi Metropolis, Nigeria

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***Abstract*—** *In many countries around the world, including Nigeria, access to potable water has become a mirage. Thus, exploitation of groundwater through the construction of hand-dug wells has become a major source of drinking water for majority of the populace. The need to assess the quality of water from this source has become imperative because of the health impacts on individuals. The physico-chemical characteristics of water from 166 hand-dug wells in Wadata area of Makurdi metropolis, Nigeria, were assessed during the dry season of 2017. Chloride(Cl^-), Nitrate(NO_3^-), Sulphate(SO_4^{2-}), Ammonium(NH_4^+), Phosphate(PO_4^{3-}), Total hardness (TH), Total dissolved solids (TDS) and turbidity were determined using standard analytical methods. Sodium (Na), Calcium (Ca), Magnesium (Mg), and Potassium (K) were determined using Flame Atomic Absorption Spectrophotometer (PG 990 model). As part of the physical parameters, TDSranged from 19.20 to 8932.20 mg/L while Turbidity also ranged from 1.00 to 12.00NTU. Chemical parameters were in the range of: Cl^-(14.18 to 751.54 mg/L); NO_3^- (11.00 to 62050.00mg/L); SO_4^{2-} (6.70 to 321.70mg/L); PO_4^{3-}(12.30 to 1093.25mg/L); $NH4^+$(2.60 to 321.40mg/L); TH(76.80 to 5467.80mg/L). Metalloids were in the range of: Na (2.90 to 78.60 mg/L); Ca (0.00 to 654.00mg/L); Mg (3.70 to 67.80 mg/L); K (4.00 to 238.00 mg/L).Many hand-dug wells in the area are contaminated based on the physicochemical parameters tested as values were above the acceptable limits prescribed by Standard Organization of Nigeria (SON) for drinking water.This information is important to all stakeholders including regulatory authorities in environment and those saddled with the responsibilities of preventing and controlling water borne disease outbreaks.*

***Keywords*— Environment, Hand-dung wells, Health, Physicochemical properties Water quality.**

I. INTRODUCTION

Generally, groundwater, surface water (rivers, streams and ponds), atmospheric water (rain-water, snow) and springs are the main sources of water available to people. The quality of these water bodies vary widely depending on the location and environmental factors [1]. The major source of groundwater is precipitation that infiltrates the ground and moves through the soil and pore spaces of rocks. Other sources include water infiltrating from lakes and streams, recharge ponds and water treatment systems. As groundwater moves through soil sediments and rocks, many impurities such as disease-causing micro-organisms are filtered [2]. In developing countries such as Nigeria many water resources are unhealthy because they contain harmful physical, chemical and biological agents. To maintain good health, water should be safe to drink and meet the local and international standards. To monitor the water resources and ensure sustainability, national and international criteria and guidelines established for water quality are being used [3]. In Benue State, despite efforts made by Government to provide potable water to urban and rural areas, a large percentage of the water supply schemes are malfunctioning, forcing consumers to use unprotected sources that pose health hazards. Annual report from national dailies show marked episodes of epidemic in Wadata area of Makurdi metropolis arising from water problem [4].The trend of uncontrolled and haphazard construction of groundwater facilities, particularly hand-dug wells, in the residential areas with refuse dumps within the vicinity, is a great health concern, as this may contribute significantly to adverse impact on the aquifer as a result of overdependence and over-abstraction with attendant negative effects. To safeguard the health of the residents, it is imperative that the quality of the water from hand-dug wells be ascertained. The overall goal of the study was therefore, to determine the potability of water from the hand-dug wells located in Wadata area of Makurdi metropolis.

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II. METHODOLOGY

Study Area

Makurdi, the study area, is situated on Long 8^o 10^1N and 8^o 45^1N; and Lat.7^o 1^1E and7^o 45^1E in the Southern Guinea Savanna of Nigeria. The town is drained by the River Benue which bisects it into two parts – North and South banks. Other minor rivers that drain the town, and in turn empty their water in the River Benue include; Rivers Idye, Genabe, Unudu, Kpege and Kereke [5]. These rivers are highly seasonal and dry up in the dry season with some stagnant pools in their channels in the dry season. Due to the general low relief of Makurdi town, large portions of the area are water-logged and flooded during heavy rainstorm. Two major climate seasons are recognized; the dry season which is between November and March, and the wet season which starts in April and then ends in October with a short break in mid-August. The average annual precipitation is above 220mm and serves as a major source of groundwater replenishment. Temperature ranges between 21.3^{o}C and 32.8^{o}C [6].

Water sampling

One hundred and sixty-six (166) water samples were collected from the study area (Fig.1), in September, 2016, using multi-stage sampling techniques. Fifty-five (55) samples from protected wells (**p**); 55 from semi-protected well(**s**); and 56 from un-protected wells(**u**), using two-litre rubber bottles which have been previously washed with 10% nitric acid (HNO_3) and 1:1HCl for 48hours. The rubber bottles were labeled and immediately, few drops of HNO3 were added in order to prevent loss of metals and the growth of any micro-organisms. Turbidity of the water samples were also measured at the time of collection.

Laboratory analysis

Turbidity and nitrate were determined using direct reading spectrophotometer (DR/2000) made by HACH Company. Total dissolved solids was determined using TDS kid Model 50150 made by HACH. Total water hardness (TH) was determined using Hardness EDTA titration. Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-} and NH_4^+ ions were determined using standard analytical methods. Metalloids: Na, Ca, Mg, K were determined using Atomic Absorption Spectrophotometer (ASS) Model PG 990. All instruments were calibrated before use.

III. RESULTS AND DISCUSSIONS

Table 1 presents the total dissolved solids, hardness and turbidity of water samples. Total dissolved solids (TDS) ranged from 19.20 to 8932.20 mg/L with a mean of 540.48 mg/L. TDS values were above permissible level of 500mg/l by SON, for drinking water. The high values observed may be as a result of intrusion or runoff of wastes from the surroundings into the wells[7,8,9]. Turbidity ranged from 1.00 to 12.00 NTU with a mean of 4.67 NTU. Turbidity positively correlated with TDS, sulphate and phosphate. High turbidity may be as result of runoff of wastes from the surroundings which increased the amount of dissolved solids and suspended materials in the water. Total hardness (TH) ranged between 76.80 mg/L and 5467.80 mg/L with a mean of 567.08 mg/L. TH exceeded SON threshold of 150 mg/L for drinking water. TH correlated significantly and positively with Cl^- and NO_3^- (at 0.05 level) and SO_4^{2-} (at 0.01 level). High values may be as a result of high chloride, sulphate, and ammonium ions present in the water samples. TH also correlated weakly with Na^+ and Ca^{2+}. This implies that the presence of these ions in water might have contributed positively to water hardness. The hardness observed in water in the study area may therefore, be permanent hardness. Sodium correlated significantly and positively with nitrate (at 0.05level).This implies that the two ions might have contributed to water hardness. Magnesium correlated significantly and positively with nitrate (at the 0.01 level), implying that the ions might have formed salt in water. Ca^{2+} correlated significantly and positively with Cl^- ions (at 0.05 level), implying that the two ions might have formed salt in the water. Statistical analysis using at 5% significant level indicated that there was significant variation in total hardness within the wells. High values may be attributed to run-off of materials from the surroundings of the wells which increase the amount of dissolved inorganic materials in the water [10].

Table.1: Total dissolved solids, hardness and turbidityof watersamples

Water Parameter	Unit	Min.	Max.	Mean	Std.	SON (2007) Guideline
TDS	mg/L	19.2	8932.2	540.48	765.7	500
Turbidity	NTU	1	12	4.67	2.67	5
TH	mg/L	76.8	5467.8	567.08	559.49	150

TH=Total hardness; TDS=Total dissolved solids; SON=Standard organization of Nigeria
Min=minimum; Max=Maximum; Std=Standard deviation

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Table 2 presents the chloride, nitrate and sulphate contents of water samples from hand-dug wells. Chlorides are usually in water in the form of sodium chloride. This may impact a salty taste to water. When present in concentrations more than 200mg/L the taste may be objectionable to some consumers. Results showed that chlorides ranged from 14.18mg/l to 751.54 mg/L with a mean of 246.62 mg/L. Chloride concentration was very high though not all samples exceeded permissible level of 250 mg/L by SON for drinking water. Statistical analysis at 5% significant level indicated that there were significant variations in the amount of chloride ions within the wells. The high values observed for standard deviation (std), may be attributed to high variations in the amount of the ions at different sample collection points. The high levels of chloride ions might be attributed to high infiltration of dissolved inorganic substances and runoff during rainy season [9].

Nitrates in water can cause methamoglobinaemia in infants less than six months old [11]. Nitrate ranged between 11.00mg/L and 62050.00 mg/l with a mean of 160.11 mg/L. The level of nitrate ions exceeded the permissible level for drinking water. Thus, babies in the study area may be exposed to serious health hazards. High nitrate concentration observed may be attributed to ingress of animal and human wastes from open septic or sewage systems [7, 8]. Statistical analysis at 5% significant level indicated that there were significant variations in the concentration of nitrate ions in the water within the wells. The high nitrate concentration may also be attributed to high rate of decomposition of organic materials and infiltration of dissolved compounds[12]. Nitrate ions correlated positively and significantly with total hardness, sodium, and magnesium ions, implying that the presence of nitrate ions in water might have contributed positively to the formation of water hardness [10]. Sulphate is an important constituent of hardness with Ca and Mg. At concentrations above 300mg/L, sulphate produces an objectionable taste and unwanted laxative effects in water. Sulphate ranged between 6.70mg/L and 321.70mg/L with a mean of 63.50mg/L. These levels are lower than the permitted values set by SON. Statistical analysis at 5% significant level indicated that there were significant variations of sulphate in water within the wells. The presence of sulphate in the water may be attributed to infiltration of dissolved inorganic compounds into the wells [7].

Table.2: Chloride, nitrate and sulphate contents of water samples from hand-dug wells

Water Parameter	Unit	Min.	Max.	Mean	Std.	SON (2007) Guideline
Cl^-	mg/L	14.18	751.54	246.62	136.16	250
NO_3^-	mg/L	11	62050	160.11	128.5	50
SO_4^{2-}	mg/L	6.7	321.7	63.5	64.11	100

SON=Standard organization of Nigeria; Min=minimum; Max=Maximum; Std=Standard deviation

Table 3 describes the phosphate and ammonium contents of water samples from the hand-dug wells.Phosphate ranged between 12.30mg/L and 1093.25mg/L with a mean of 189.99 mg/L. The high value of standard deviation may be as a result of variation in the values observed from different sample collection points. The level of phosphate ions exceeded SON threshold for drinking water. Statistical analysis at 5% significant level indicated that there were significant variationsin phosphate ions in water within the wells. The high values of phosphate may be as a result of decomposition of organic matter and from runoff, surface catchment and interactions between water and sediments from dead plants and animals that remained at the bottom of the wells. Ammonium ranged between 2.60 mg/L and 321.40mg/L with a mean of 44.20 mg/L. The values exceeded SON threshold for drinking water. Statistical analysis at 5% significant level indicated that there were significant variations in the level of ammonium ions in water within the wells. The high values observed may be as a result of decomposition of organic matter from the surroundings and runoff water which infiltrated the wells[7].

Table.3: Phosphate and ammonium contents of water samples from hand-dug wells

Water Parameter	Unit	Min.	Max.	Mean	Std.	SON (2007) Guideline
PO_4^{3-}	mg/L	12.3	1093.25	189.99	174.5	5
NH_4^+	mg/L	2.6	321.4	44.2	31.01	< 1.00

SON=Standard organization of Nigeria; Min=minimum; Max=Maximum; Std=Standard deviation

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Table 4 presents the sodium, calcium, magnesium and potassium contents of water samples. The values of sodium ions ranged from 2.90 mg/L to 78.60 mg/L with a mean of 10.19 mg/l. The level of sodium ions did not exceed SON threshold for drinking water. Statistical analysis at 5% significant level indicated that there were significant variations in the amount of sodium in water within the wells. Sodium ions in water can cause water hardness.The values of calcium ranged between 0.0mg/l and 654.00 mg/L with a mean of 213.90 mg/L. Calcium ions exceeded WHO standards (50mg/L) for drinking water. Statistical analysis at 5% significant level indicated that there were significant variations in the level of calcium ions in the water within the wells. Magnesiumions showed a range of 3.70 mg/L and 67.80 mg/L with a mean of 10.72 mg/L. The results showed magnesium levels above standards by SON. Statistical analysis at 5% significant level indicated that there were significant differences in the level of magnesium ions in water within the wells. The presence of Mg ions in water may be attributed to runoff and infiltration of dissolved materials from the surroundings. Potassium showed a range of 4.00mg/L to 238.00 mg/L with a mean of 75.72 mg/L. The levels are above WHO standards (1–2mg/L) for drinking water. Statistical analysis at 5% significant level indicated that there was no significant difference within the wells. The presence of K ions in water may be attributed to runoff and infiltration of dissolved waste materials from the surrounding dumpsite.

Table.4: Sodium, calcium, magnesium and potassium contents of water samples

Water Parameter	Unit	Min.	Max.	Mean	Std.	SON (2007) Guideline
Na	mg/L	2.9	78.6	10.19	8.79	200
Ca	mg/L	0	654	213.9	164.86	100
Mg	mg/L	3.7	67.8	10.72	6.14	0.2
K	mg/L	4	238	75.72	41.82	10

SON=Standard organization of Nigeria; Min=minimum; Max=Maximum; Std=Standard deviation

Figure 1 clearly compared the mean values of water parameters and SON minimum threshold as standards. Based on this, average nitrates, phosphates, ammonium, total hardness, calcium, potassium and ammonium contents in water samples were above the SON limits. Average turbidity, chloride, sulphide, magnesium and sodium were still within or lower than the minimum threshold.

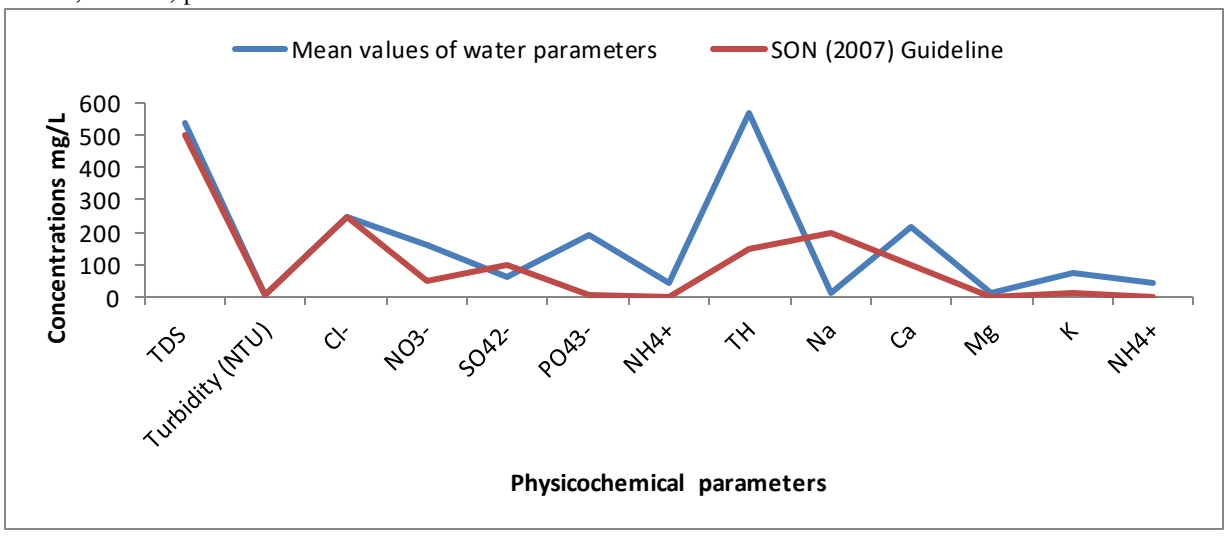

Fig.1: Line plot of mean water parameters and SON standard guideline

SON=Standard organization of Nigeria

IV. CONCLUSIONS

The overall results showed that the water from the hand-dug wells is contaminated with the physical and chemical parameters analyzed. The water is therefore, not safe for drinking, cooking and laundry purposes. The inhabitants are therefore, at risk of harmful chemical ions in water which may lead to endocrinal damage, dental decay or dental caries in babies, oxygen starvation in the brain of infants

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leading to 'blue baby' syndrome, kidney or gall bladder stones, disruption of heart and muscular function, water hardness and irritation of the mucous membranes. The need to reduce contamination has a direct relationship with contraction of harmful chemical ions which may lead to water-borne diseases. To achieve this, there is the need to treat the well water before use, either by boiling and filtration or by chemical sterilization or both. Periodic water quality monitoring and incorporation of household water treatment practices with hand-dug well water are recommended. Improved sanitation and hygiene practices by the residents in the area are indispensable.

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Modeling Adsorption and Transport of Chrome VI onto Iron Oxide-Coated Sand filter media

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Abstract**—** *Natural decay of pollutants is rarely considered in mass transport equation. This paper develops a mathematical model of a filter based on iron oxide-coated sand for the removal of chromium in groundwater and surface water. The natural decay of chromium was analyzed using a zero-order decay reaction. The advection dispersion equation for this purpose is solved analytically using the Laplace transform method and numerically by the Fourth order Runge kutta method to determine the spatio-temporal distribution of the pollutant through the filter. The control parameters are the adsorption coefficient, the initial concentration and the chromium degradation coefficient. It has been proven that the chromium degradation coefficient strongly affects the concentration values of pollutants inside the filter. The results of this work should be used to manufacture low-cost filters based on iron oxide-coated sand.*

Keywords**— *Filter, Sand, Iron oxide, Chromium, Adsorption, Advection-dispersion, Laplace.***

I. INTRODUCTION

Water is an essential component in the universe and plays an important role in the proper functioning of ecosystems [1]. Despite this, access to drinking water is an increasingly recurrent challenge worldwide and it is estimated that nearly eight hundred and eighty (880) million people lack safe drinking water at home [2, 3]. Water pollution results mainly from poorly planned urban and rural development, industrial discharges, agricultural discharges, geological and environmental activities, domestic activities etc., it is a serious problem because it affects our lives [4]. In addition, the World Health Organization (WHO) estimates that 80% of the diseases that affect the population are directly carried by water: 400 million people are permanently affected by gastroenteritis, 160 million from malaria and 30 million onchocerciases [5]. The industry uses heavy metals in various fields because they have a high electrical conductivity; however, these heavy metals have some toxicity to humans, leading in particular toxic effects on the nervous system, carcinogenic effects on the blood and the bone marrow and kidney disorders.

Chromium is one of the most widely used metals in industries, since it has enough advantages for tanneries, textile, wood processing, agribusiness... Chromium VI is the most problematic form of chromium since in this form chromium is very toxic and very soluble in water. This solubility gives it great mobility in ecosystems. Today, following a lack of compliance with laws or accidents, a large number of industrial sites are polluted by chromium. Therefore, the development of low-cost, easy-to-maintain technologies to reduce this pollutant in drinking water sources to an acceptable level for consumption is an interesting research topic.

The methods implemented today to treat discharges polluted by chromium (VI) are generally aimed at recovering this metal, in order to reuse it and thus reduce its eco-toxicological impact. In parallel, physicochemical methods attempt to eliminate Cr (VI) and electrochemical methods attempt to reduce Cr (VI) to Cr (III). Iron oxide-coated sand has proven its effectiveness for the elimination of various heavy metals (As, Cd, Cr, Pb, ...) at an elimination rates close to 100%. In this study, we develop a mathematical model to understand and predict the adsorption of chromium VI in a fixed column bed made witch iron oxide-coated sand. As Chromium undergoing a natural decay, the discussion will be focus on the effect of the degradation coefficient λ on the spatio-temporal variation of the pollutants inside the filter.

II. MATERIAL AND METHODS

2.1 Preparation of iron oxide-coated sand

The iron oxide-coated sand was prepared using a procedure similar to that of Bailey et al. (1992) [6]. The author used washed and dried river sand with a weight of 200 g and a geometric size of 0.49 mm [7]. It is obtained by mixing for 2

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min, 80 ml of a solution of ferric nitrate 2M (Fe (NO_3) $39H_2O$) to washed river sand and dried. He then placed the mixture in a drying oven at 110 ° C for 14 hours. He finally places the mixture in distilled water until the runoff is cleaned at 105 ° C and stored in closed bottles [7].

2.2 Mathematical description

The linear dispersion advection-equation is used to describe the transport of pollutants in this fixed column bed (Fig. 1). The equation was derived to predict aqueous concentrations of contaminants over time and space in the porous medium. It is written as follows: [8]

$$\rho \frac{\partial q}{\partial t} + \theta \frac{\partial c}{\partial t} = \theta D \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x} \quad (1)$$

Where ρ is the density, θ the water content, D the hydrodynamic dispersion coefficient, V: average Darcy velocity and q the equilibrium adsorbed concentration which is described as follows [8]:

$$q = kc^n \quad (2)$$

Where k is the adsorption capacity and n the adsorption intensity.

In the absence of the iron oxide-coated sand which is the adsorbent, Cr (VI) ion disappeared naturally from the solution. Given the fact that chromium is a naturally decreasing pollutant, this disappearance rate was assumed to be at zero order as follows:

$$-dc/dt = \lambda; c = c_0 \text{ at } t = 0 \quad (3)$$

Where λ is the zero order rate constant or chromium degradation coefficient, c_0 is the initial Cr(VI) concentration and c the Cr(VI) concentration at the time t.

By adding a decay term λc to the equation above according to Runkel, 1996 and by considering a linear adsorption isotherm (n = 1), after a few transformations, we obtain:

$$\frac{\partial c}{\partial t} = d \frac{\partial^2 c}{\partial x^2} - u \frac{\partial c}{\partial x} - \lambda c \quad (4)$$

The appropriate boundary conditions for the model are given by:

$$c(x,0) = 0 \quad , x \geq 0;$$
$$c(0, t) = c_0, t \geq 0; \quad (5)$$
$$c(\infty, 0) = 0 \quad , x \geq 0$$

2.3 Analytical solution of the mathematical model

The analytical solution is obtained using Laplace transform. It is use to transform one or more partial derivatives of the differential equation into algebraic expressions. We call Laplace transform of the function G denoted L (G), the function of complex variable P defined by:

$$L[G(t)] = \bar{G}(p) = \int_0^\infty e^{-Pt} G(x,t)\, dt \quad (6)$$

Where L denotes the Laplace transform, G (t) is a function of time, $\bar{G}(P)$ is a function of the transformed space, it corresponds to the Laplace value. The operational property of the transformation that eliminates a partial derivative of the equation is as follows:

$$L \frac{\partial G(t)}{\partial t} = p\bar{G} - G(0) \quad (7)$$

Where G (0) is the value of G at time t = 0. To reduce equation (4) to a more familiar form, we take:

$$c(x,t) = \Gamma(x,t) exp\left[\frac{ux}{2d} - \frac{u^2 t}{4d} - \lambda' t\right] \quad (8)$$

Let $\emptyset(x,t) = \frac{ux}{2d} - \frac{u^2 t}{4d} - \lambda' t$

By substituting equation (8) in equation (4), we obtain:

$$\frac{\partial \Gamma}{\partial t} = d \frac{\partial^2 \Gamma}{\partial x^2} \quad (9)$$

The initial and boundary conditions (5) become:

$$c(x,0) = 0 \quad , x \geq 0$$
$$c(0, t) = c_0 \, exp\left(\frac{u^2 t}{4d} + \lambda' t\right), t \geq 0 \quad (10)$$
$$c(\infty, 0) = 0 \quad , x \geq 0$$

The Laplace transform of equation (9) is:

$$L\left(\frac{\partial \Gamma}{\partial t}\right) = d \frac{\partial^2 \Gamma}{\partial x^2} \quad (11)$$

Therefore, it's reduced to an ordinary differential equation

$$\frac{\partial^2 \Gamma}{\partial x^2} = \frac{P}{d} \bar{\Gamma} \quad (12)$$

The solution of the problem is:

$$\Gamma(x,t) = \frac{c_0}{2} exp\left[\frac{u^2 t}{4d} + \lambda' t\right]\left[e^{2\varepsilon} erfc\left(\alpha + \frac{\varepsilon}{\alpha}\right) + e^{-2\varepsilon} erfc\left(\alpha - \frac{\varepsilon}{\alpha}\right)\right] \quad (13)$$

Substituting (13) in equation (8), the solution becomes:

$$\frac{c}{c_0} = \frac{1}{2} exp\left[\left(\frac{ux}{2d}\right) e^{-2\varepsilon} erfc\left(\alpha - \frac{\varepsilon}{\alpha}\right) + e^{2\varepsilon} erfc\left(\alpha + \frac{\varepsilon}{\alpha}\right)\right] \quad (14)$$

Where, $\alpha = \frac{x}{2\sqrt{dt}}$, $\varepsilon = \sqrt{\left(\frac{u^2}{4d} + \lambda'\right)}\left[\frac{x}{2\sqrt{d}}\right]$, $H = \frac{2\lambda' d}{u^2}$ and $\Gamma = \sqrt{1 + 2H}$

Finaly:

$$\frac{c}{c_0} = \frac{1}{2}\left\{exp\left[\frac{ux}{2d}(1 - \Gamma)\right] erfc\left(\frac{x - ut\Gamma}{2\sqrt{dt}}\right) + exp\left[\frac{ux}{2d}(1 + \Gamma)\right] erfc\left(\frac{x + ut\Gamma}{2\sqrt{dt}}\right)\right\} \quad (15)$$

2.4 Numerical solution of the mathematical model

The numerical solution allows to take into account more complex heterogeneous systems, and they can be thus more realistic and flexible to simulate the real conditions. It is not very obvious to obtain an analytical solution of linear PDE such as equation (4). However, there is an approximate solution for linear PDEs obtained from different numerical methods. Therefore, to solve the EDP of pollutant transport in porous media, we use numerical methods. The partial discretization method, where the finite difference approximation is used to obtain the discrete form of the spatial derivative. Then, the finite space difference diagram in space is used to have discrete shape spatial derivation equations of equation (4). Finally, the fourth order Runge Kutta system (RK4) is used to solve equation (4). This

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numerical technique is used to solve the ordinary differential equation expressed as follows:

$$\frac{\partial c}{\partial t} = g(t, c) \quad (15)$$

The RK4 algorithm is written as follows:

$k_1 = \Delta t\ g(t, c(t))$

$k_2 = \Delta t\ g(t + \frac{1}{2}\Delta t, c(t) + \frac{1}{2}k_1)$

$k_3 = g(t + \frac{1}{2}\Delta t, c(t) + \frac{1}{2}k_2)$ (16)

$k_4 = \Delta t\ g(t + \Delta t, c(t) + k_3)$

$c(t + \Delta t) = c(t) + \frac{1}{6}(k_1 + 2\ k_2 + 2\ k_3 + k_4)$

Where, Δt is the time step, c(t) is the concentration of the pollutant at time t, c(t + Δt) is the concentration at time (t + Δt). The partial discretization method of the finite difference approximation of first-order spatial derivatives is:

$$\frac{\partial c}{\partial x} = \frac{c_i^j - c_{i-1}^j}{\Delta x} \quad (17)$$

The discrete form of derivative of the second order is:

$$\frac{\partial^2 c}{\partial x^2} = \frac{c_{i+1}^j - 2c_i^j + c_{i-1}^j}{\Delta x^2} \quad (18)$$

The indices (i) and (j) represent the discretization nodes along (x) and (t) respectively. Δx is the step of space. Thus, equation (4) can be written in the following reduced form:

$$\frac{\partial c_i^j}{\partial t} = g\left(t, x, c_{i+1}^j, c_i^j, c_{i-1}^j\right) \quad (19)$$

where ,

$$g\left(t, x, c_{i+1}^j, c_i^j, c_{i-1}^j\right) = \frac{d}{\Delta x^2}\left(c_{i+1}^j - 2c_i^j + c_{i-1}^j\right) - \frac{u}{\Delta x}\left(c_i^j - c_{i-1}^j\right) - \lambda' c_i^j$$

$$g\left(t, x, c_{i+1}^j, c_i^j, c_{i-1}^j\right) = d_1\left(c_{i+1}^j - 2c_i^j + c_{i-1}^j\right) - d_2\left(c_i^j - c_{i-1}^j\right) - \lambda' c_i^j$$

With $d_1 = \frac{d}{\Delta x^2}$, $d_2 = \frac{u}{\Delta x}$

We define $x = i\Delta x$, $t = j\Delta t$ with (i=0, 1,2…, N_x) and (j=0, 1,2, … , Nt)

The discrete version of the initial condition associated with equations (4) and (5) is expressed as follows:

$$c_i^0 = c_0 \ if\ i = 0 \quad (20)$$

$$c_i^0 = 0 \quad if\ i \neq 0$$

Equations (20) express a column without initial contamination. The discrete form of the boundary conditions is: $c_i^0 = c_0$, c_0 is the initial concentration of the pollutant.

III. RESULTS AND DISCUSSION

3.1 Spatial and temporal variation of pollutants

Figure 1 represents the spatial evolution of the concentration of the pollutant (chromium) as a function of the depth x for the different times t = 1 day, t_1 = 5 days and t_2 = 30 days. c/c_0 is the ratio of the simulated value of the pollutant concentration to a depth x. The analysis of this figure shows that at a time t the concentration of the pollutant decreases exponentially inside the filter. In general, this concentration increases with the time. Considering 40% of the initial chromium concentration as a guideline, we found that the depths of the column *x* should be x_1 = 10 cm for t_1 = 1day, x_2= 20 cm for t_2= 5 days and x_3 = 25, 5 cm for t_3 = 30 days. Figure 2 shows the time evolution of the concentration of the pollutant at the filter outlet for different depth values of the filter: x_1= 20cm, x_2= 25 cm and x_3= 30 cm. It is found that the concentration of the pollutant increases with time and decreases as the depth of the filter increases.

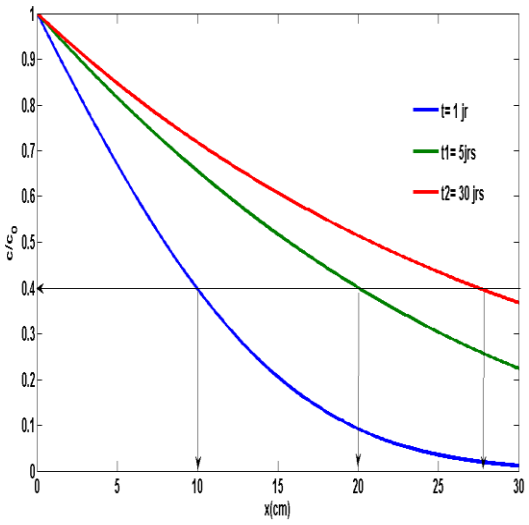

Fig. 1: Evolution of chromium concentration in space for x = 30 cm

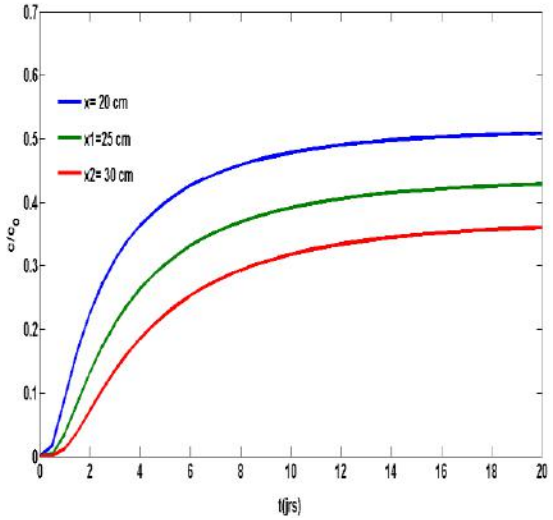

Fig. 2: Evolution of the chromium concentration over time for t = 20 days

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3.2 Effect of the decay rate of Chromium

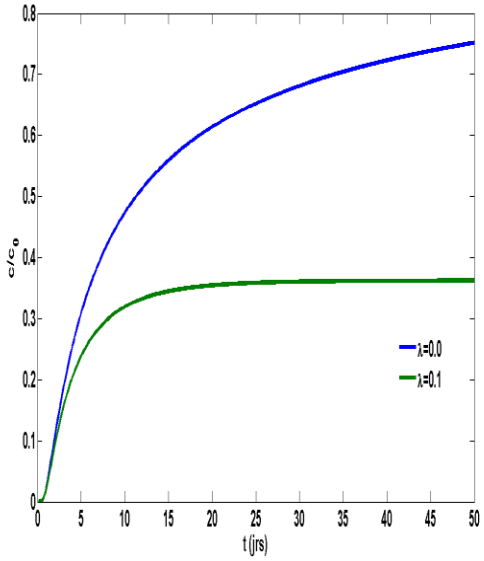

Fig. 3: the temporal evolution of the chromium concentration for the values of λ = 0.0 and λ = 0.1 at t = 10 min and x = 30 cm.

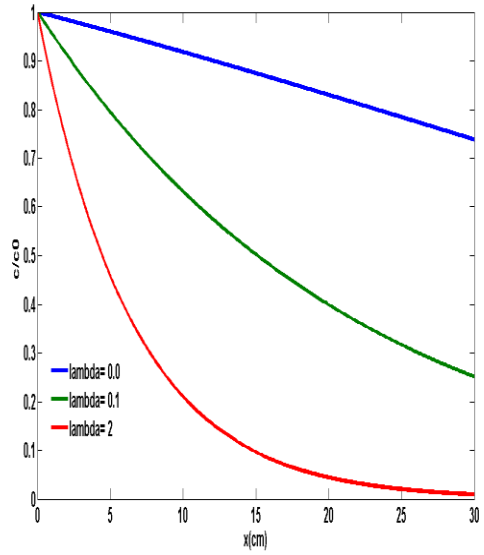

Fig. 4: the spatial evolution of the chromium concentration for the values of λ = 0.0 and λ = 0.1 at t = 10 min and x = 30 cm.

Figure 3 represents the temporal evolution of the pollutant concentration in the filter with different values of the decay rate λ = 0.0 and λ = 0.1. We find that in the absence of this degradation coefficient i.e. when λ = 0, the concentration of the pollutant evolves rapidly inside the filter, in the presence of the latter, the concentration evolves slowly.

Figure 4 represents the spatial evolution of the concentration in the filter with different values of the decay rate λ = 0.0, 0.1 and λ = 2. The analysis of this figure reveals an exponential decay of the concentration inside the filter. The larger the lambda, the higher the concentration of the pollutant decreases rapidly inside the filter until reaching the asymptote c = 0. Therefore, the natural decay of pollutants plays an important role in wastewater treatment since it allows to avoid material waste. It is necessary for the design of industrial sorption columns

3.3 Validation of Analytic and numerical approach

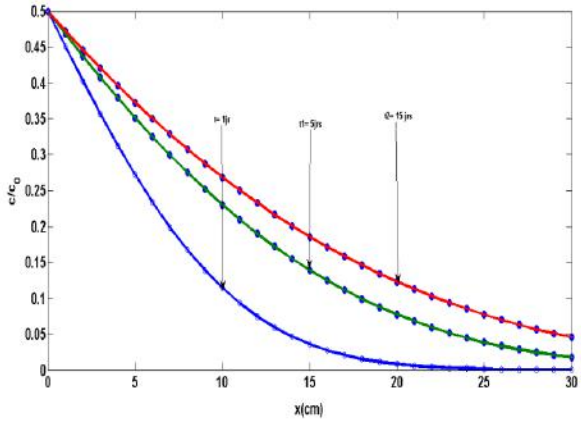

Fig.6: spatial variation of Concentration

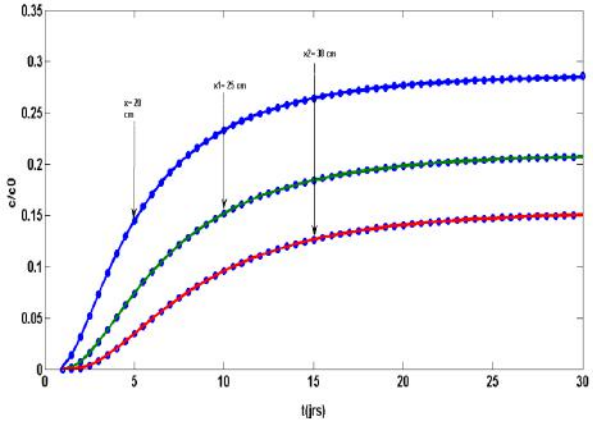

Fig.7: temporal variation of concentration

Numerical solutions of dispersion equation are generated using the RK4 algorithm. For the stability criterion, we took space step and temporal step Δx = 1 and Δt = 1 respectively. These results were obtained for different values of the filter depth x_1 = 20 cm, x_2 = 25 cm and x_3 = 30 cm and for several times t_1 = 1 day, t_2 = 5 days and t_3 = 30 days. After analysis, it appears on Figure 6 that the concentration of the pollutant decreases exponentially with time inside the water filter. Figure 7 shows that the concentration of the pollutant increases at the exit of the filter. The variation of the

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concentration of pollutants into filter is due to the concentration at the filter entrance and to the characteristics of the material (iron oxide-coated sand) used. Finally, the results obtained shows that these two methods (analytical and numerical) are in good agreement.

3.4 Spatio-temporal evolution of the concentration of the pollutant

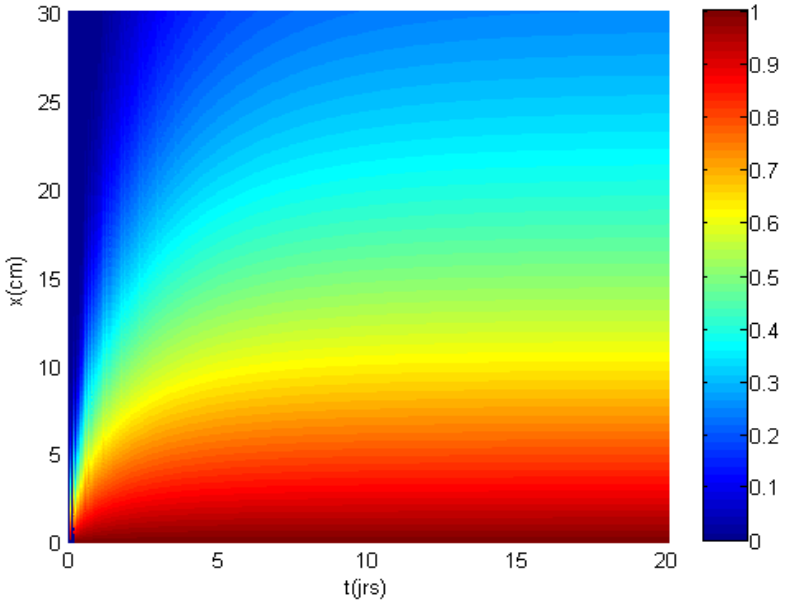

Fig.8: Spatio-temporal evolution of the pollutant concentration in the filter for x = 30 cm and t = 20 days

Figure 8 shows the spatio-temporal evolution of the concentration of the pollutant in the filter. The x-axis is associated to the time taken by the pollutant in the filter, and the y-axis represents the depth of the column. This figure is subdivided into three phases. The initial phase which starts from 0 to 30% of the initial concentration characterized by the blue color, the unsaturated phase from 30 to 70%, and the saturated phase which start from 70 to 100% characterized by the red color. Figure 8 shows the concentration of pollutant increase with time and decrease with the column depth. These results could be used to design equipment for chromium removal in aqueous solution.

IV. CONCLUSION

The degradation coefficient is an important factor which control the design of membrane filter to remove chromium VI in water. This coefficient must be known to optimize chromium VI adsorption in an iron oxide-coated sand based filter on a technical scale. For adsorption of chromium VI on iron oxide-coated sand, analytical and numerical data obtained from simulating a fixed bed adsorber showed that iron oxide-coated sand was very effective in removing chromium VI from an aqueous solution. It is shows that the degradation coefficient of chromium λ has a significant effect on the spatio-temporal evolution of the concentration of the pollutant. This coefficient should be taken into account in models in order to avoid to overestimate the concentration of the pollutant in reactive media. A clear improvement is observed for a material having a high adsorption capacity and a high first order degradation coefficient. Affordable water treatment filters could be constructed using iron oxide-coated sand which is an effective, inexpensive material for the removal of water pollutants or for the treatment of effluents from industries.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Cattle Density Analysis for Development of Cattle Farming in Coconut Land Area in South Minahasa Regency, Indonesia

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Abstract*—In an effort to develop livestock in a region in a developing country, it is necessary to analyze the potential of the area and density of livestock for the development of livestock business in a development area. This study aims to determine the potential of the region regarding cattle livestock density consisting of: economic density, farming density and regional density for the development of beef cattle farming in coconut plantations in South Minahasa Regency. The method used to carry out the analysis used livestock density analysis and analysis of regional potential using the primary and secondary data needed. The results of the study can be concluded based on the calculation of the combination of economic, farming and region densities, it is known that the sub-districts that receive the first priority in the development of beef cattle are the Sinonsayang and Amurang Barat sub-districts including the southern part of South Minahasa Regency, because this region can develop cattle farming and forage for both grasses and legumes in coconut fields which are mostly located in this region, which can be applied in an integrated manner through the coconut and beef cattle (coco-beef) integration system model. Whereas in Tatapaan Subdistrict and other sub-districts in the North and East regions of South Minahasa Regency it is not a priority for cattle development, because this region is more dominant with clove plantations and food crops such as paddy fields and other food crops.*

Keywords*—*cattle density, coconut land, farming.

I. INTRODUCTION

North Sulawesi Province, Indonesia sets coconuts as one of the leading commodities. The area of coconut plantation in North Sulawesi is around 270 thousand hectares (Marbun, 2014). Most of the coconut area is only cultivated in monoculture even though it has the potential of integration with livestock (Polakitan, 2012 and Salendua et al., 2018), even the land under coconut trees is only overgrown with vegetation for wild pastures both grass and local legume that grows wild, although the yield and quality of these types of forages are low and some of them are low edible for cattle, but due to lack of forages then the farmers are forced to feed or provide feed for local species (Osak, et al. 2018).

The potential of livestock in South Minahasa Regency is quite adequate, especially cattle, where according to BPS data in 2016 there were 17,345 (BPS, 2017), as one of the largest areas and has the potential for cattle development because of the availability of land in the coconut area which can be used as land for forages and feed crops. Potential areas for developing cattle in South Minahasa Regency are in the area of extensive coconut plantations, which is the influence of the potential of the region on the capacity and density of cattle in the South Minahasa Regency.

Through this research, it is expected to obtain findings and innovations, which consist of: (1) the density of cattle that is divided into three types, namely economic density, farming density and regional density; (2) the potential for developing forage crops in coconut fields; and (3) the potential of the region for the development of beef cattle business in coconut land areas in South Minahasa Regency. The research problems are as follows: (1) How is the level of cattle density divided into three types, namely economic density, farming density and regional density in South Minahasa Regency; (2) How wide is the potential of the development area of cattle in the coconut plantation area in South Minahasa Regency.

The potential for developing livestock production, including farming the cattle-plant integration system, especially with plantation crops in Indonesia, is very large, supported by the potential of land resources for agricultural development, which is 100.7 million ha, which can feed biomass for cattle about 1-3 head / ha throughout the year. If not utilized, agricultural waste will become a problem and obstacle in agribusiness, because at the time of harvest it is wasted and becomes a polluter (BBLSBP, 2009). In addition, cow manure can be processed into bioslurry and biogas, according to Osak and Hartono (2016) that contribute to environmental

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sustainability is reduction in greenhouse gas emissions (GHG). Greenhouse gas emissions cause of global warming lately. Global warming is warming of the earth's atmosphere, due to accumulation principally of carbon dioxide and methane. Meanwhile, diversified systems according to FAO (2001) consist of components such as crops and livestock that coexist independently from each other.

II. MATERIALS AND METHODS

This research was carried out on cattle farms in South Minahasa Regency, North Sulawesi Province, Indonesia, where 3 (three) sample sub-districts were selected which had the most cattle population in the East, Central and West regions, namely Sub District of Tatapaan (North region), Sub District of West Amurang (Central region) and Sub District of Sinonsayang (Southern region). The data used in this research includes primary data and secondary data. Sources of data collected are primary data from farmers and secondary data from relevant agencies that are related to this research.

Economic valuation methods use primary data obtained from direct observation in the field, with the method of in-depth interviews with respondents based on the questionnaire that has been prepared in accordance with the objective of the study (Hidayatullah, et al., 2011). Determination of sample villages and sample farmers was carried out by purposive sampling. The sample villages were selected by the criteria of the village which had a large cattle population, while the sample farmers with the criteria of having cattle and coconut plantations.

This study analyzed the potential for developing cattle based on livestock density using the analytical method used by Ashari et al., (1995), where livestock density is divided into three types, namely (i) economic density; (ii) farming density; and (iii) regional density, based on other statistical and secondary data, the formula can be seen in Table 1.

Table 1. Formulas and Criteria for Cattle Density

No.	Description	Formula	Criteria
1.	Economic density	$\frac{\Sigma \text{ cattle population (AU*) x 1000}}{\Sigma \text{people pupulation}}$	▪ Very density (>300) ▪ Normal Density (100-300) ▪ Middle density (50-100) ▪ Rarely density (<50)
2.	Farming density	$\frac{\Sigma \text{ cattle population (AU)}}{\text{The area of arable land (ha)}}$	▪ Very density (>2) ▪ Normal Density (1-2) ▪ Middle density (0,25-1 ▪ Rarely density (< 0,25)
3.	Regional density	$\frac{\Sigma \text{ cattle population (AU)}}{\text{Territory area (km}^2\text{)}}$	▪ Very density (>50) ▪ Normal density (20-50 ▪ Middle density (10-20) ▪ Rarely density (<10)

*) AU= animal unit.

III. RESULTS

The environment and climate in South Minahasa supports the development of tropical agriculture. In general, commodity crops and plantations cultivated in this area are food crops (rice, corn, potatoes, cassava, sweet potatoes, peanuts and soybeans), horticultural plants (carrots, beans, spices and onion stems), fruit plants (banana, pineapple, orange, avocado, papaya, mango, durian and rambutan), and plantation crops (coconut, cloves, and vanilla).

South Minahasa Regency has the highest planting area of coconut which reaches 45,041 ha, with total production per hectare of 49,375 tons. Corn is a food crop that has the largest harvest area among other types of food crops, which is as much as 20,882 ha with a total production of 3.94 tons per hectare.

Coconut plants have the largest planting area in South Minahasa Regency, because this regency is one of the centers of coconut production in North Sulawesi province. Coconut plant area has great potential for cattle development, because the area can be planted with grass and legumes for forage under coconut trees. Productivity of Pennisetum purpureum cv. Mott in coconut field that has been fertilized with cattle manure based compost, produces the yield of fresh forage per year can reach 661,947.64 kg per hectare per year, then the stocking rate per hectare of land under coconut trees can be given to around 45.34 heads of cattle a year (Osak et al., 2018)

The development of cattle in the research area can be done by taking into account the existing values and density criteria of livestock. Determination of values and criteria is based on potential economic density, farming density, and regional density in a region. The results of the study on the value and density criteria of cattle in South Minahasa Kabupaen the results of the analysis can be seen in Table 2.

Table 2. Values and Levels of Cattle Density in South Minahasa Regency

Sample Sub District	Economic density		Farming density		Regional density	
	Value	Level	Value	Level	Value	Level
Tatapaan	92,75	Rarely	0,26	Rarely	11,10	Medium
Amurang Barat	236,24	Medium	0,21	Rarely	14,25	Medium
Sinonsayang	143,33	Medium	0,05	Rarely	5,91	Rarely

The economic density of the sample sub-districts in South Minahasa Regency is in the rarely and medium level categories, farming densities generally include level rarely, and regional density including still rarely and medium density. This shows that economic density for cattle is still rarely and medium density compared to the population. If seen from the density of farming, which is generally still rarely density, so it still has a great opportunity to continue to be developed, whereas it is

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seen from the available arable land area that is still classified as rarely and medium density so it is still possible to accommodate cattle to be developed.

South Minahasa Regency is still feasible to develop its potential for cattle. But the data and assessment for each sub-district will produce different information on each density in the economy, farm and regional density. Districts that are economically included are still rarely in Tatapaan Subdistrict, while West Amurang and Sinonsayang Sub Districts are of medium level density for cattle. For farming densities, the three sample sub-districts are included in the rarely density criteria, so that cattle are still possible to be developed.

When viewed from the density of the region, there is no densely populated sub-district, where the District of West Amurang and Tatapaan are classified as medium density density areas, while the Sinonsayang District is still rarely density. This shows that the three sample sub-districts still have potential in developing cattle. This information can provide an alternative in the development of cattle should be done more intensively, so that the potential of the area is used more optimally and the use of coconut plantation crops can be suggested by application to farmers, done by groups of farmers and coconut plantation companies especially Sinonsayang District which has several companies coconut plantations both state-owned companies (BUMN or Badan Usaha Milik Negara in Indonesian) and private companies have business land use rights (HGU or Hak Guna Usaha in Indonesian).

The results of other studies reported that the development of beef cattle can be done through increasing land potential, human resources, and feed patterns. Mukson et al. (2008), that the factors that influence the development of beef cattle by 92.3% are influenced by land area, availability of forage, labor, and capital. This shows that the extent of land availability and the potential of agricultural waste produced is an opportunity that can be utilized for the development of beef cattle farming including in South Minahasa Regency.

Based on the value of the key characterization criteria, the assessment of the criteria for the density of cattle for all sample sub-districts fulfills the requirements for the development of beef cattle. This assessment shows that all sample sub-districts in Tatapaan, West Amurang and Sinonsayang meet the criteria for cattle development.

Based on all the factors analyzed, it is known that all sub-districts in South Minahasa Regency have the potential to develop beef cattle. The results of the calculation of a combination of economic, farming and regional densities are known that the sub-districts that receive the first priority in the development of beef cattle are the Sinonsayang and Amurang Barat sub-districts which belong to the southern part of South Minahasa Regency because this area can develop cattle farming and fodder both grasses and legumes in coconut fields are mostly located in this region which can be applied in an integrated manner through a model of the integration of cattle and coconuts (coco-beef). Whereas in Tatapaan Sub District and other sub-districts in the North and East regions of South Minahasa Regency it are not a priority for cattle development, because those regions are more dominant with clove plantations and food crops such as paddy fields and other food crops.

The results of the analysis above are based on the potential of effective coconut land. However, this condition needs to be supported by forage technology. The results showed that the land under the coconut trees had not been utilized so that cattle only consumed agricultural waste and grass that grew wild. This phenomenon also occurs in other regions, that utilization of vacant land for plantations has not been maximized.

Land under coconut trees in South Minahasa Regency can be utilized for forage development. This approach shows the development of cattle through the integration of coconut-cattle in the research area. The integrated farming system approach can increase productivity and profitability compared to conventional agriculture. The integrated farming system approach causes improvements in household nutrition, income and job creation. The importance of the role of integrated crop-livestock systems for sustainable development, and that integrated crop-livestock systems, implying a diverse range of integrated ecological, biophysical, socio economic conditions, have been a foundation of agriculture for hundreds of years (FAO, 2010).

Livestock management integrated with livestock-crop integration systems, both technically and economically feasible to be developed. This system produces additional work for family labor, and minimizes the risks associated with conventional agricultural systems. Integrated management shows livestock development which leads to the concept of sustainable livestock which includes important components of socio-economic and environmental aspects. Studies that have been carried out on the assessment of sustainable integrated farming are in accordance with quantitative environmental standards and socio-economic benchmarks.

IV. CONCLUSION

The combination of economic, farming and regional densities is known that the sub-districts that receive the first priority in the development of beef cattle are Sinonsayang and Amurang Barat sub-districts including the southern part of South Minahasa Regency, because this region can develop good cattle farming and forage

grass and legumes in coconut fields which are mostly located in this region through a system of integration of coconuts and beef cattle (coco-beef integration system). Whereas in Tatapaan Subdistrict and other sub-districts in the North and East regions of South Minahasa Regency it is not a priority for cattle development, because this region is more dominant with clove plantations and food crops such as paddy fields and other food crops.

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Analysis of Utilization of Traditional Medicine for the Treatment of Malaria among Rural Farmers in Abia State, Nigeria

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***Abstract*—** *The study investigated the level of utilization of traditional medicine for treatment of malaria among rural households in Abia State, Nigeria. Specifically, the study described socio -economic characteristics of the respondents; ascertain the extent of utilization of traditional medicine among the respondents and determine the factors influencing level of usage of traditional medicine in the area. Data for the study were collected from (180) respondents using structured questionnaire and analyzed using both descriptive and inferential statistics. The result showed that 55.0% of the respondents were males, average age of 58.45 years, 81.2% were married and 45.0% had secondary education. The study further revealed that respondents had high access to medicinal plants from market (mean = 2.35) and traditional medical practitioners (mean = 2.36). Tobit regression result showed that coefficient of age, years of education, income and farming experience influenced access to traditional medicine at 1% probability level. The study concluded that there was moderate utilization of traditional medicine for treatment of malaria in the study area, and recommended ensuring appropriate information on traditional medicine efficacy for effective utilization by the farmers in the study area.*

***Keywords*— Utilization , Traditional Medicine and Rural Farmers.**

I. INTRODUCTION

Agriculture is the mainstay of the Nigerian economy. It employs about two-thirds of the country's total labour force, contributing about 45 % of GDP and providing the means of livelihood for over 70 % of the population (IFAD, 2009). Agriculture is also a major source of raw materials for agro-allied industries (Oboh et al, 2009). It is carried out mainly in the rural areas by rural farmers who play several roles in an agrarian country like Nigeria, given their capacity for job creation and local food production (Asenso-Okyere, 2011)

About 90 % of Nigearia's food is produced by small-scale farmers who cultivate small plots of land and depend mostly on rainfed agriculture rather than on irrigation systems. Agricultural development provides suitable conditions for breeding of anopheles mosquitoes which is the vector of plasmodium parasite (Asenso-Okyere,et al 2011). Agriculture and health being bi-directionally linked, perpetuate poverty in rural areas, where up to 80 % of the population are farmers and live below the poverty line. Food, social services and infrastructure are limited to the population (IFAD, 2009), they tend to be vulnerable to malnutrition, illiteracy and poor health condition especially in the absence of modern, adequate and functional primary health care facilities. In Nigeria, malaria is endemic and it occurs throughout the year. Traditional medicine is the sum of the total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of the health, as well as to prevent diagnose, improve or treat physical and mental illness (WHO, 2000). Traditional knowledge plays a significant role in life style of the members of the local communities and hence an essential resource for any human development process. They form the basis of decisions pertaining to food security, human and animal health, education, natural resources management and other vital activities

According to Federal Ministry of Health (2005), there are over 100 people at risk of malaria. Nigeria has the third highest rate of maternal mortality in the world (1100 per 100,000) after Sudan and Democratic Republic of Congo. The major cause of this being ill-health of which malaria scourge is distinguished (USAID, 2008) Attitude toward malaria as a disease is important in understanding health-seeking behaviour and utilization of preventive methods.

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Many rural people do not trust Western method medicine, preferring instead to use traditional ways which is an alternative or non-conventional mode of treatment involving the use of herbs in a non-orthodox manner, as well as the process of consulting herbalists, mediums, priests, witchdoctors, medicine men and various local deities (Mafimisebi and Oguntade, 2010). Good knowledge, attitude and practices of any public health disease by individuals and communities seems necessary if effective treatment and preventive measures are to be realistic (Iwueze et. al., 2013). These treatments are usually incorrect or sub-optimal since they are most times administered without proper diagnosis (Okeoluwapo et. al., 2008).

Malaria is usually first treated at home with herbal teas and baths prepared with neem, pawpaw, guava, and eucalyptus leaves. Analysis of "what respondents will do first" during malaria attack showed that only 35.5 percent of respondents will use synthetic antimalarial drugs, 13.4 percent will use local herbs, while 27.3 percent will go to the hospital, and 18.2 percent will just pray (Asenso-Okyere et. al.,2009). Utilization of traditional medicine is complex and varies among member states. It is important to take into consideration the utilization pattern of a location in other to ensure that the information developed will be effective and appropriate to the specific country location and their culture . Reliance on herbs as the first treatment action for malaria was also noted in South-east Nigeria upon which this paper intends to ascertain the extent of utilization of Traditional Medicine for the Treatment of Malaria among

Rural Farmers in Abia State through the following specific objectives

1 ascertain the extent of utilization of traditional medicine among the respondents,

2 identify the problems associated with traditional medicine practice and utilization

HO_1: There is a significant relationship between some socio-economic characteristics of the respondents and utilization of traditional medicine.

HO_2: There is no significant difference between farmers' level of access and utilization of traditional medicine

II. METHODOLOGY

The study was conducted in Abia State, Nigeria. Abia State is located within latitudes 400N – 470 N of equator and longitude 70 – 80E of the Greenwich Meridian NRCRI, 2010). The state has a total land area of about 5,410sq kilometers, with a human population of about 2,881,380 (NPC, 2017). Abia State shares a common boundaries with Rivers State in the South, Imo in the West, Ebonyi and Enugu States in the North and Akwa Ibom and Cross River States in the East. Abia State is made up of 17 Local Government Areas (LGAs) and three agricultural zones namely Aba, Ohafia and Umuahia. Most people especially the rural dwellers are engaged in subsistence farming, producing such arable crops as cassava, yam, cocoyam, maize, vegetables, melon, banana/plantain sweet potatoes and rice. The study was conducted in Bende and Ohafia Local Government Area in Abia State. Both LGAs lies within approximately latitudes 5'34" North of the equator and longitudes 7'38" East of the Greenwich Meridian. It covers an area of about 304.45sqKm with 192,111 people (FRN, 2007; NPC, 2006), among which 128,074 people are females and 64,034 are males, according to the NPC state and Local Government Census figure. The major occupation of this people is farming, with other occupations like civil services, and businesses. It is a low-land with a heavy rainfall of about 2,400mm between May and October, which favours agricultural activities. These Local Government Area is blessed with several inland water channels and seasonal run-offs. It has a tropical climate with average temperature of 7.20c from January to April and over 21.10c from September to December. The great variety of plants in L.G.A reflects its rich soil and topography. Major food and cash crops produced include cassava, yam, plantain and oil palm. Other economic activities engaged by the people include, handcrafts and petty businesses. (Imo Bulletin, 2006).

Multi-stage random sampling technique was used in selecting 180 (one hundred and eighty) respondents in the study area. In the first stage, two local government areas were purposively selected from Abia State, namely Bende, and Ohafia. In the second stage, one autonomous community was selected from each of the Local Government Area. In the third stage, two (2) villages were randomly selected from each of the selected autonomous communities making a total of eight (6) villages. In the third stage, 30 respondents were randomly selected from the villages selected. This will give a sum total of eighty (180) respondents which made up the sample size for the study, Both descriptive and inferential statistics was used to analyze the data collected for the study

MODEL SPECIFICATION

The model for the mean decision used in this work is specified thus:

$$\bar{X} = \frac{\sum fx}{n}$$

Where,

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$\sum$ = Summation of the frequencies
Xs = Assigned scores to different categories
n = number of sample
$\overline{X}$ = Arithmetic mean
Hypothesis 1 and 2 were analyzed using Tobit regression estimates which is specified thus:
$Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7 \ldots Xn, e)$
Tobith model expressed thus;
$1_i = B^T X + e_i$……(1)
Y_i = if 1 = T
Where,
Y_i = represent a limited dependent variables which simultaneously measures the level of access and utilization of traditional medicine.
T = observed threshold
1^x = the underlying content variables indexing the levels of access and utilization
Xi = the vectors of independent variables
X1 = Age of respondents (years)
X2 = marital status (married = 1, single = 0)
X3 = level of education (number of years spent in school)
X_4 = Gender (male = 1, female = 0)
X_5 = Occupation
X_6 = Household size (number of persons)
X_7 = Income (Naira)

1.1 LEVEL OF UTILIZATION OF TRADITIONAL MEDICINE

Table.1. 1: Distribution of the respondents based on their level of utilization of medicinal medicine

Level of utilization	Sum	Std Dv	Mean	Rank
Drinking neem water squeezed out From the leaves to cure malaria.	157.0015'	0.77040	1.96	5th
Steam bathing and inhalation of boiled leaves (roots) bark concoction from roots and leaves to cure malaria.	148	0.74799	1.85	6th
Drinking of boiled concoction from roots and leaves to cure malaria.	197	0.54988	2.46	1st
Drinking of tree bark, roots and alcohol concoction to cure malaria.	164	0.78276	2.10	4th
Drinking of boiled concoction made from unripe fruits to cure malaria.	185	0.68610	2.31	2nd
Rubbing of traditional lotions and concoctions to cure malaria.	174	0.80779	2.18	3rd
Combining medical therapy with traditional medicine.	86	0.30914	1.08	7th
Total mean			**13.94**	
Grand mean			**2.00**	
Benchmark mean			**2.00**	

Source: *Field survey, 2018*.

The result Table 1.1 showed distribution of the respondents based on their level of utilization of medicinal medicine in the study area. Generally, the respondents had high level of utilization of medicinal plants in the study area. The respondents highly utilized medicinal plant in the areas of drinking of boiled concoction from roots and leaves to cure malaria (mean = 2.46), drinking of boiled concoction made from unripe fruits to cure malaria (mean = 2.31), rubbing of traditional lotions and concoctions to cure malaria (mean = 2.18), drinking of tree bark, and roots and alcohol concoction to cure malaria (mean = 2.10).

The result is plausible owing to the fact that traditional medicine is accessible, affordable, culturally acceptable, socially sanctioned and easy to prepare with little or no side effects. Most rural households prefer traditional medicine to the exorbitantly priced conventional health care services. Adesina (2014) and Alarm (2011) were of the notion that 75-80% of the population in Africa uses traditional medicine for their needs.

1.2 PROBLEMS ASSOCIATED WITH TRADITIONAL MEDICINE

Table.1.2: Distribution of the respondents based on the problems associated with traditional medicine

Problems	Frequency	Percentages
Poor and inadequate medical facilities.	53	66.2
Limited access and use of these medicines.	37	46.2
Poor quality assurance	25	31.2
There are very few practitioners in area.	55	68.8

The practitioner is not easily accessible.	35	43.8
There are very few healers in attendance.	54	67.5
The drugs have no dosage	52	65.0
Drugs are not usually available.	28	35.0
There is no provision from practitioners to visit very sick patient at home.	41	51.2
High transport cost to traditional healers.	51	63.8
Traditional healers are not well trained or skilled	42	52.5
Lack of knowledge and ignorance	43	53.8
Traditional healers are not well trained/ skilled.	38	47.5
Unhygienic nature of traditional medicine.	44	55.0
At times they are out of reach of patients.	37	46.2

Source: Field survey, 2018
***Multiple responses recorded**

Table 1.2 showed the distribution of the respondents based on the problems associated with traditional medicine in the study area. The result showed that the respondents perceived some challenges associated with medicinal plants. These challenges were that there are very few practitioners in area (68.8%), very few healers in attendance (67.5%), poor and inadequate medical facilities (66.2%), drugs have no dosage (65.3%), high transport cost to traditional healers (63.2%), unhygienic nature of traditional medicine (55.0%), lack of knowledge and ignorance (53.8%), traditional healers are not well trained or skilled (52.5%) among others.

Hypothesis

Table.1.3: Regression estimates of the relationship between socioeconomic characteristics of the respondents and level of utilization of medicinal plants in the study area

Variables	***Parameters***	**Estimate**	**Std. Error**	**T – value**
ge	X_1	.026	.025	2.104**
Gender	X_2	1.553	.499	9.701***
Marital	X_3	-.331	.888	.139
Years of education	X_4	.170	.133	1.634
Household size	X_5	.035	.121	.085
Farm size	X_6	.070	.188	.139
Occupation	X_7	1.659	.811	4.187***
Income	X_8	3.022E-7	.000	6.014***
Farming experience	X_9	.030	.025	1.440
Pseudo R-Square		0.573		
-2 Log Likelihood		264.103		
Chi-Square		617.663***		

Source: ***Field survey, 2018***
Keys: * indicates significance at 1%**

Table 1.2 showed Tobit regression estimate of the relationship between selected socioeconomic characteristics and the utilization of medicinal plants in the study area. The Pseudo R-Square of 0.573 indicated that 57.3% of the variations in the dependent variable were accounted for while others were due to error. The chi-square value was statistically significant at 1% level of probability indicating the Tobit regression line of best fit. The coefficients of age, gender, occupation and income positively influenced the intensity of utilization of medicinal plants in the study area. Furthermore, the coefficient of age was statistically significant at 1% level of probability. This result implies the older person will have high intensity and probability of utilization of medicinal plants.The coefficient of gender was statistically significant at 1% level of probability. The result implies that male respondents had more intensity of

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utilization of medicinal plants than their female counterparts in the study area.The coefficient of occupation was statistically significant at 1% level of probability. The result implies that farmers had more intensity of utilization of medicinal plants than non farmers' counterparts in the study area. This may be attributed to the fact farmers are more familiar with these medicinal plants than non farmers.

The study therefore rejected the null hypothesis which stated that there is no significant relationship between some socio-economic characteristics of the respondents and utilization of traditional medicine and concluded otherwise.

Hypothesis 2

Table.1: 4 Z – test comparative analysis of the difference in the access and utilization of medicinal plants

Variables	Mean	Std. Deviation	Std. Error Mean	Df	Z - Test
Access	1.9625	.27759	.03104		
Use	1.9911	.24517	.02741		
Access – Use	-.02857	.28408	.03176	79	0.900

No significant difference

The result in table 1.4. showed Z-test of the difference in the levels of access and utilization of traditional medicine in the study area. The result showed the mean value of access 1.9625 and mean utilization value of 1.9911 with mean difference of 0.02857. The difference obtained implied that there is no statistically significant difference in the levels of access and utilization of medicinal plants in the study area.

III. CONCLUSION AND RECOMMENDATIONS

The study concluded that there was moderate high utilization of traditional medicine in the treatment of malaria in the study area. It is therefore important to ensure that the appropriate information is made available to the consumers to enable effective utilization which entails maximizing the benefit of traditional medicine while minimizing the risks Production of traditional malaria drug with labeled instruction, dosage and expiry date, so as to ensure adequate utilization. Traditional doctors should ensure that the drugs are produced in a hygienic environment. Women and youths should be encouraged to utilize traditional medicine in the treatment of malaria in the study area.

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Access of Traditional Medicine for the Treatment of Malaria among Rural Farmers in Abia State, Nigeria

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Abstract— *The study investigated the level of access and utilization of traditional medicine for treatment of malaria among rural households in Abia State, Nigeria. Specifically, the study described socioeconomic characteristics of the respondents; ascertained the level of access to traditional medicine; ascertained the extent of utilization of traditional medicine and determined factors influencing access and utilization of traditional medicine in the study area. Data for the study were collected from (80) respondents using structured questionnaire and analyzed using both descriptive and inferential statistics (Tobit regression). The result showed that 55.0% of the respondents were males, average age of 58.45 years, 81.2% were married and 45.0% had secondary education. The study further revealed that respondents had high access to medicinal plants from market (mean = 2.35) and traditional medical practitioners (mean = 2.36). The respondents highly utilized traditional medicine with grand mean of 2.0. Tobit regression result showed that coefficient of age, years of education, income and farming experience influenced access to traditional medicine at 1% probability level. Coefficients of gender, age, occupation and income at 1%, 5%, 1% and 1% probability level respectively influenced utilization of traditional medicine in the study area. The study concluded that there was moderate access and high utilization of traditional medicine for treatment of malaria in the study area, and recommended ensuring appropriate information on traditional medicine for effective access and utilization.*

Keywords— Access, Traditional Medicine and Rural Farmers.

I. BACKGROUND OF THE STUDY

Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plants, animals and minerals based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being (WHO, 2002). Traditional medicine variously known as ethno-medicine, folk medicine, native healing or complementary and alternative medicine is the oldest form of health care system that has stood the test of time. It is an ancient culture-bound method of healing that humans have used to cope and deal with various diseases that have threatened their existence and survival (Abdullahi, 2011). Consequently, different societies have evolved different forms of indigenous healing methods that are captured under the broad concept of traditional medicine, example, Chinese, India and African traditional medicines. This explains the reason there is no universally accepted definition of term (Cook, 2009).

The World Health Organization (WHO) defines traditional medicine as the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (WIPO Publication No. 993). Traditional medicine, according to the World Health Organization (2002) is the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance, relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or written. There are strong indications that traditional healthcare systems are still in use by majority of the people, not only in Africa, but across the world (Cook, 2009). Examples of traditional healers are herbalists, diviners, faith healers, traditional surgeons, etc. All these traditional healers need information to support their work. Traditional medical practice illustrates the medical knowledge practices, which improved for several

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centuries ago within a variety of societies before the era of modern Allopathic or Homopathic Medicine began (Alam, 2011). He went further to say that among non-industrialized societies, the use of herbal medicine to heal diseases is almost universal. People from countries in Latin America, Asia, Africa, even North America are still using herbal products to fulfill their regular health-related necessitates. Adesina (2014) supported Alam (2011) notion by showing that nearly 75-80 percent of the population in Africa uses traditional medicine for their needs. Owing to the fact that traditional medicine is accessible, affordable, culturally acceptable, socially sanctioned and easy to prepare with little or no side effects, most people prefer it to the exorbitantly priced health care services. There are some hazardous side effects of this medication and their limitations in the domain of holistic health, especially in African society.

The effects of poor health go far beyond physical pain and suffering. The combination of high level of poverty, inadequate public health infrastructure and high cost of private health care services have confirmed the larger proportion of both urban and especially the rural populace to patronize traditional medicine. It is estimated that over 70% of West Africans rely on traditional medicine or health care services for treatment on both communicable and non-communicable diseases (WHO, 2012). According to the World Health Organization (WHO), traditional medicine usage for example, up to 80% of people in Africa and Asia use traditional health care services, in China, 50% of total medical consumption and 90% of Germans, 70% Canadians and 60% Swedes have used a natural remedy at some time (WHO, 2008; Hanssen *et al*., 2005).

Traditional medicine is also an affordable source of health in many countries. It is firmly embedded in the belief systems and can be termed culturally compatible (WHO, 2002). In developed countries, higher income and higher education are guiding factors of patient preference for traditional medicine. Due to difficulties in assessing modern health care services, ethnic minorities in developed societies who are disadvantaged both economically and socially, use traditional medicine as first health care choice, making it non-complementary (Bodeker *et al*., 2007). Increase in chronic diseases, awareness about limitations of modern medicine, proven efficacy of traditional medicine systems in selected conditions, emerging interest in holistic preventive health-integrated approach to medical education and increasing awareness among physicians are some of the reasons for renewed interest in traditional medicine (Bodeker *et al*., 2002). Higher quality of care by traditional medicine practitioners have also been reported as an important reason for increasing health seeking and consumer satisfaction of traditional medicine.

II. PROBLEM STATEMENT

Traditional medicine is popular throughout the world. In some Asian and African countries, 80% of the populations depend on traditional medicine, including for primary healthcare (WIPO publication No. 993). Many modern drugs and vaccines are based on natural resources and associated traditional knowledge. Growing commercial and scientific interest in traditional medicine systems has led to calls for traditional medical knowledge to be better recognized, respected, preserved and protected. According to Kleinman (2002), health-seeking arenas can be classified into three: home level, informal and professional sector. Of this home level covers 75% and in every 1000 illness episodes, 750 never get outside of family sector and are managed through household means. The remaining 25% is divided among professional sector, such as hospitals, clinics of biomedicine or western medicine, Chinese medicine, etc. Many simple primary health care problems like fever, upper respiratory tract infections, hepatitis, anaemia, arthritic conditions and certain gynaecological conditions can be managed at household level through simple herbal home remedies (Hariramamurthi *et al*., 2007). The provision of safe and effective traditional medicine therapies could become a critical tool to increase access to healthcare. In 2004, the South African health minister, Manto Tshabalalu Msimang, suggested that the use of African traditional medicines may eventually replace antire trovirals in the treatment of HIV and AIDS (WHO, 2003). There are both benefits and risks associated with the use of traditional medicine. Therefore, it is important to ensure that the appropriate information is made available to the consumers to enable effective utilization which entails maximizing the benefits of traditional medicine while minimizing the risks. This inspired this study which is on access and utilization of traditional medicine among rural households in Abia State, Nigeria.

OBJECTIVES OF THE STUDY

Specifically, the objectives were:

1. describe the socio-economic characteristics of the respondents in the study area,
2. ascertain the extent of utilization of traditional medicine among the respondents,
3. determine the factors influencing level of access of traditional medicine,

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HO_1: There is a significant relationship between some socio-economic characteristics of the respondents and utilization of traditional medicine.

III. METHODOLOGY

The study was conducted in Abia State, Nigeria. Abia State is located within latitudes 400N – 470 N of equator and longitude 70 – 80E of the Greenwich Meridian NRCRI, 2010). The state has a total land area of about 5,410sq kilometers, with a human population of about 2,881,380 (NPC, 2017). Abia State shares a common boundaries with Rivers State in the South, Imo in the West, Ebonyi and Enugu States in the North and Akwa Ibom and Cross River States in the East. Abia State is made up of 17 Local Government Areas (LGAs) and three agricultural zones namely Aba, Ohafia and Umuahia. Most people especially the rural dwellers are engaged in subsistence farming, producing such arable crops as cassava, yam, cocoyam, maize, vegetables, melon, banana/plantain sweet potatoes and rice.

Methodology

The study was conducted in Ohafia agricultural zone in Abia state, Nigeria. Bende and Ohafia LGA lies between co-ordinates latitude 5.617° North and longitude 7.833° East. They has about 245,987 thousand people (population census, 2006). Bende and Ohafia L.G.A are among the Local Government Areas that make up the seventeen (17) L.G.A of the State (FRN, 2007; NPC, 2006), among which 128,074 people are females and 64,034 are males, according to the NPC State and Local Government Census figure. The major occupation of this people is farming, with other occupations like civil services, and businesses. It is a low-land with a heavy rainfall of about 2,400mm between May and October, which favors agricultural activities. The average annual temperature is 26.1° and the average annual rainfall is 2147mm. These Local Government Area are blessed with several inland water channels and seasonal run-offs. It has a tropical climate with average temperature of 7.20c from January to April and over 21.10c from September to December. The great variety of plants in these L.G.A reflects its rich soil and topography. Major food and cash crops produced include cassava, yam, plantain and oil palm. Other economic activities engaged by the people include, handcrafts and petty businesses. (Abia Bulletin, 2006). Objectives and 1, 2 and 3 were analyzed with descriptive statistics such as frequency, percentage. While the Hypothesis was realized using Tobit regression model,

MODEL SPECIFICATION

The model for the mean decision used in this work is specified thus:

$$\overline{X} = \frac{\sum fx}{n}$$

Where,

$\sum$ = Summation of the frequencies

Xs = Assigned scores to different categories

n = number of sample

$\overline{X}$ = Arithmetic mean

Hypothesis 1 were analyzed using Tobit regression estimates/model which is specified thus:

$Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7 \ldots Xn, e)$

Tobit model expressed thus;

$1_i = B^T X + e_i \ldots\ldots\ldots\ldots\ldots\ldots(1)$

Y_i = if 1 = T

Where,

Y_i = represent a limited dependent variables which simultaneously measures the level of access and utilization of traditional medicine.

T = observed threshold

1^x = the underlying content variables indexing the levels of access and utilization

Xi = the vectors of independent variables

X1 = Age of respondents (years)

X2 = marital status (married = 1, single = 0)

X3 = level of education (number of years spent in school)

X_4 = Gender (male = 1, female = 0)

X_5 = Occupation

X_6 = Household size (number of persons)

X_7 = Income (measured in naira from major occupation)

E = error term

Variable	Frequency (n = 80)	Percentages	Mean
Gender Male	44	55.0	
Female	36	45.0	
Age			
20-30	4	5.0	
31-40	12	15.0	
41-50	24	30.0	
51-60	37	46.25	

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61-70	3	3.75	58.45 years
Marital status			
Single	15	18.8	
Married	65	81.2	
Educational attainment			
No Formal Education	11	13.7	
Primary Education	17	21.2	
Secondary Education	36	45.0	
Tertiary Education	16	20.0	
Household size			
1-3	7	8.75	
4-6	40	50.0	
7-9	26	32.5	
10 -12	7	8.75	6 person
Household farm size			
0.5 -1.0	19	23.8	
1.1 - 1.5	40	50.0	
1.6 - 2.0	12	15.0	
2.1-2.5	9	11.25	2.1 hectares

The distribution of respondents according to gender was presented in Table 1. The results showed that majority (55.0%) of the respondents were male while 45.0% were females.

1.2 Age

The distribution of respondents based on their age was shown in Table 1. The result showed that about 46.25% of the respondents were within the age ranges of 50 – 60 years, 30.0% were within the age ranges of 41 – 51 years, 15.0% were within 31 – 40 years, 5.0% were within 21 – 30 years while 3.75% were within age ranges of 61 – 70 years. The result further showed a mean age of 58.45 years indicating the respondents were relatively old but still active..

1.3 Marital status

The marital status of the respondents in the study area was presented on Table 1. The result showed that majority of the respondents (81.2%) were married while 18.8% were still single. This result implied that married people accessed and utilized medicinal plants more than tv here single counterparts.

1.4 Educational attainment

The result on the educational attainment of the respondents showed that majority (45.0%) of the respondents had secondary education. The result further revealed that 21.2% had primary education, 20.0% had tertiary education while 13.7% had no formal education. The result implied that most of the respondents were educated and have good knowledge of medicinal plants.

1. 5 Household size

Majority (50.0%) of the respondents had 4 – 6 persons in their households. Furthermore, 32.5% had 7 – 9 persons in their household while 8.75% of the respondents had 1 - 3 and 10 -12 persons in their household. The result also show an average number of 6 persons in household size of the respondents in the study area. The result implied that there was relatively a large household size in the study area which provides the needed labour to their households.

1.6 Farm size

The distribution of respondents in the study area based on the farm size was shown in Table 1. The result showed that majority (50.0%) of the respondents had farm size of 1.1 – 1.5 hectares. The result further showed that about 23.8% had 0.5 – 1.0 hectares, 15.0% had 1.6 – 2.0 hectares while 11.25% had 2.1 – 2.5 hectares. With an average farm size of 2.1. The result implied that respondents had small farm sizes in the study area.

1.7. Income

The distribution of respondents based on their income was shown in table 1. The table showed that the average income of the respondents was ₦168,398.5 indicating that on the average, the respondents earned small income annually from their livelihood/farming activities hence most of the respondents were farmers. Furthermore, the result revealed that majority (43.7%) of the respondents earned ₦ 201,000- ₦ 300,000 annually, about 15.0% earned ₦301,000- ₦400,000 annually, while 12.5% and 11.2% earned ₦10,000 – 100,000 and ₦401,000-500,000 annually respectively.

1.8 Farming experience

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The distribution of respondents based on their farming experience was showed in table .1. The result revealed that majority 83.8% of the respondents had farming experience of 11 – 20 years. About 11.0% had farming experience of 21 – 30 years while 5.0% had 1 – 10 years of farming experience. The average years of farming experience was 15.94 years. This result implied that the respondents were well experience in farming activities and is therefore more likely to be aware of a lot of medicinal plants.

1.9 Membership to agricultural organization

The result of membership to agricultural organizations showed that the majority (52.5%) of the respondents were members to agricultural organization in the study area while 47.5% were not. Membership to agricultural organization will definitely influence the respondent's awareness, access and utilization of medicinal plants in their location.

1.2 LEVEL OF ACCESS TO TRADITIONAL MEDICINE

Table.1.2: Distribution of the respondents based on their level of access to medicinal medicine

Level of access	Sum	Std Dv	Mean	Rank
Buying from the market	188	0.57589	2.35	2nd
From the bush	158	0.77908	2.00	3rd
From traditional medicine	189	0.71589	2.36	1st
From native doctor	95	0.50551	1.19	5th
Friends / Family	155	0.75211	1.94	4th
Total mean			9.81	
Grand mean			1.96	
Benchmark mean			2.00	

Source: *Field survey, 2016.*

The result in Table 1.2 showed the distribution of the respondents based on their level of access to medicinal plants in the study area. The result revealed that generally, respondents had moderate access to medicinal plants in the study area. Furthermore, the result showed that that the respondents had access to medicinal plants from traditional medicine (mean = 2.36), buying from the market (mean = 2.35), and from the bush (mean = 2.00). This result implied that the respondents accessed medicinal plants from various sources. A large proportion of Nigerians, especially those living in rural areas continue to patronize traditional medicine because traditional healers are considered successful in curing a number of illnesses (Mafimisebi and Oguntade, 2010).

1.3 Determine the factors influencing level of access of traditional medicine

Table.1.3: Distribution of the respondents based on the determination of the factors influencing level of access of traditional medicine

Problems	Frequency	Percentages
Poor and inadequate medical facilities.	53	66.2
Limited access and use of these medicines.	37	46.2
Poor quality assurance	25	31.2
There are very few practitioners in area.	55	68.8
The practitioner is not easily accessible.	35	43.8
There are very few healers in attendance.	54	67.5
The drugs have no dosage	52	65.0
Drugs are not usually available.	28	35.0
There is no provision from practitioners to visit very sick patient at home.	41	51.2
High transport cost to traditional healers.	51	63.8
Traditional healers are not well trained or skilled	42	52.5
Lack of knowledge and ignorance	43	53.8
Traditional healers are not well trained/ skilled.	38	47.5
Unhygienic nature of traditional medicine.	44	55.0
At times they are out of reach of patients.	37	46.2

Source: Field survey, 2016

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***Multiple responses recorded**

Table 1.3 showed the distribution of the respondents based on the problems associated with traditional medicine in the study area. The result showed that the respondents perceived some challenges associated with medicinal plants. These challenges were that there are very few practitioners in area (68.8%), very few healers in attendance (67.5%), poor and inadequate medical facilities (66.2%), drugs have no dosage (65.3%), high transport cost to traditional healers (63.2%), unhygienic nature of traditional medicine (55.0%), lack of knowledge and ignorance (53.8%), traditional healers are not well trained or skilled (52.5%) among others.

1.4 HYPOTHESES TESTING

Table.1.4: Regression estimates of the relationship between socioeconomic characteristics of the respondents and level of access to medicinal plants in the study area

Variables	*Parameters*	Estimate	Std. Error	t- values
Age	X_1	.043	.026	2.751***
Gender	X_2	.129	.488	.069
Marital	X_3	-.915	.918	.993
Years of education	X_4	.620	.152	16.573***
Household size	X_5	.029	.124	.054
Farm size	X_6	-.601	.200	0.038
Occupation	X_7	.321	.817	.154
Income	X_8	-5.203E-6	.000	-3.844***
Farming experience	X_9	0.750	.027	7.993***
Pseudo R-Square		0.652		
-2 Log Likelihood		413.528		
Chi-Square		221.951***		

Keys: * indicates significant at 1%**

Table 1.4 showed Tobit regression estimate of the relationship between selected socioeconomic characteristics and level of access to medicinal plants in the study area. The Pseudo R-Square of 0.750 indicated that 75.0% of the variations in the dependent variable were accounted for while others were due to error. The chi-square value was statistically significant at 1% level of probability indicating the Tobit regression line of best fit. The coefficients of age, years of education, income and farming experience influenced the intensity of access to medicinal plants in the study area.

The coefficient of age was statistically significant at 1% level of probability. This result implies the older person had high intensity of access to medicinal plants as age increases with level of access. The coefficient of education was statistically significant at 1% level of probability. This result implies that increase in level of education will lead to a corresponding increase in the intensity and probability of access to medicinal plants. The coefficient of income was statistically significant at 1% level of probability and negatively related. This inverse relationship implies that increase in level of education will lead to a corresponding decrease in the intensity and probability of access to medicinal plants. The coefficient of farming experience was statistically significant at 1% level of probability and positively related. This result implies that increase in farming experience will lead to a corresponding increase in the intensity and probability of access to medicinal plants. The study therefore rejected the null hypothesis which stated that there is no significant relationship between some socio-economic characteristics of the respondents and access to traditional medicine and concluded otherwise.

IV. CONCLUSION AND RECOMMENDATIONS

The study concluded that there was moderate access to traditional medicine in the treatment of malaria in the study area. It is therefore important to ensure that the appropriate information is made available to the consumers to enable effective utilization which entails maximizing the benefit of traditional medicine while minimizing the risks. From the problems associated with traditional medicine is the problem of very few practitioners in the area, poor and

inadequate medical facilities, drugs have no dosage, unhygienic nature of traditional medicine and lack of knowledge and ignorance, Proper orientation should be carried out such as workshop training, public enlightenment and radio advert, for the safety of natural drug utilization to that of chemical drugs utilization as well as encourage access to traditional medicine.Women and youths should be encouraged to access traditional medicine in the treatment of malaria.

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Effects of Rainfall Seasonality on Scarabaeinae dung Beetles in an Agriculture Habitat in South Western Ghats

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Abstract*—Scarabaeinae dung beetles are a ubiquitous group of beetles that are detritivores and provides important ecological services to the ecosystem. They are biological indicators and their community structure is affected by physical and biotic characteristics of the environment. Rainfall is an important parameter that affects these beetles. Rainfall seasonality of dung beetles in an agriculture habitat in South Western Ghats was studied. Cow dung baited pitfall traps were used to trap dung beetles in a banana plantation during the northeast monsoon, summer and southwest monsoon season. The study showed that agriculture activities affected dung beetle community attributes in the three seasons. Abundance, species richness and diversity was lowest in the northeast monsoon season characterized by moderate rainfall and ideal environmental conditions. This was a result of agricultural practices in the region, where banana cultivation activities peaks in the northeast monsoon season, which makes conditions unfavorable for dung beetles to colonize the agriculture habitat during this season.*

Keywords*— *Scarabaeinae, agriculture habitat, rainfall seasonality

I. INTRODUCTION

Scarabaeinae dung beetles are a ubiquitous group of beetles that are detritivores, feeding on mammalian dung, carrion and vegetable matter (Hanski and Cambefort, 1991). Through their feeding habits, they provide important ecosystem services such as nutrient recycling, soil aeration, secondary seed dispersal, reduce population of disease causing flies and helminth parasites (Nichols *et al*., 2008). Scarabaeinae beetles are important indicator species as their community structure is affected by changes in microclimate factors of the environment, vegetation and mammal abundance (Nichols *et al*., 2007).These beetles are also affected by seasonal changes in rainfall (Hanski and Cambefort, 1991), with differences in abundance and guild structure between dry and wet seasons (Andresen, 2005). Rainfall provides humidity to the soil and triggers the emergence and/or the onset of activity in the beetle species (Doube, 1991; Halffter, 1991; Hanski and Cambefort, 1991). Rainfall also affects the quantity and quality of dung available to the beetles by affecting vegetation growth and abundance of mammals (Cambefort and Walter, 1991; Estrada *et al*., 1999).

Western Ghats in the Indian subcontinent is a biodiversity hotspot facing many threats to its ecosystem through deforestation, non-timber product harvest, construction of dams and roads (Kumar, 1993; Jha *et al*., 2000; Shahabuddin and Prasad, 2004). Large tracts of forest in the South Western Ghats region has been converted into plantations and agriculture habitats (Joy, 1991; Nair, 1991; Latha and Unnikrishnan, 2007). The Western Ghats strongly influences the rainfall pattern of Kerala state in the Indian subcontinent. The Kerala state is a strip of land running almost in North–South direction and is situated between the Arabian Sea on the West and the ranges of Western Ghats and Nilgiri Hills on the East, both running parallel to each other. The region is characterised by three seasons, the heavy rainy periods of southwest monsoon, moderate rainy periods of northeast monsoon and the hot summer season with scanty rains (Nathan, 2000).

There is limited information on the effects of habitat conversion and rainfall seasonality on dung beetle community attributes in South Western Ghats. In the present study, species richness, abundance and diversity of dung beetles in the southwest monsoon, northeast monsoon and summer seasons in an agriculture habitat was studied.

II. MATERIALS AND METHODS

2.1 Study site

The study region, Nelliampathi is located at a height of 467 to 1572 m above sea level and is spread over a total area of 82 sq km at a distance of about 52 km from Palghat town in Kerala state (Fig. 1). The study was carried out in Kaikatty located at 10^0 31'N and 76^0 40'E, at an elevation of 960 msl. The temperature of the region varied between 15^0C-30^0C and

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annual rainfall exceeds 3000 mm (Nair, 1991). The study habitat consisted of 920 acres of government owned agriculture land where orange, sapodilla, banana and other fruit trees are grown. The traps were set in the part of the agriculture habitat with banana plantation.

2.2 Sampling

Dung beetles were collected on a seasonal basis in May (summer season), September (southwest monsoon season) and December (northeast monsoon season) during the 2007-2008 study period. Each collection effort involved placing ten baited pitfall traps containing 200g cow dung as bait, placed 50 m apart in the study habitat. The trap contents were collected at 12 h intervals (6:00-18:00h and 18:00-6:00h) for each collection effort. Collected beetles were preserved in 70% alcohol overnight and later identified to species levels using taxonomic keys and by verifying with type specimens available in the Coleoptera collections of St. Joseph's College, Devagiri, Calicut.

2.3 Analysis

Since the data was not normally distributed, non-parametric statistics Kruskal-Wallis H test was used to test the significant levels of variation in overall abundance of beetles, Shannon diversity (H') and abundance of individual species of dung beetles with seasons. Differences with a p-value <0.05 was compared using Mann-Whitney Test. The beetles were classified as seasonal beetles if they showed significant variation in abundance with seasons, and aseasonal if they did not show significant variation in abundance with seasons. Singletons were considered as rare and excluded from seasonality studies.

III. RESULTS

A total of eight species and 44 beetles were collected during northeast monsoon season, 17 species and 194 beetles in the summer season and 17 species and 105 beetles in the southwest monsoon season (Fig. 2, Table 1). Abundance of dung beetles showed significant variation with seasons ($H= 22.373$, $df=2$, $p=<0.001$). Pairwise comparison of abundance showed significant variation between northeast monsoon and summer ($p=<0.001$), northeast monsoon and southwest monsoon ($p=<0.001$), but there was no significant variation in abundance between summer and southwest monsoon ($p= 0.177$). The Shannon diversity value (H') for northeast monsoon season was 1.49, summer was 2.12 and southwest monsoon was 2.28. Diversity did not vary significantly with seasons ($H=4.450$, $df=2$, $p=0.108$).

Eight species, all tunnelers showed significant seasonality, *Caccobius meridionalis*, *Catharsius molossus*, *Copris repertus*, *Onthophagus furcillifer*, *O. laevis*, *O. manipurensis*, *O. pacificus* and *Paracopris davisoni* (Table 1). *Caccobius meridionalis*, *Catharsius molossus* and *Onthophagus furcillifer* showed highest abundance in summer; *Copris repertus* and *Onthophagus pacificus* showed highest abundance in southwest monsoon. *Onthophagus laevis* and *Paracopris davisoni* were present only in southwest monsoon season; *O. manipurensis* was present only in summer. Eleven species were aseasonal tunnelers. Seasonality in six species could not be determined due to rarity in collection (Fig. 3; Table 1).

IV. DISCUSSION

Low abundance, species richness and diversity recorded during the northeast monsoon season in the agriculture habitat in Nelliampathi is in contrast to results observed in the forest (Latha, 2019; Vinod, 2009) and agriculture habitat (Venugopal *et al*., 2012; Vinod, 2009) of the South Western Ghats region, where high abundance, species richness and diversity was observed in the northeast monsoon season. Dung beetle seasonality studies done in other parts of the world also show that activity is greatest during moist and minimal during dry periods and the abundance of scarab beetles increases strongly after heavy rainfall (Walter, 1985; Doube *et al*., 1991; Hanski and Krikken, 1991; Andresen, 2005; Batista *et al*., 2016).

After the heavy rainy periods of the southwest monsoon season, northeast monsoon season is characterized by ideal climatic conditions and plant growth. This is the time when agricultural activities reach a peak. Low abundance, species richness and diversity observed in the northeast monsoon season in the agriculture habitat of Nelliampathi region is directly related to the agricultural practices. Banana cultivation in the region is done annually and periods of heavy monsoon and severe summer is avoided for planting. This is to avoid the high temperatures of the summer season and heavy rains of the monsoon season which are detrimental to the crop. In the northeast monsoon period agricultural activities such as ploughing, tilling, planting, fertilizing is at its peak and these activities are unfavorable to the beetles and hence low abundance, diversity and species richness was recorded during this season. Cattle dung is the main source of dung in the agriculture habitat of the region (Latha and Sabu, 2018; 2019). During northeast monsoon season while banana cultivation peaks, cattle is not allowed into the field which in turn lowers the dung resource availability for dung beetles, leading to low abundance, species richness and diversity of beetles. While in summer after harvesting banana, the vegetation is cut and domestic cattle is allowed to graze in the fields, and there is greater availability of dung resource for the dung beetles.

Eight species all tunnelers showed significant seasonality. *Caccobius meridionalis, Catharsius molossus, Copris repertus, Onthophagus furcillifer* and *O. manipurensis* which showed peak abundance in summer can be considered as heliophiles, able to tolerate the high temperature and sunlight exposure of the summer season in the open agriculture habitat. Decades of anthropogenic disturbance that has heavily degraded the forests in the region has led to the establishment of heliophiles and synanthropic dung beetle species in the region that are able to survive in the summer season in the open agriculture habitat (Latha and Sabu, 2018; 2019). *Copris repertus, Onthophagus pacificus, O. laevis, Paracopris cribratus, P. davisoni* which showed peak abundance in southwest monsoon season are able to tolerate the heavy rains characteristic of that period.

V. CONCLUSION

Dung beetles in the agriculture habitat responded differently to rainfall seasonality when compared to forest beetles in the same region. Agricultural practices affected dung beetle's response to seasonality. Northeast monsoon season with favourable climatic conditions showed low abundance, diversity and species richness. Agriculture activities did not provide favourable conditions for dung beetles in the field during the northeast monsoon period and also affected the availability of dung which led to the low species richness, abundance and diversity during that season.

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Fig. 1 (A) Study site Nelliampathi in South Western Ghats in Kerala; (B) Agriculture habitat in Nelliampathi.

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*Table 1: Seasonal abundance (NEM=Northeast monsoon, S=Summer, SWM=Southwest monsoon); and seasonality (SE=Seasonal, AS=Aseasonal, *=Seasonality not determined) of dung beetle species in an agriculture habitat in Nelliampathi in South Western Ghats during the 2007-2008 study period.*

Species	NEM	S	SWM	Seasonality
Caccobius gallinus	0	5	0	SE
Caccobius meridionalis	19	63	6	SE
Caccobius ultor	0	0	3	AS
Catharsius molossus	0	7	5	SE
Copris repertus	0	10	17	SE
Liatongus indicus	1	0	0	*
Onitis subopacus	0	1	0	AS
Onthophagus amphicoma	1	2	0	AS
Onthophagus andrewesi	1	0	0	*
Onthophagus bronzeus	0	1	1	AS
Onthophagus ensifer	1	8	3	AS
Onthophagus fasciatus	13	38	23	AS
Onthophagus favrei	0	2	3	AS
Onthophagus furcillifer	5	28	11	SE
Onthophagus insignicollis	0	0	2	AS
Onthophagus laevis	0	0	4	SE
Onthophagus manipurensis	0	8	0	SE
Onthophagus pacificus	0	2	11	SE
Onthophagus porcus	0	1	0	*
Onthophagus rectecornutus	0	0	1	*
Onthophagus turbatus	0	10	2	AS
Paracopris cribratus	0	1	6	SE
Paracopris davisoni	0	0	6	SE
Tibiodrepanus setosus	3	7	0	AS
Tibiodrepanus sinicus	0	0	1	*

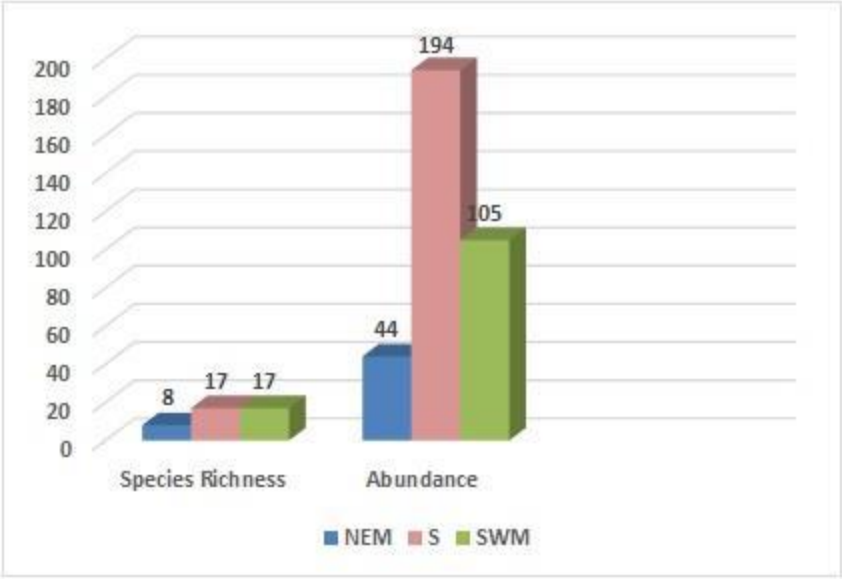

Fig. 2: Dung beetle species richness and abundance in northeast monsoon (NEM), summer (S) and southwest monsoon (SWM) seasons in an agriculture habitat in Nelliampathi in South Western Ghats during the 2007-2008 study period.

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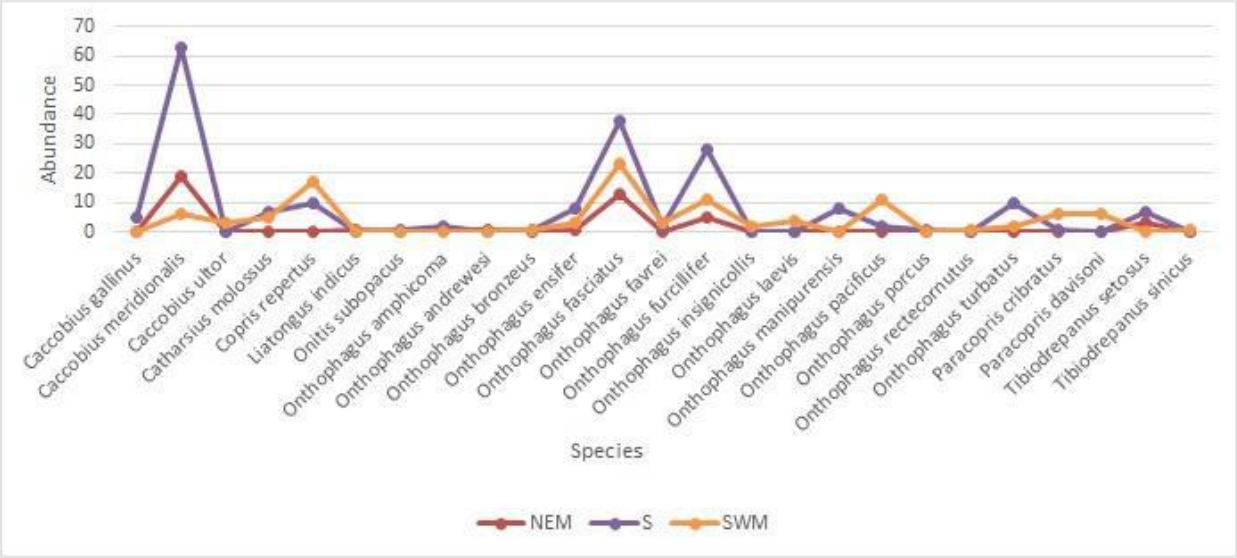

Fig. 3: Seasonal abundance of dung beetle species in an agriculture habitat in Nelliampathi in South Western Ghats during the 2007-2008 study period (NEM =northeast monsoon, S= summer, SWM=southwest monsoon).

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Effects of Rainfall Seasonality on Scarabaeinae dung Beetles in an Agriculture Habitat in South Western Ghats

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Abstract*—Scarabaeinae dung beetles are a ubiquitous group of beetles that are detritivores and provides important ecological services to the ecosystem. They are biological indicators and their community structure is affected by physical and biotic characteristics of the environment. Rainfall is an important parameter that affects these beetles. Rainfall seasonality of dung beetles in an agriculture habitat in South Western Ghats was studied. Cow dung baited pitfall traps were used to trap dung beetles in a banana plantation during the northeast monsoon, summer and southwest monsoon season. The study showed that agriculture activities affected dung beetle community attributes in the three seasons. Abundance, species richness and diversity was lowest in the northeast monsoon season characterized by moderate rainfall and ideal environmental conditions. This was a result of agricultural practices in the region, where banana cultivation activities peaks in the northeast monsoon season, which makes conditions unfavorable for dung beetles to colonize the agriculture habitat during this season.*

Keywords*— *Scarabaeinae, agriculture habitat, rainfall seasonality

I. INTRODUCTION

Scarabaeinae dung beetles are a ubiquitous group of beetles that are detritivores, feeding on mammalian dung, carrion and vegetable matter (Hanski and Cambefort, 1991). Through their feeding habits, they provide important ecosystem services such as nutrient recycling, soil aeration, secondary seed dispersal, reduce population of disease causing flies and helminth parasites (Nichols *et al*., 2008). Scarabaeinae beetles are important indicator species as their community structure is affected by changes in microclimate factors of the environment, vegetation and mammal abundance (Nichols *et al*., 2007).These beetles are also affected by seasonal changes in rainfall (Hanski and Cambefort, 1991), with differences in abundance and guild structure between dry and wet seasons (Andresen, 2005). Rainfall provides humidity to the soil and triggers the emergence and/or the onset of activity in the beetle species (Doube, 1991; Halffter, 1991; Hanski and Cambefort, 1991). Rainfall also affects the quantity and quality of dung available to the beetles by affecting vegetation growth and abundance of mammals (Cambefort and Walter, 1991; Estrada *et al*., 1999).

Western Ghats in the Indian subcontinent is a biodiversity hotspot facing many threats to its ecosystem through deforestation, non-timber product harvest, construction of dams and roads (Kumar, 1993; Jha *et al*., 2000; Shahabuddin and Prasad, 2004). Large tracts of forest in the South Western Ghats region has been converted into plantations and agriculture habitats (Joy, 1991; Nair, 1991; Latha and Unnikrishnan, 2007). The Western Ghats strongly influences the rainfall pattern of Kerala state in the Indian subcontinent. The Kerala state is a strip of land running almost in North–South direction and is situated between the Arabian Sea on the West and the ranges of Western Ghats and Nilgiri Hills on the East, both running parallel to each other. The region is characterised by three seasons, the heavy rainy periods of southwest monsoon, moderate rainy periods of northeast monsoon and the hot summer season with scanty rains (Nathan, 2000).

There is limited information on the effects of habitat conversion and rainfall seasonality on dung beetle community attributes in South Western Ghats. In the present study, species richness, abundance and diversity of dung beetles in the southwest monsoon, northeast monsoon and summer seasons in an agriculture habitat was studied.

II. MATERIALS AND METHODS

2.1 Study site

The study region, Nelliampathi is located at a height of 467 to 1572 m above sea level and is spread over a total area of 82 sq km at a distance of about 52 km from Palghat town in Kerala state (Fig. 1). The study was carried out in Kaikatty located at 10^0 31'N and 76^0 40'E, at an elevation of 960 msl. The temperature of the region varied between 15^0C-30^0C and

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annual rainfall exceeds 3000 mm (Nair, 1991). The study habitat consisted of 920 acres of government owned agriculture land where orange, sapodilla, banana and other fruit trees are grown. The traps were set in the part of the agriculture habitat with banana plantation.

2.2 Sampling

Dung beetles were collected on a seasonal basis in May (summer season), September (southwest monsoon season) and December (northeast monsoon season) during the 2007-2008 study period. Each collection effort involved placing ten baited pitfall traps containing 200g cow dung as bait, placed 50 m apart in the study habitat. The trap contents were collected at 12 h intervals (6:00-18:00h and 18:00-6:00h) for each collection effort. Collected beetles were preserved in 70% alcohol overnight and later identified to species levels using taxonomic keys and by verifying with type specimens available in the Coleoptera collections of St. Joseph's College, Devagiri, Calicut.

2.3 Analysis

Since the data was not normally distributed, non-parametric statistics Kruskal-Wallis H test was used to test the significant levels of variation in overall abundance of beetles, Shannon diversity (H') and abundance of individual species of dung beetles with seasons. Differences with a p-value <0.05 was compared using Mann-Whitney Test. The beetles were classified as seasonal beetles if they showed significant variation in abundance with seasons, and aseasonal if they did not show significant variation in abundance with seasons. Singletons were considered as rare and excluded from seasonality studies.

III. RESULTS

A total of eight species and 44 beetles were collected during northeast monsoon season, 17 species and 194 beetles in the summer season and 17 species and 105 beetles in the southwest monsoon season (Fig. 2, Table 1). Abundance of dung beetles showed significant variation with seasons ($H= 22.373$, $df=2$, $p=<0.001$). Pairwise comparison of abundance showed significant variation between northeast monsoon and summer ($p=<0.001$), northeast monsoon and southwest monsoon ($p=<0.001$), but there was no significant variation in abundance between summer and southwest monsoon ($p= 0.177$). The Shannon diversity value (H') for northeast monsoon season was 1.49, summer was 2.12 and southwest monsoon was 2.28. Diversity did not vary significantly with seasons ($H=4.450$, $df=2$, $p=0.108$).

Eight species, all tunnelers showed significant seasonality, *Caccobius meridionalis*, *Catharsius molossus*, *Copris repertus*, *Onthophagus furcillifer*, *O. laevis*, *O. manipurensis*, *O. pacificus* and *Paracopris davisoni* (Table 1). *Caccobius meridionalis*, *Catharsius molossus* and *Onthophagus furcillifer* showed highest abundance in summer; *Copris repertus* and *Onthophagus pacificus* showed highest abundance in southwest monsoon. *Onthophagus laevis* and *Paracopris davisoni* were present only in southwest monsoon season; *O. manipurensis* was present only in summer. Eleven species were aseasonal tunnelers. Seasonality in six species could not be determined due to rarity in collection (Fig. 3; Table 1).

IV. DISCUSSION

Low abundance, species richness and diversity recorded during the northeast monsoon season in the agriculture habitat in Nelliampathi is in contrast to results observed in the forest (Latha, 2019; Vinod, 2009) and agriculture habitat (Venugopal *et al*., 2012; Vinod, 2009) of the South Western Ghats region, where high abundance, species richness and diversity was observed in the northeast monsoon season. Dung beetle seasonality studies done in other parts of the world also show that activity is greatest during moist and minimal during dry periods and the abundance of scarab beetles increases strongly after heavy rainfall (Walter, 1985; Doube *et al*., 1991; Hanski and Krikken, 1991; Andresen, 2005; Batista *et al*., 2016).

After the heavy rainy periods of the southwest monsoon season, northeast monsoon season is characterized by ideal climatic conditions and plant growth. This is the time when agricultural activities reach a peak. Low abundance, species richness and diversity observed in the northeast monsoon season in the agriculture habitat of Nelliampathi region is directly related to the agricultural practices. Banana cultivation in the region is done annually and periods of heavy monsoon and severe summer is avoided for planting. This is to avoid the high temperatures of the summer season and heavy rains of the monsoon season which are detrimental to the crop. In the northeast monsoon period agricultural activities such as ploughing, tilling, planting, fertilizing is at its peak and these activities are unfavorable to the beetles and hence low abundance, diversity and species richness was recorded during this season. Cattle dung is the main source of dung in the agriculture habitat of the region (Latha and Sabu, 2018; 2019). During northeast monsoon season while banana cultivation peaks, cattle is not allowed into the field which in turn lowers the dung resource availability for dung beetles, leading to low abundance, species richness and diversity of beetles. While in summer after harvesting banana, the vegetation is cut and domestic cattle is allowed to graze in the fields, and there is greater availability of dung resource for the dung beetles.

Eight species all tunnelers showed significant seasonality. *Caccobius meridionalis, Catharsius molossus, Copris repertus, Onthophagus furcillifer* and *O. manipurensis* which showed peak abundance in summer can be considered as heliophiles, able to tolerate the high temperature and sunlight exposure of the summer season in the open agriculture habitat. Decades of anthropogenic disturbance that has heavily degraded the forests in the region has led to the establishment of heliophiles and synanthropic dung beetle species in the region that are able to survive in the summer season in the open agriculture habitat (Latha and Sabu, 2018; 2019). *Copris repertus, Onthophagus pacificus, O. laevis, Paracopris cribratus, P. davisoni* which showed peak abundance in southwest monsoon season are able to tolerate the heavy rains characteristic of that period.

V. CONCLUSION

Dung beetles in the agriculture habitat responded differently to rainfall seasonality when compared to forest beetles in the same region. Agricultural practices affected dung beetle's response to seasonality. Northeast monsoon season with favourable climatic conditions showed low abundance, diversity and species richness. Agriculture activities did not provide favourable conditions for dung beetles in the field during the northeast monsoon period and also affected the availability of dung which led to the low species richness, abundance and diversity during that season.

VI. ACKNOWLEDGEMENT

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Onthophagus insignicollis	0	0	2	AS
Onthophagus laevis	0	0	4	SE
Onthophagus manipurensis	0	8	0	SE
Onthophagus pacificus	0	2	11	SE
Onthophagus porcus	0	1	0	*
Onthophagus rectecornutus	0	0	1	*
Onthophagus turbatus	0	10	2	AS
Paracopris cribratus	0	1	6	SE
Paracopris davisoni	0	0	6	SE
Tibiodrepanus setosus	3	7	0	AS
Tibiodrepanus sinicus	0	0	1	*

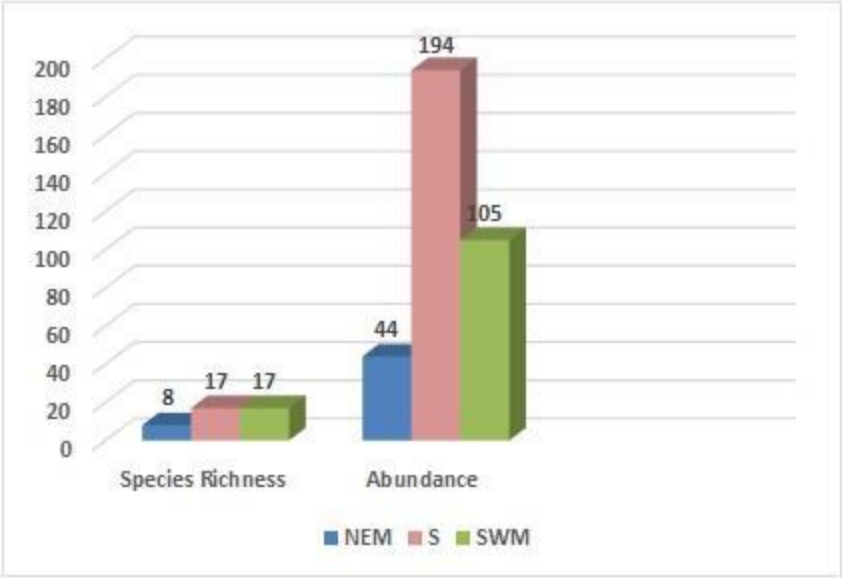

Fig. 2: Dung beetle species richness and abundance in northeast monsoon (NEM), summer (S) and southwest monsoon (SWM) seasons in an agriculture habitat in Nelliampathi in South Western Ghats during the 2007-2008 study period.

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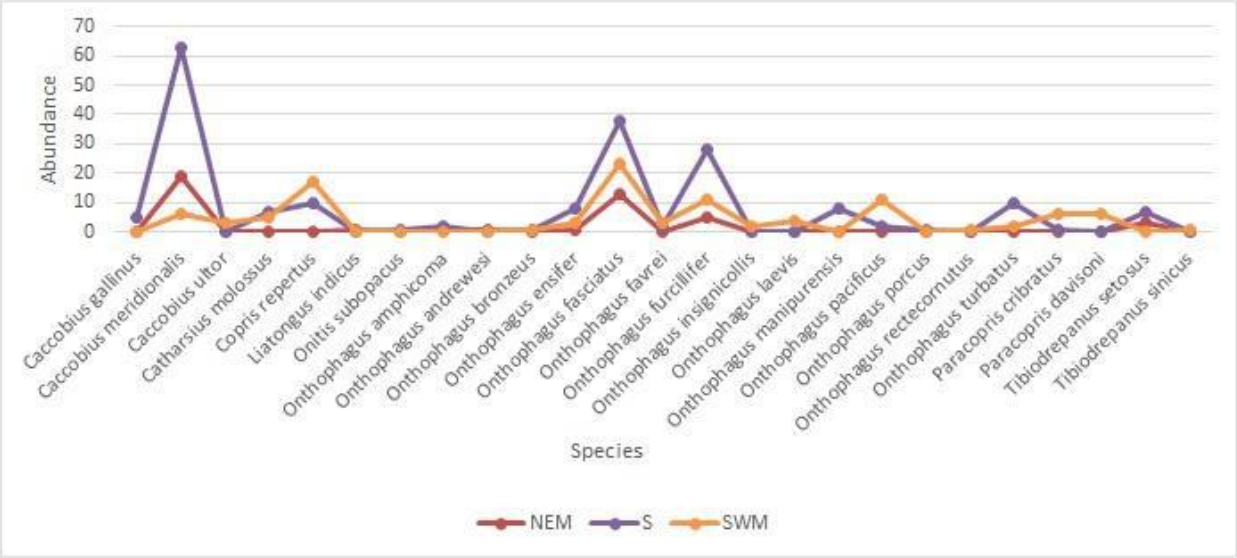

Fig. 3: Seasonal abundance of dung beetle species in an agriculture habitat in Nelliampathi in South Western Ghats during the 2007-2008 study period (NEM =northeast monsoon, S= summer, SWM=southwest monsoon).

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The Impacts of Striped Snakehead (*Channa striata* Bloch) Fish Farming in Net Cages on Social, Economic and Environmental aspects in Bangkau Village, Hulu Sungai Selatan

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Abstract*—The purpose of this study was to analysis and identify the impacts of striped snakehead (Channa striata Bloch) fish farming in net cages on socio economic and environmental aspects. This study was a survey research. Location determination in Bangkau Village, Hulu Sungai Selatan Regency, South Kalimantan Province, Indonesia was done purposively because this area was a center for cultivating striped snakeheads in Hulu Sungai Selatan Regency. The collection of respondent data in this village was carried out in a census of 20 people from the whole population of cultivated striped snakeheads in net cages. The effect on social was done by identifying the effect of social aspect, the economic aspect determined by analysis used was the calculation ofprofit (π) and payback period (PP), while the environmental aspects, it was done by identifying the measurement of water quality. The results showed that from the social aspect, this business influenced the use of labor in its business, benefits obtained from the business of cultivating this striped snakeheads varied between 627,433.33 IDR to 9,789,533.33 IDR per year, while the payback period was 2.23 years. This cultivation effort from the environmental aspects of water quality is still within the class 3 water quality classification tolerance limit.*

Keywords*—*Striped Snakehead, Net Cages, Social Economic and Environmental Effect.

I. INTRODUCTION

Marine and fisheries development in Indonesia is an inseparable part of overall economic development and must support the realization of an advanced, resilient and efficient economy characterized by the ability to prosper the lives of fish farmers and fishermen while at the same time enhancing their ability and independence in promoting the development of the fisheries sector and marine. Bangkau Village is one of the villages in Kandangan Subdistrict, Hulu Sungai Selatan Regency, South Kalimantan Province Indonesia. Livelihoods in general are as fishermen and farmers and a small part as fish farmers in net cages, Bangkau village is geographically classified as swamp area.

One effort to realize an increase in the welfare of fish farmers and fishermen is to increase the production and productivity of fisheries businesses to achieve self-sufficiency in protein-sourced food so as to increase income while improving nutrition for all family members. In this case, Striped snakehead (*Channa striata* Bloch) fish farming can be an option in increasing family income.

*Channa stirata*in local fish known as “haruan and gabus”. English is known as the common snakehead, snakehead murrel, chevron snakehead and striped snakehead. The snakehead name refers to the shape of the head that resembles a snake's head. While the scientific name is *Channa striata* Bloch (Weber, M & Beaufort, 1912).

The potential of aquaculture resources is quite large with various types of fish and economically valuable biota that allow it to be cultivated, but its utilization has not been fully maximized so that the contribution to development and the economy in general and the improvement of living standards of fish farmers in particular is not optimal. The potential that is so broad should be used effectively and efficiently in fish farming.

Utilization of swamp land for fisheries is still dominated by capture fisheries activities whose productivity tends to decrease, along with the increasing population growth, the need for protein sourced from fish also increases while the production of fragrant fish tends to continue to even decline in production. Capture fisheries production,

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especially the production of fragrant cork fish in Hulu Sungai Selatan Regency can be seen in table 1 as follows:

Table 1. Data on Haruan Fish Production in Hulu Sungai Selatan Regency in 2011-2017

Description	Production year						
	2011	2012	2013	2014	2015	2016	2017
Total Production (Tons)	1.126,7	1.105,5	1.094,8	1.211,2	1.107,3	1.099,3	1.089,4

Source: Processed Capture Fisheries Statistics Report (2018)

Destructive fishing is one of the causes of reduced stock of cork fish in public waters. Fresh fish in this area is a type of fish that has a high economic value so that the demand for fish availability will be directly proportional to the selling price in the market, the higher the demand with the decreasing stock will increase the selling price, but when the stock is high, the price also down, this results in haruan fish being one of the fish that causes inflation in the South Kalimantan area (BPS, 2018).

To find out the social, economy and environmental impacts from the cultivation activity of striped snakeheads fish farming in net cages, it is necessary to carry out this research.

II. METHODOLOGY

2.1 Place and Time of research

The location of this research was conducted in Bangkau Village, Kandangan Subdistrict, Hulu Sungai Selatan Regency, South Kalimantan Province, Indonesia, selected purposively or deliberately by considering the villages that had the most cultivators of striped snakehead in stepped net cages. This research was conducted starting from November 2018 to February 2019.

2.2 Population and Sample

The population of this research was fish farmers who carried out of striped snakeheads fish farming in net cages who also worked as fishermen in Bangkau village, Kandangan Subdistrict, Hulu Sungai Selatan Regency, South Kalimantan Province, Indonesia.

2.3 Data Collection Technique

Data collection methods used in this study was survey and interview methods using questionnaires (Sugiyono, 2008). Primary and secondary data were recorded both in the form of the results of interview questionnaires with respondents as well as existing data on government agencies or institutions associated with this research.

2.4 Data Analysis Method

Primary and secondary data that had been collected were made in the form of tabulation, then the data were processed using several quantitative descriptive analysis tools to explain the social, economic and environmental effects wich include financial anaysis and payback period analysis, and qualitative descriptive analysis tools to explain the general description social, economic and environmental influences.

2.4.1. The Influence of Striped Snakehead Fish Farming on Social Effects.

The social effect of striped snakehead fish farming in net cages is identifying how much labor is used and its to have were recorded in the form of the results of interview questionnaires.

2.4.2. The Influence of Striped Snakehead Fish Farming on Economic Effects.

The economic effects of striped snakeheads fish farming in net cages is said to have a profit if the total value of revenue is greater than the total expenditure. This profit analysis (Izmaniar, H,.et. al, 2018) can be formulated by:

$\Pi = TR - TC$

where: π = Profit (IDR)

TR = Total Revenue (IDR)

TC = Total Cost (IDR)

Whereas to calculate the Payback Periodthe formula is:

$$PP = \frac{investment}{Profit}$$

2.4.3.The Influence of Striped Snakehead Fish Farming on Environmental Aspects.

The environmental effects caused by striped snakehead fish farming activities in net cages were in the form of impacts environmental aspects and for the striped snakehead populations were carried out by measuring in situ water quality wich includes pH, temperature and dissolved oksygen (DO), fiber water quality measurement compared to water Quality Classification Class 3 based on PP No. 82 Year 2001.

III. RESULTS AND DISSCUSION

3.1 Characeristics of Respondents

The characteristics of respondents were showed in table 1 as follow :

Table.1: The Characteristics of Respondents

No	Respondent	Ages (Years)	Fish Farming Experiences (Years)
1	Ambri	53	10
2	Pidi	45	8
3	Ahmad Kusasi	53	10

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4	Abdu Samad	43	8
5	Kaspul Anwar	55	20
6	M. Arifin	25	3
7	Lasa	55	10
8	Sarman	45	13
9	Ipin	34	4
10	Abd. Rahman	45	10
11	Samsuri	50	10
12	Raslan	60	15
13	Jadri	50	10
14	Rusdi	50	15
15	Abdul Azis	45	10
16	Jakfar	56	15
17	Nayan	55	20
18	Ruslan A	60	20
19	Ruslan	53	18
20	Darkani	40	5

Experience of Fish Cultivation. Cultivated striped snakehead farmers who had longer experience tended to be more successful than those who were not experienced. The relationship of experience with the amount of profit can be seen in the following graph:

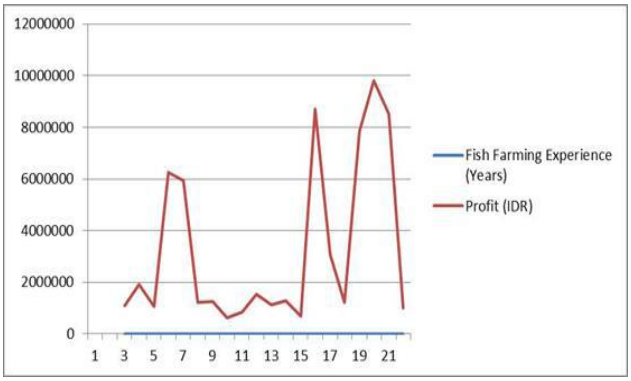

Fig.1: Graph of Comparison of Experience Relationships with the Number of Profit (Processed Primary Data, 2019)

Figure 1 above shows that there is a tendency for the longer the experience of the fish farmers in carrying out the business, the greater the profit generated, therefore the experience of the striped snakehead cultivation business is one of the supporting factors in the business, the longer a person conducts fish farming business, the better to overcome and anticipate problems that arise in fish farming.

3.2 The Influence of Striped Snakehead Fish Farming on Social Effects

The social effects were taken from data interviews with the use of labor as following table :

Table.2: The use of labors in striped snakehead fishfarming

No.	Respondent	Use of Labor
1	Ambri	1
2	Pidi	1
3	Ahmad Kusasi	1
4	Abdu Samad	1
5	Kaspul Anwar	1
6	M. Arifin	1
7	Lasa	1
8	Sarman	1
9	Ipin	1
10	Abd. Rahman	1
11	Samsuri	1
12	Raslan	2
13	Jadri	1
14	Rusdi	1
15	Abdul Azis	1
16	Jakfar	1
17	Nayan	1
18	Ruslan A	2
19	Ruslan	3
20	Darkani	1

Source: Results of Primary Data Processing (2019)

Table.3: The use of labors in striped snakehead fishfarming

No	Type	TotalUse of Labors (persons)	Total Wages (IDR)
1.	The Use of Labors	24	2.400.000,00

From the social aspect, this business influenced the use of labor in its business, both in the form of its own labors and family member labors. The use of labors started from the preparation, maintenance and harvest stages.

3.3 The Influence of Striped Snakehead Fish Farming on Economic Effects

The economic effects were taken form data as following tables. The analysis of the profitability of the business of enlarging embedded net cage can be calculated by several components as follows:

Table.4: Components and Average Total Cost of striped snakehead Fish Farming In Net Cages

No	Type of Cost	Average Cost (IDR/ person / year)	Percentage (100%)
1.	Fixed Cost	852,066.67	65.60

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2.	Variable Cost	389,300.00	34.40
	Total	**1,298,841.67**	**100.00**

Source: Results of Primary Data Processing (2019)

The Average farmers profits can be seen in the table as follow :

Table.5: Profit and Payback Period of Fish Farmers

No.	Components	Value (IDR/Fish Farmer
1.	Income	4.162.500,00
2.	Costs	1.608.265,48
3.	Profit per Year	2.554.234,52
4.	Payback Period	2,23

Source: Results of Primary Data Processing (2019)

In Table 4 , it is explained that the average total cost incurred for the cultivation business of striped snakehead in the cage is 1,298,841.67 IDR per year, which consists of the average fixed costs of 852,066.67 IDR / farmer / year and the average amount of the variable cost of 389,300.00 IDR / farmer / year. In Table 5, it is explained the average profit and payback period from the bussines of this fish farming. This business produced profits that varied between 627,433.33 IDR (lowest) up to 9,789,533.33 IDR (highest), and the return period of capital for both investment and fixed costs was 2.23 years. According to the Decree of the Governor of South Kalimantan Number 188.44 / 0570 / KUM/2018 concerning the Determination of the Minimum Wage of the Province of South Kalimantan in 2019, the Minimum Wage of the Province of South Kalimantan is 2,651,781.95 IDR if it is compared to the Provincial Minimum Wage there are still 13 people below the standard, while those above the Minimum Wage are as many as 7 respondents (Table 6), this is related to the level of experience of farmers who have been explained in the previous sub-chapter (Figure 1), that the higher the experience in cultivating striped snakehead then the higher the tendency to increase profits / income is. The average payback period of investment in striped snakehead cultivation in this embeded net cage is 2.23 indicating that this business can return capital with a minimum business average of 2.23 years. In the third year there will be real returns from the return on investment costs incurred during maintenance, there are even 6 farmers who have a payback period of under 1 year, meaning that there is a possibility that this business can be returned in just one production period and the farmers have even obtained the real benefit of this business if they are able to manage the business of cultivating the striped snakehead diligently and seriously.

3.4 The Influence of Striped Snakehead Fish Farming on Environment Effects

The environment effects (Muslim, et. al., 2018) were showed in Table 6 as follow :

Table.6: Measurement Results of In Situ Water Quality in Maintenance Media

No	Location	pH		Temperature (°C)		DO (mg/l)	
		Outside	Inside	Outside	Inside	Outside	Inside
1.	RT.01	6,7	6,68	28,4	27,6	4,06	4,10
2.	RT.02	6,76	6,71	28,9	28,5	3,95	3,97
3.	RT.03	6,8	6,75	28,0	28,3	4,51	4,50
Average		6,75	6,71	28,43	28,13	4,17	4,19
Standard Class 3*)		6 – 9		Deviation 3		Min 3	

*) Water Quality Classification Based on PP No. 82 of 2001

Whereas from the aspect of the business environment this did not have a harmful and polluting effect on the environment, because all inputs did not use hazardous materials and the products are fish that are safe for public consumption. This business utilized fish stomach waste which was used as alternative feed so that it was no longer wasteful but could also reduce production costs.

Measurement of in situ water quality both from the parameters of temperature, pH and DO did not show a significant difference between the quality of water that was in maintenance media and outside the maintenance media, and still within the tolerance limit of Class 3Water Quality required according to its designation, namely for fishery, agriculture and livestock activities

IV. CONCLUSION

Based on the results of the research, conclusions can be taken as follows:

1. Based on the results of the profit analysis, the stiped snakehead fish farming proved to be profitable. The benefits obtained by farmers varied between 627,433.33 IDR to 9,789,533.33 IDR per cultivating season, compared to the South Kalimantan Regional Minimu Wage 7 people were above the South Kalimantan Minimum Wage of 2,651,781.95 IDR, while 13 people were still below the Provincial Minimum Wage. While the results of the calculation of Payback Period (PP) of 2.23 indicated that to be able to return the cost of investment mode this business must run at least 2.23 years.
2. The striped snakehead fish farming in stepped net cages influenced the socially, economically, and from environmental aspects in the form of water quality of save the striped snakehead the maintenance media is

still within the tolerance limit of Class 3 Water Quality Classification for fishery, agriculture and livestock activities..

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Yield analysis and adaptation for *Bacillus thuringiensis* (Bt) and non-*Bacillus thuringiensis* (Bt) Cotton varieties in the kingdom of Eswatini

Daniel Khumalo

Abstract**—** *Cotton in Eswatini contributes 2.1 % of the country's Gross Domestic Product owing to low cotton yield due to high pest pressure. Eswatini farmers grow Alba QM 301 a conventional non Bt variety which is affected by bollworm. Cotton is no longer profitable and farmers are quitting the industry, yet it is the only source of livelihood in drought prone areas of Eswatini. Countries like India and South Africa have replaced conventional cotton with high yielding Bt or genetically modified cotton.The study analyses yield and adaptation of Bt cotton under rain fed condition.Bt cotton hybrid was evaluated under field condition for adaptation and yield performance in 2016 and 2017 season. Two Bt cotton varieties JKCH 1947 Bt and JKCH 1050 Bt were tested against the local variety Alba Plus QM 301 and JKC 724 both Non Bt (NBt).JKCH 1947 recorded significantly higher seed cotton yield per ha of 3070 kg/ha on the first year. It was closely followed by JKCH 1050 with a yields of 2955 kg/ha.The number of boll per plant was also significant higher compared the control. Alba Plus QM 301 and JKC 724 both Non Bt (NBt) recorded the lower yields of 2066 and 821 kg/ha respectively, under the same condition with less number of bolls per plant. Similar observations were recorded on the second year, JKCH1947 and JKCH 1050 recording 1765kg/ha and 1865kg/ha respectively. A similar trend was observed onthe number of bolls per plant,higher number of bolls were recorded in JKCH 1050 Bt followed by JKCH 1947 Bt. Alba Plus QM 301 NBt and JKC 724 NBt recorded fewer boll in both years. All varieties showed good adaptability to local environment with good plant stand.*

Keywords**— Bt cotton, rain fed conditions, seed cotton.**

I. INTRODUCTION

This paper is about introducing genetically modified cotton in the Kingdom of Eswatini. Discussions in the paper are guided by management processes of introducing a new product or new technology in a market. In the Kingdom of Eswatini, agriculture plays a major role in the economy; it's a major source of food, and also employs more than 60% of the country's population (ISAAA, 2014; Thomson, 2012). Eswatini's agriculture is mainly dependent on sugar cane, cotton and forestry.Cotton is the second biggest cash crop after sugarcane in Eswatini. It is an important cash crop for most Swazis who live on drought prone areas and smallholder farmers who are reliant on the crop for their livelihood (Central Bank of Swaziland, 2013). Eswatini farmers are still entirely reliant on conventional hybrid cotton seeds. Hybrid cotton seeds have long been used in the industry as the sole means for cotton production.

Genetically modified cotton is a variety of cotton that has been modified through a biotechnological process in order to achieve a higher yield. Bollworm resistant, *Bacillus Thuringiensis* (BT) cotton is the most popular genetically modified cotton seed used throughout the world. Genetically modified cotton was first introduced in the early 1990s and has since been adopted by major cotton producing countries such as the USA, India, China and South Africa (James, 2011). The genetically modified cotton seeds are engineered via a biotechnological process to reproduce the soil bacterium *Bacillus Thuringiensis* in a crystal form in order to exterminate certain types of insects and pests which damage the cotton crop and reduce farmer's yields (Craig *et al*., 2008). The new genetically modified seed has outstripped its traditional hybrid counterparts in terms of yield (Brookes & Barfoot, 2013).

In Eswatini, the cotton industry is currently facing a decline in production and this has affected the textile industries which relied on Eswatini cotton as their main source of inputs. Most textile industries have closed due to the shortage of cotton. The few textile factories that are operational survive through importing cotton supplement locally depressed supplies for the daily operations. The government of Eswatini has to revive the cotton industry by introducing a new product in the market. The purpose of this paper is to analyse yield and adaptability of genetically modified cotton in Eswatini. The paper will compare two Bt cotton varieties against the locally grown conventional cotton variety by evaluating the agronomic characteristics of the varieties under condition of the in the Kingdom of Eswatini.

The cotton industry in Eswatini is currently facing many challenges. The country's largest cotton ginnery which is under the stewardship of the Swaziland Cotton Board

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(SCB) and located at Big Bend, has a capacity to handle 25 000metric tons of cotton. Currently, a mere 10% of the ginnery's capacity is being utilised owing to unavailability of inputs and decreased cotton production, among other reasons (Mavuso, 2014). The cotton industry is solely dependent on conventional hybrid cotton seeds. This product has been used by all cotton farmers for the past two decades (Cotton Board, 2014). However, the hybrid cotton seed has reached their decline phase and this is characterised by a rapid decrease in the yield of this product. The decrease in cotton production threatens the 90 ginnery employees' jobs at the Big Bend ginnery (Cotton Board, 2014).

Hybrid cotton that is currently grown by Eswatini farmers is no longer producing high yield as it used to do in the past years. The product has reached a decline phase which is characterized by high production cost, low yields, and heavy pesticides application requirements. From a management point of view a product in decline phase needs to be phased out and replaced because it will be fool hardy to rejuvenate the product (Kotler, 2012). Cotton acreage has drastically been reduced from 20,000 hectares to merely 3000 hectares (Cotton Board, 2013). Correspondingly, the number of cotton farmers in Eswatini has also decreased from 9000 to 3000 in the past 6 years (Cotton Board, 2013). The sector has a potential capacity to create employment directly and indirectly through the textile industry, ginning, spinning, and weaving of fabric respectively, has gone down. This has been aggravated by labour migration from rural areas to the cities (Thomson, 2012). The country has to find strategies of filling the demand gaps created by dwindling cotton production over the years and cheaper technology to continue producing enough cotton to meet increasing demand. Opportunities that are not utilised when they arise will always be taken up by one's competitors (Bryman,et. al. 2014). It is the researchers' conviction that the introduction of genetically modified cotton seed is one of the viable options to tackle the cotton industry's prevailing challenges. There is an urgent need to test the Genetically modified (Bt) technology under local condition and adopt genetically modified (Bt) cotton technology to replace hybrid cotton seed.

In an effort to address the problem in the cotton industry of Eswatini it was essential to conduct agronomic trials of genetically modified (Bt) cotton and test its adaptability under Eswatini soil and weather conditions. This study aimed analyzing yields and the agronomic traits of two Bt cotton hybrids, JKCH 1947 Bt and JKCH 1050 Bt against a popular control variety Alba Plus QM 301(non Bt) NBt and an inbred JKC 724 NBt for yield potential and adaptability. The field experiment was conducted over 2 year, 2016 and 2017 season.

II. MATERIALS AND METHODS

Field experiment was conducted at two years on the same site in Eswatini during 2016and 2017 planting season. The trials focused on agronomic and yield performance of two Bt cotton hybrids (JKCH 1947 Bt and JKCH 1050 Bt), inbred (JKC 724 Non Bt) developed and owned by JK Agri Genetics Limited and the control was a locally grow conventional variety (Alba Plus QM 301 Non Bt). The Lowveld Experimental Station (LES) is located in the Lowveld region (26° 57.95S, 31° 31.52E; 89m asl), with mean temperatures ranging between 26.4 to 30.5°C and annual rainfall of 450 mm. The soils are M-series, which are sandy loam, well drained and fertile (Murdoch, 1968). The experiment used a randomized complete block design (RCBD) with six replications. Gross plot size of experiment was 4 rows of 6 metres length planted at an inter-row spacing of 90 cm and 25 cm between plants. Whereas, the net plot constituted of 2 middle rows with each row having 20 plants thus a total of 40 plants for the net plot.

Observations were recorded on six randomly selected plants from each variety per replication for the characters viz., plant height (cm), no. of lateral branches, no. of lateral branhes (≥ 4 bolls), days to 50% flowering, no. of damaged bolls, no. of bolls/plant, damaged bolls (%),50 bolls dry weight (g), ginning out turn (%) and cotton yield (Kg/Ha). Out of all the bolls per plant, fifty bolls were randomly selected and weighed using a digital balance. Thereafter, the seed cotton yield per plot was estimated after picking the cotton from the whole plot and adding the weight of the collected bolls. The values were up scale from kg/plot to kg/ha for each cotton strain and replication. Field management was done general agriculture practice in the cotton industry of Eswatini. Multiple foliar sprays were applied on control variety Alba Plus QM 301 NBt and inbred JKC 724 NBt to manage cotton bollworm infestation. No foliar sprays were applied on Bt cotton hybrids.

Statistical Analysis

All data were expressed as mean with standard deviation. Agronomic and yield traits data from the cotton varieties were pooled and analysed using one way ANOVA. Analysis of variance was performed by using the ANOVA procedure of the SAS software (version 9.3 for windows). Significant differences between varieties agronomic and yield traits means were determined by Fischer's Least Significant Difference Test at the level of $p \leq 0.05$.

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III. RESULTS AND DISCUSSION

Table.1: Year 1 agronomic traits of Bt and Non-Bt cotton results

Cotton Variety/Hybrid	Plant Height (cm)	No. of Lateral Branches	No. of lateral Branhes (≥ 4 bolls)	Days to 50% Flowering	No. of Damaged Bolls	No. of bolls/plant	Damaged Bolls (%)
Alba Plus QM 301 NBt	83.4a[1]	9.5a	2.5b	106.5a	20.5a	58.8b	35.6
JKC 724 NBt	48.1b	7.7b	3.0ab	110.5a	16.2a	56.2b	29.0
JKCH 1947 Bt	87.7a	9.4a	3.8ab	84.8b	6.7b	92.0a	7.6
JKCH 1050 Bt	78.0a	9.8a	4.2a	86.3b	6.1b	90.2a	6.6

[1] Means with the same letters within the same columns are non-significant with Fischer's Least Significant Differences (LSD) test

Table.2: Year 1 yield components of Bt and Non-Bt cotton results

Cotton Variety/Hybrid	50 Bolls Dry Weight (g)	Ginning Out Turn (%)	Cotton Yield (Kg/Ha)
Alba Plus QM 301 NBt	283.3a[1]	44.7a	2066b
JKC 724 NBt	207.2b	40.8c	1173b
JKCH 1947 Bt	311.5a	43.1b	3070a
JKCH 1050 Bt	294.7a	43.3b	2955a

[1] Means with the same letters within the same columns are non-significant with Fischer's Least Significant Differences (LSD) test

Table.3: year 2 Agronomic traits of Bt and Non-Bt cotton results

Cotton Variety/Hybrid	Plant Height (cm)	No. of Lateral Branches	No. of lateral Branhes (≥ 4 bolls)	Days to 50% Flowering	No. of Damaged Bolls	No. of Bolls/Plant	Damaged Bolls (%)
Alba Plus QM 301 NBt	131.0a[1]	12.52a	2.56c	158.6b	1.0a	41.2b	2.4
JKC 724 NBt	89.0b	11.03a	2.36c	165.a	1.2a	37.3b	2.7
JKCH 1947 Bt	148.0a	12.58a	3.67b	101.5c	0.3a	65.5a	0.5
JKCH 1050 Bt	131.0a	12.92a	5.39a	106.1c	0.5a	67.2a	0.7

[1] Means with the same letters within the same columns are non-significant with Fischer's Least Significant Differences (LSD) test

Table.4: Year 2 yield components of Bt and Non-Bt cotton results

Cotton Variety/Hybrid	50 Bolls Dry Weight (g))	Ginning Out Turn %	Cotton Yield (Kg/Ha)
Alba Plus QM 301 NBt	220.3a[1]	45.7ab	1337b
JKC 724 NBt	172.2b	44.0b	821c
JKCH 1947 Bt	226.8a	46.3a	1765a
JKCH 1050 Bt	218.8a	47.3a	1817a

[1] Means with the same letters within the same columns are non-significant with Fischer's Least Significant Differences (LSD) test

Early flowering was observed in hybrids JKCH 1947 BT (85 days) and JKCH 1050 Bt (86 days) compared to the control variety Alba Plus QM 301 NBt (106 days. Damaged cotton bolls were prominent in Alba Plus QM 301 NBt (35.6%) compared to minimum damaged bolls in JKCH 1947 Bt and JKCH 1050 Bt almost (7.0%) each

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(Table 1). Industrially acceptable ginning out turn percentage (GOT%) ranged between 43.1 to 44.7% in cotton varieties except JKC 724 NBt with 41.0%. Yield indicated that out of the four varieties tested, JKCH 1947 Bt (3070 Kg/ha) and JKCH 1050 Bt (2955 Kg/ha) produced significantly superior seed cotton yield compared to control variety Alba Plus QM 301 NBt (2066 Kg/ha) on the first year. The same trend was observed on the second year. The lowest yielding variety was inbred JKC 724 NBt (1173 Kg/ha). Bigger boll size was observed in JKCH 1947 Bt (312 g/50 bolls) followed by JKCH 1050 Bt (295g/50bolls) and Alba Plus QM 301 NBt (283g/50 bolls) (Table 2). Agronomic performance of Bt cultivars may vary substantially from their non-Bt counterparts (Jenkins et al., 1997)Significantly higher number of bolls were recorded in JKCH 1050 Bt (67.0) followed by JKCH 1947 Bt (66.0) compared to Alba Plus QM 301 NBt (41.0). The hybrids Bt varieties JKCH 1947 Bt and JKCH 1050 Bt (106 days) were significantly faster in boll formation taking (102 days and 106 respectively. Alba Plus QM 301 NBt took the longest time of 159 days. Control entry Alba Plus QM 301 NBt (% boll damage 2.4%) had more boll damage compared to minimum boll damage in JKCH 1947 Bt (0.5%) and JKCH 1050 Bt (0.7%) (Table 3). Industrially acceptable ginning out turn (%) was observed in JKCH 1050 Bt (47.3%), JKCH 1947 Bt (46.3%) and control variety Alba Plus QM 301 NBt (45.7%). JKC 724 NBt exhibited a low GOT% (44.0 %). Based on the weight of 50 balls per variety, Hybrid JKCH 1947 Bt (227 g/50 bolls) had bigger boll size followed by Alba Plus QM 301 NBt (220g/50 bolls) and JKCH 1050 Bt (218 g/50 bolls). Significant differences were again observed on yield of the four cotton varieties trials on the second with JKCH 1050 Bt (1817 Kg/ha) and JKCH 1947 Bt (1765 Kg/ha) compared to control variety Alba Plus QM 301 NBt (1337 Kg/ha).The lowest yield was observed in inbred JKC 724 NBt (821 Kg/ha) (Table 4).

IV. DISCUSSION

Yield is dependent on many component characters, such as boll weight, number of bolls per plant and harvest index. Bt cotton hybrids produce increased seed cotton yield over their non-Bt counter parts and check hybrids, Bt cotton hybrids recorded more than 100% increased seed cotton yield over non-Bt and control hybrids (Anon, 2002).

The Increased yield is attributed to the Bt-genotypes in JKCH 1947 Bt and JKCH 1050. Bolls in the Bt hybrid varieties were protected, while only those that survived the pest pressure were harvested under the local Alba Plus QM 301NBt and the inbred JKC 724 NBt. The two Bt-genotypes eliminate shedding of bolls due to bollworm infestation. Alba Plus QM 301NBt and the inbred JKC 724 NBt suffered from significant boll worm damage. This culminated to the higher seed cotton yield on Bt-genotypes over the local checks.

Since cotton is grown under rain fed condition, the number of days to flowering became is important to cotton producers. The earlier the cotton flowers it the earlier is the maturity time and exposure to heat unit required for crop maturity compared to late flowering varieties. This contributed to high-yielding ability, JKCH 1947Bt and JKCH 1050Bt in both years of experiment. Bt hybrids recorded significantly higher yield than the corresponding non-Bt hybrids. Early-maturity and high-yielding ability is double benefit to the rain fed farmers. The early maturity provide farmers with drought escape toward climate change. Quick trait will help the hybrids to escape from terminal moisture stress in the season. This makes the cultivar to be preferred under rain fed areas (Hofs et al., 2006).

Numbers of bolls per plant play a vital role in determining final yield of a cotton variety. This is influenced directly or indirectly by the growing conditions and its genetic ability to perform in the given environmental condition (Luqman et al., 2015. The statement by Luqman et al. 2015 clearly correspond to the observation of this study where in both years, the Bt cotton cultivars expressed a higher mean number of bolls per plant compared to the local variety and the Non Bt hybrid in both locations. The results obtained from the field trials corroborated those of a trial by Sudha et al. 2011 in Govankoppa village in India.

V. CONCLUSION

This study analyzed the yield performance of two Bt cotton varieties in Eswatini over a period of 2 years based on rain fed conditions. Cultivation of hybrid Bt cotton did not only give a significantly higher yields but also realized significantly reduced insecticidal usage, hence giving security to farmers about the cotton yield. The study conducted over two year's clearly depicted good adaptability of both Bt (JKCH1050 and 1947) cotton hybrids to Eswatini environment. The varieties were early maturing and high-yielding. Adoption of these Bt varieties can help empower Swazi cotton farmers to embrace and benefit from product of modern biotechnology. It is therefore concluded that the two Bt varieties(JKCH1050 and 1947) be released to farmers for commercial growing.

RECOMMENDATION

It is recommended that the kingdom of Eswatini commercialize the growing of Bacillus thuringiensis cotton to cotton growers.

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It further recommended that further studies be conducted on the four region of Eswatini on the performance and adaptation of this technology.

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Plant Diversity in Various Agroforestry System Based on Cocoa in Pasaman, West Sumatra

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Abstract**—** *In Sumatra, cocoa has been cultivated by small holders in diverse agroforestry systems. But recently, companion shade trees are being removed in hopes of reaching higher cocoa yield. This study tests a hypothesis, that high cocoa productivity is compatible with shade and diversity if farmers apply good cocoa management. The study was conducted throughout 2018 in Sontang village, Pasaman district, West Sumatra. We aimed to compare cocoa yield, tree diversity, and ethnobotanical value in 3 shade management systems (low/medium/high). Twelve 20 x 20 m plots were sampled random lyunder each shade, resulting in 36 plots covering 1.44 ha. Plant diversity was measured by species inventories and usefulness was determined based on ethnobotanical interviews. The medium shade also showed the highest crop diversity and highest usefulness. The study concludes that the medium shade harbors suitable level of tree diversity with a positive impact on cocoa yield. We recommend training farmers in cocoa management while sustaining medium shade as a productive, useful and bio diverse system.*

Keywords**— Cocoa, Agroforestry System.**

I. INTRODUCTION

Cacao (Theobroma cacao L.) is an estate crop commodity that has been growing rapidly and has an important role in the national economy, especially as a employment provider, the main source of income for the majority of the population in some provinces, as well as the third largest foreign exchange supply after rubber and palm oil. According to Dampa (2003), cocoa is the prior commodity of the estate agency based on the following considerations: a) biologically, cocoa require a shade trees that make them suitable to be developed under other plants, and also make this commodity as a new alternative income for farmers b) economically, cocoa including the commoditythat could produces yield in medium term period than others annual crops; c) in terms of price, cocoa bean is one of the most profitable product, d) in the way of cultivation, cocoa doesn't require an appropriate technology on farming practise, so that it would be suitable to be cultivated by smallholder farmers.

Cocoa plantations developed through agroforestry systems and then managed properly will ensure the continuity of the structure and ecological processes in it. Interactions between ecosystem structure allows for a variety of ecological processes, including biomass production and nutrient cycling (nutrient cycle). Cocoa-based agroforestry systems can provide a range of ecosystem services (ecosystem services), among others, contribute to maintaining the organic matter content of the soil, thereby improving soil fertility (Mendez., 2006), minimize erosion, preventing the development of pests and diseases, and reduce the weed population. Mixing plants in a cropping system greatly affect nutrient cycles that occur in an ecosystem.

Based on the above it is necessary to investigate the level of plant diversity contained in the model farm management cocoa agroforestry good (simple shade) and complex (multi-level) that have an impact on the productivity of cocoa compared to systems plantation management in non agroforestry (monoculture)

II. MATERIAL AND METHOD

This study was performed using comparative survey. Implementation of the survey begins from field observations (cacao agroforestry with various systems), making the coordinates of the garden, the determination of sample plots and observations as well as the selection of the cocoa farmers to be used as subjects in this study. Determination and election observation plots cocoa farmers carried out by random sampling. Observation of the diversity of plants is done by counting all the species of plants and the number of individuals of each species in research plots. To determine the species diversity calculated using diversity index of Shannon-Wiener (Kent and Paddy, 1992; Smith and Wilson, 1996; Spellerberg and Fedor, 2003).

$$\mathbf{H}' = -\sum_{i=1}^{N} (pi)(\ln pi)$$

H '= Shannon Wiener Index
N = the total number of species to-i
Pi = The proportion of species to-i
Kriteria levels of biodiversity are:

relatively high when H> 3.5, while if H '= 1.5 to 3.5 and lower when H' <1.5

III. DISCUSSION

Plant diversity in cacao agroforestry system

Agroforestry is important for the habitat of various species of plants, animals and a variety of beneficial microorganisms, so as to conserve biodiversity in an ecosystem. Agroforestry can increase the biodiversity, in the absence of agroforestry may have a lot of species that are extinct. A landscape dominated by intensive farming still requires the presence of many natural species, especially those related to biodiversity in the soil (Hairiah et al., 2002). In addition, agroforestry can provide a useful contribution to the economy is agricultural land, as agroforestry can be a useful place to stay, for example pollinators, predators of agricultural pests.

In this study, the diversity of plants is counted in 3 pengelolalao system cocoa plantations, namely cocoa farm run complex agroforestry systems, cacao agroforestry systems managed by simple and cocoa plantations managed by non-agroforestry system. The diversity of crops is calculated with an inventory of all kinds of useful plants that exist in the cocoa farm then also calculated the index of diversity and species richness index, in order to know the level of diversity of each cocoa farm management systems.

Table 1. Diversity of species of plants in various cocoa farm management system

a. Plant diversity in general

No.	Local name	Latin name	Plot Type		
			AF.Komplek	AF.Sederhana	Non.AF
1	areca nut	*Areca cathecu L.*	34	10	8
2	Rubber	*haveabrasiliensis*	25	11	0
3	tuba root	*Derris elliptica.*	4	0	8
4	Chili	*Frustences Capsicum L.*	48	108	67
5	petai	*Parkiaspeciosa*	2	2	0
6	betel Forest	*Piper aduncum L.*	1	2	2
8	Papaya	*Carica papaya L.*	14	11	21
9	Langsat	*Lansiumdomesticum*	8	3	0
10	Durian	*Duriozibethinus*	17	9	3
11	Lime	*Citrus aurantifolia*	4	0	0
12	Avocado	*Perseaamericana*	3	6	0
13	Ginger	*Zingiberofficinale*	5	3	2
14	Banana	*Musa paradisiaca*	55	76	26
15	JambuBol	*Syzygiummalaccense L*	3	0	0
16	acid Sundai	*Citrus hystrix*	1	0	0
17	Turmeric	*Curcuma longa L.*	12	0	0
18	Mango	*Mangiferaindica L.*	1	1	0
19	Gliricidia	*Gliricidiasepium*	10	12	4
20	Sijungkat	*Lactuca sativa*	6	58	5
21	Rice-rice	*Sauropusandrogynus*	17	21	4
22	Ranti	*Solanumamericanum*	1	1	6
23	jengkol	*Archidendronpauciflorum*	2	1	0
24	Coffee	*Coffearobusta*	5	2	6
25	Sweet shoots	*Manihot esculenta*	53	69	4
26	Rimbang	*Solanumtorvum*	4	9	2

27	galangal	*Alpiniagalanga L.*	6	3	7
28	Cinnamon	*Cinnamomumburmannii*	6	0	0
29	Dab-dab	*Erythrina Sp*	1	0	0
30	Moringa	*Moringa oleifera*	0	1	1
31	Taro	*Colocasia esculenta L.*	0	6	0
32	Pepper	*Piper ningrum L.*	0	1	0
33	rambutan	*Nepheliumlappaceum L.*	0	1	0
34	Serai	*Cymbopogon citratus*	0	10	0
35	Lime	*Citrus hystrix*	0	1	2
36	Pineapple	*Ananascomosus L.*	0	0	0
37	petai china	*Leucaena leucocephala*	0	0	1
38	sugar palm	*Arengapinnata*	0	0	2
		Total	**17**	**32**	**15**

b. Shade tree Diversity

No.	Latin name	species	Σ Individuals	Σ Individuals	ΣIndividu
1	*Areca cathecu L.*	areca nut	34	10	8
2	*haveabrasiliensis*	Rubber	25	11	0
3	*Parkia speciose*	petai	2	2	0
4	*Carica papaya L.*	Papaya	14	11	21
5	*Lansiumdomesticum*	Langsat	8	3	0
6	*Duriozibethinus*	Durian	17	9	3
7	*Persea Americana*	Avocado	3	6	0
8	*Musa paradisiaca*	Banana	55	76	26
9	*Syzygiummalaccense L*	JambuBol	3	0	0
10	*Mangiferaindica L.*	Mango	1	1	0
11	*Gliricidiasepium*	Gliricidiya	10	12	4
12	*Archidendronpauciflorum*	jengkol	2	1	0
13	*Coffearobusta*	Coffee	5	2	6
14	*Cinnamomumburmannii*	Cinnamon	6	0	0
15	*Erythrina Sp*	Dab-dab	1	0	0

No.	Latin name	species	Σ Individuals	Σ Individuals	ΣIndividu
17	*Leucaena leucocephala*	petai china	0	0	1
16	*Nepheliumlappaceum L.*	rambutan	0	1	0
18	*Arengapinnata*	sugar palm	0	0	2
	Total		**186**	**145**	**71**

Based on Table 1 above it can be seen that the diversity of plants ditemuakan in the system agroforestry cocoa in Nagari Sontang, Padang District gelugur include 342 individual plants of 28 species found in the system agroforestry complex, followed by 436 total individual plants of 27 species found in simple agroforestry system and the latter with 189 total individual plants of 20 species of plants are found non agroforestry systems of cacao (Figure 1).

From the above it can be concluded that the total of individual plants is highest in the system agroforestry simple (436 individual plants), compared to a system of agroforestry complex (342 individual plants), this was due to the system of agroforestry simple overgrown with various kinds of crops and wild plants under the cocoa plant vegetation or cover crops. The high degree of shade in complex agroforestry system can reduce the diversity of undergrowth. While the total of the lowest individual plants contained in the non-agroforestry system as many as 189 people because it is dominated by a few shade trees and seasonal plants under the cocoa plant vegetation.

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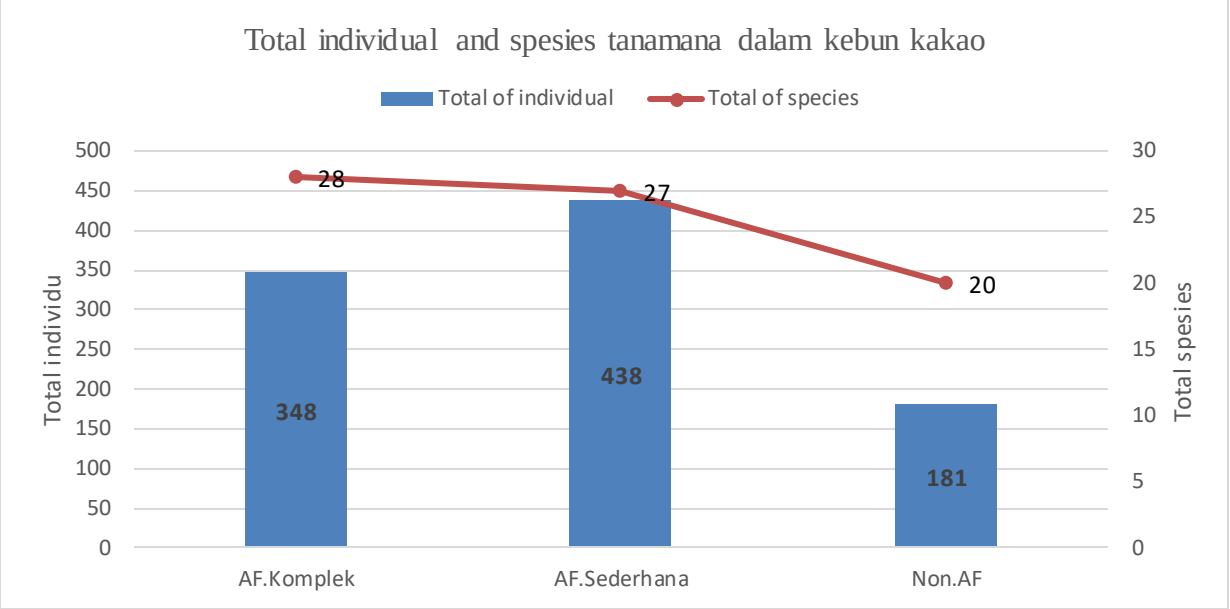

Fig.1: Diagram of plant species diversity in cacao

The index of plant diversity depends the number of individuals of the species that are present in a system. In Figure 2 below tetera that the highest diversity index contained in the simple agroforestry system that is equal to 2.02, followed by a complex agroforestry system with diversity index value of 1.93. Non agroforestry garden systems have the lowest diversity index is 1.56. Mangurran (1998) explains that the Diversity Index (H ') is highly correlated with species richness (number of individuals of each species) at a specific location, but is also influenced by the distribution of species abundance. The higher the index value H ', the higher the plant biodiversity, ecosystem productivity, pressures on ecosystems and ecosystem stability.

The low index of diversity of systems in non-farm management of agroforestry is because the system is only dominated the sidelines a few plants that are generally only as limiting as areca gardens (*Areca cathecu L.)*,and some herbaceous habitus plants like papaya (Carica papaya L), banana (Musa paradisiaca) and some herbaceous plants such as moringa habitus (Moringa oleifera) and Gliricidia (Gliricidia sepium).Setiarno (1998) states that an area dominated by certain types then the area has a low species diversity and in the community the interaction between species is low.

Wealth index type depending on the number of species found in an area / ecosystem. In figure 2 shows that the highest number of plant species is dominated by a complex system of agroforestry as many as 28 species, followed by a simple agroforestry systems and last as many as 27 species of 20 species on non-agroforestry system. The higher the number of species in an ecosystem, the more tinggu also index the species richness of plants, it can be seen in Figure 4 below where the wealth index is highest plant species in complex agroforestry system amounted to 4.24 and in the simple agroforestry system amounted to 4, 06, while the non-agroforestry system 3,23.

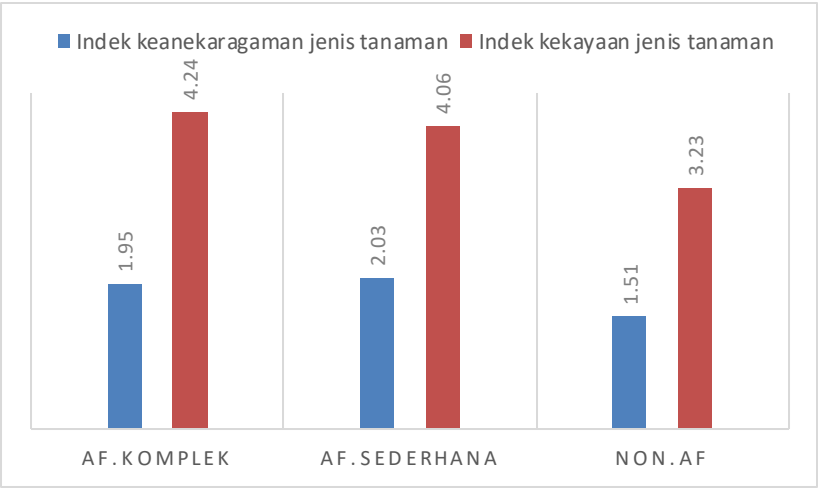

Fig.2: The index of diversity and richness of plant species in the cocoa farm

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Richness is the number of types (species) in a community. The more the number of species is found, the index is also getting bigger fortune. Margalef richness index, divide the number of species by natural logarithm function which indicates that the increase of the number of species varies inversely with the number of individuals. It also shows that it is usually in a community / ecosystem has many species will have the least amount of individuals in each species.

IV. CONCLUSION

Based on experiments that have been conducted found some conclusions that

1. The highest plant diversity index of 2.03 as found in complex agroforestry systems, while the highest species richness index of 4.24 found in simple agroforestry systems
2. Farm management system with diversity cocoa agroforestry cover crops can provide environmental services (ecosystem services) to the development of the cocoa plant.

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Effect of Nitrobenzene on Sweet Cucumber *(Cucumis sativus* L.*)* Yield and Yield Quality under Green House Condition

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***Abstract* —** *Sweet cucumber (Cucumis sativus L.) is one of the most important vegetable crops grown extensively throughout the world especially in the temperate countries. Poor fruit-set was believed to be one of the major barriers to the tropical adaptation of sweet cucumber. Nitrobenzene is a combination of nitrogen and plant growth regulators, extracted from sea weeds that act as plant energizer, flowering stimulant and yield booster in crop production. The objectives of the present study were to examine the effect of nitrobenzene on sweet cucumber yield to evaluate the optimum dose of nitrobenzene for economically better yield. The study was conducted at a farmer poly tunnel located in Athgala (WU1). The experiment was laid out in a Completely Randomize Design (CRD) with four treatments randomized in three replicates. The treatments were T_1 – Control (without Nitrobenzene), T_2 – Nitrobenzene 10%, T_3 – Nitrobenzene 15%, T_4 – Nitrobenzene 20%. Plants were established in drip-fertigated bags in the Poly tunnel and standard crop management practices were done throughout the study. Nitrobenzene was sprayed to the seedlings 20 and 35 days after sowing. Albert solution, 6: 30: 30 fertilizer mixture 20: 20 fertilizer mixture and $Ca(NO_3)_2$ were used as recommended fertilizers. Measurements were taken on growth, flowering, Fruit setting and postharvest stages. The data obtained were subjected to the Analysis of Variance (ANOVA) procedure of Statistical Analysis System (SAS) 9.1. Duncan's New Multiple Range Test (DNMRT) was performed to compare the differences among treatment means at p=0.05. The highest values of plant growth parameters, reproductive parameters, yield parameters and postharvest parameters were observed in T4, i.e. 20% Nitrobenzene applied treatments. On the other hand the lowest values were recorded from T1 (control of the experiment). Specially, advanced flowering and fruit setting, number of flowers per plant and total yield per plant were recorded from T4, i.e. 20% Nitrobenzene applied treatments. So, 20% nitrobenzene applied plants showed superior results in contrast to other nitrobenzene levels with enhancing flowering, fruit setting, yield qualities as well as postharvest performances.*

***Keywords*— Sweet cucumber, Nitrobenzene concentrations, Flowering, Fruit setting, Poly tunnel.**

I. INTRODUCTION

Cucumber *(Cucumis sativus* L.) is an important vegetable and one of the most popular members of the family Cucurbitaceae [1]. It is a sub-tropical vegetable crop that grows successfully under conditions of high light, high humidity, high soil moisture, temperature and fertilizers in green-houses[2]. However, greenhouse cucumber farmers often encounter problems regarding the agronomy of the crop due to existing gaps in the local knowledge base. Cucumber demands high temperatures and soil moisture for satisfactory yield. An unfavorable climatic conditions cause problems, such as the reduction of female flowers [3], delay in fruit growth [4] and mineral disorders [5] reduced the quality and quantity of the yield. Premature fruit yellowing or light-coloured fruit is associated with low nitrogen (low EC), high temperatures, over-maturity, low light levels and high humidity (low vapour pressure deficit). Increasing the amount of light reaching the fruit, reducing the number of fruit per plant and increasing the concentration of fertilizer in the nutrient solution, may help to reduce the incidence of fruit yellowing [8]. Nitrobenzene is a combination of nitrogen and plant growth regulators, extracted from sea weeds that act as plant energizer, flowering stimulant and yield booster [7]. Nitrobenzene produces best results in combination with plant growth regulators, which have capacity to increase flowering in plant and also prevent flower shedding. It is specially recommended for vegetable crops and flowering plants [8]. Nitrobenzene 20% w/w is a new generation plant energizer and yield booster of low cost

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PGRs compared to others. Nitrobenzene is quickly absorbed into the plants. It influences the bio chemical pathway of the plants to uptake more nutrients from the soil. It also increases the nutrient use efficiency thus improves the vegetative growth. Induces profuse flowering and helps in the retention of the flowers and fruits [9]. On the other hand, Nitrobenzene improves the organoleptic factors and keeping quality of the produce, which increases the harvestable yield of any crops [10]. As a further improvement step for greenhouse fruit set of sweet cucumber, Nitrobenzene can be adopted. Four sprays of nitrobenzene during 40, 55, 80 and 105 days after sowing (DAS) improve the yield up to 40% (Jeyakumar, 2005). Unfortunately, very limited researches have been carried out regarding the use of nitrobenzene on sweet cucumber varieties in Sri Lanka. Therefore, this research was designed to study the effect of nitrobenzene on sweet cucumber yield and the quality. Furthermore it was expected to assess the most effective nitrobenzene concentration to reduce cost of production in order to improve the profit.

II. MATERIALS AND METHODS

1.1 Experimental design and treatments

The experiment was laid out in a Completely Randomize Design (CRD) with four treatments randomized in three replicates. Here we used commercially available "Bloom Flower- 20% Nitrobenzene] solution. The treatments were four different concentrations of Nitrobenzene (%) applied to the seedlings to cover whole aerial parts of the plant as an aqueous spray by using a hand sprayer as given below.

Treatment	Nitrobenzene levels (%)
T1	Control (without Nitrobenzene)
T2	Nitrobenzene 10%
T3	Nitrobenzene 15%
T4	Nitrobenzene 20%

Planting materials and handling

The study was conducted at a farmer Poly tunnel located in Athgala (WU1- Wet Zone area in Up country), Sri Lanka. Plants were established in drip-fertigated bags in the Poly tunnel and standard crop management practices were done throughout the study. Nitrobenzene was sprayed at two weeks intervals after transplanting of seedlings in pots. Albert solution, 6: 30: 30 fertilizer mixture and Ca $(NO_3)_2$ were used as recommended fertilizers.

1.3 Measurements

Data were collected at one week intervals after first spraying. Measurements were taken on growth, yield and yield determining parameters with postharvest quality. Sweet cucumbers were harvested at an immature stage when they are in full size and green. The total yield per plant was measured directly in the field by using a digital balance with four digits. Ten fruits were selected randomly from each treatment and kept in normal environmental conditions and at the same time remaining five fruit samples were kept in refrigerator to determine the shelf life (days). At the same time weight loss was recorded at five days intervals by using a digital balance.

1.4 Statistical analysis

The data obtained were tabulated and analyzed subjected to the Analysis of Variance (ANOVA) procedure of Statistical Analysis System (SAS)9.1. Duncan's New Multiple Range Test (DNMRT) was performed to compare the differences among treatment means at p=0.05.

III. RESULTS AND DISCUSSION

3.1 Evaluation of plant growth parameters

Among different treatments tested the plant height, plant girth and leaf area index did not show any significant differences ($p>0.05$) between T2, T3 and T4, i.e. 10%, 15% and 20% Nitrobenzene applied treatments. However, control treatment showed the lowest values. On the other hand, the highest number of leaves per plant and number of flowers per plant was observed from the treatment T4, i.e. 20% Nitrobenzene applied treatment whilst the lowest number of flowers was observed from the control (Table 1). Nitrobenzene is a combination of plant growth regulators. So, the highest concentration of Auxin and Gibberellins is present in 20% of Nitrobenzene which influences the elongation of cells so that the plant height is increased dramatically. According to the study findings of Nickell [12] and Richard [13] that cell growth and elongation is influenced by Auxin and Gibberellins. Cytokinin and Auxin with higher ratios of Nitrobenzene influence the lateral growth of parenchyma cells in stem so that the plant girth was increased with higher concentrations of Nitrobenzene. The best concentration of Nitrobenzene is present in 20% Nitrobenzene which influence to increase flowering in plant, prevent flower shedding, enhance early flowering and plants take less time to flower so that the number of flowers is increased for the highest concentration of Nitrobenzene. The application of Nitrobenzene and their simultaneous transport to the auxiliary buds would have resulted in a better sink for the mobilization of photo-assimilates at a faster rate. This would have helped in the early transformation from the vegetative phase to reproductive

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phase. The induction of early flower bud initiation might be influenced by triggering of such metabolic processes and narrowing of the carbon: nitrogen ratio by the significant accumulation of carbohydrates. The result on earliness in flowering in this experiment goes with the study findings of Singh and Mukherjee [14].

3.2 Evaluation of yield parameters

Among different treatment tested number of fruits per plant and total yield per plant show significant differences ($p>0.05$) between T1 and T4 i.e. control and 20% Nitrobenzene. This results showed, spraying growth regulator Nitrobenzene confirm maximum yield. Nitrobenzene is quickly absorbed into the plants, which has capacity to increase flowering in plants and maximum number of fruits per plant [9]. The highest yield per plant was recorded for 20% of nitrobenzene applied treatment and lowest yield per plant was recorded in control treatment. Nitrobenzene increase the fruit weight as well as bigger fruits is produced thus the total yield increase due to its application compare to control [15]. Nitrobenzene can be used as spray or in granular form, which increases flower forming substances by altering auxin, cytokinin, gibbrellic acid and Ethylene ratio favourably tilting to a higher level of flower forming substances, thereby increasing flowers by more than 40 to 45% and yield [16].

3.2 Evaluation of postharvest parameters

The highest shelf life in room temperature and refrigerator was recorded from T4, i.e. 20% Nitrobenzene applied treatment and lowest fruit weight was recorded from T1, i.e. control treatment. According to these results, nitrobenzene can extended the shelf life in room temperature conditions. The highest weight loss in room temperature was recorded from T1, i.e. control treatment and it has significantly difference among other treatments tested. On the other hand, there was no significant difference ($p>0.05$) among treatments T2, T3, T4, i.e. 10%, 15% and 20% Nitrobenzene. But, according to the mean values of weight loss in room temperature conditions showed 20% nitrobenzene decreased the weight loss of sweet cucumber and extended the postharvest qualities. There was no significant difference ($p>0.05$) among treatments on weight loss in refrigerator. But, average results show T4, i.e. 20% nitrobenzene applied treatment was decreased the weight loss in refrigerator and extended the postharvest qualities.

Table.1: Evaluation of plant growth parameters of sweet cucumber

Treatments	Plant height (cm)	Plant girth (cm)	Leaf Area Index	Number of leaves per plant
T1	157.55^{a}	3.60^{b}	0.67^{a}	23.52^{c}
T2	160.00^{a}	3.73^{ab}	0.68^{a}	24.22^{bc}
T3	164.53^{a}	3.85^{a}	0.69^{a}	24.92^{b}
T4	171.58^{a}	3.86^{a}	0.71^{a}	26.55^{a}

Note**:** Means followed by the same letter/s along the column are not significantly different at P=0.05

Table.2: Evaluation of plant reproductive parameters of sweet cucumber

Treatments	Number of days for flowering	Number of flowers per plant	Number of days for fruit setting	Number of fruits per plant
T1	28.33^{c}	5.88^{c}	33.33^{b}	23.33^{b}
T2	28.00^{ab}	6.97^{bc}	32.33^{a}	25.00^{ab}
T3	27.33^{b}	7.75^{b}	32.33^{a}	26.00^{ab}
T4	24.33^{a}	10.08^{a}	30.33^{a}	26.67^{a}

Note**:** Means followed by the same letter/s along the column are not significantly different at P=0.05

Table.3: Evaluation of yield parameters of sweet cucumber

Treatments	Total yield per plant (kg)	Fruit weight (g)	Fruit length (cm)	Fruit girth (cm)	Pericarp thickness (cm)
T1	4.66^{c}	207.0^{a}	17.6^{a}	13.68^{b}	9.4^{a}
T2	5.12^{bc}	210.4^{a}	17.7^{a}	13.84^{b}	9.5^{a}
T3	5.51^{ab}	212.0^{a}	17.8^{a}	14.10^{b}	9.8^{a}
T4	5.81^{a}	213.6^{a}	18.1^{a}	14.52^{a}	9.8^{a}

Note**:** Means followed by the same letter/s along the column are not significantly different at P=0.05

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Table.4: Evaluation of postharvest parameters of sweet cucumber

Treatments	Refrigerator shelf life (days)	Room temperature shelf life (days)	Refrigerator weight loss (g)	Room temperature weight loss (g)
T1	18.2^{c}	7.0^{b}	20.2^{a}	72.0^{b}
T2	20.0^{b}	7.6^{b}	18.2^{a}	68.2^{a}
T3	20.6^{ab}	7.8^{b}	17.2^{a}	67.6^{a}
T4	21.4^{a}	8.6^{a}	17.2^{a}	66.2^{a}

Note**:** Means followed by the same letter/s along the column are not significantly different at P=0.05

IV. CONCLUSION

Results enumerated that the application of nitrobenzene had a significant ($p<0.05$) effect on growth, reproductive, yield and quality parameters of sweet cucumber. Nitrobenzene acts as a plant energizer, flowering stimulant and yield booster. Due to the more number of flowers it increases the yields by better quality of fruits. So, as a further improvement step for greenhouse fruit set of sweet cucumber, Nitrobenzene can be adopted. In the light of this situation application of 20% Nitrobenzene can be considered as an economically feasible treatment to get better yield from sweet cucumber under greenhouse condition. A long shelf life was observed in 20% Nitrobenzene applied treatment. Furthermore high Nitrobenzene levels showed a significant positive impact on postharvest quality of sweet cucumber with extending the shelf life.

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Potential, Characteristics and Utilization of Shrimp Pond Solid Waste as Organic Fertilizer

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Abstract**—** *The study aimed at determining the potential, characterization and utilization of super-intensive shrimp pond solid waste as a raw material for organic fertilizer, and its application in fisheries and agriculture. It was conducted at Experimental Pond Installation, Research Institute for Coastal Aquaculture and Fisheries Extension located in Punaga Village in South Sulawesi. The research involved 3 of 1,000 m^2 concrete ponds for 105 rearing period days and entailed the stocking density of 750-1,250 shrimp/m^2. The observation was carried out in the sediments on a wastewater treatment plant (WWTP) with 7,000 m^2. The variables observed in this study included sedimentation rate, total sediment, and estimation of total nutrient in the contents of solid waste such as C-organic N total. P_2O_5. K_2O. pH, water content and C/N ratio. The results showed total sediment in the following stocking 750, 1000 and 1,250 vaname shrimp/m^2 weighed 18.2, 20.3 and 21.9 tons respectively. During the shrimp cultivation, TN, TP and C accumulated in sediments increased, resulting to an upsurge in stocking density. Therefore, the solid waste may potentially be used as organic fertilizer because it has a fairly high nutrient content such as N total of 0.58%. P_2O_5 by 3.33%. K_2O by 0.8%. C-organic by 9.94%. pH 6.73, water content of 16.36% and C / N ratio by 17.14%.*

Keywords**— solid waste, organic fertilizer, super intensive, pond.**

I. INTRODUCTION

The intensive and super-intensive cultivation of shrimp decreases the quality of coastal waters in several countries, including Thailand (Hazarika et al. 2000, Lorenzen et al. 1997), Vietnam (Bui et al. 2012) and Mexico (Barraza-Guardado et al. 2013). Furthemore, it produces large amounts of waste. The waste is often in form of stool, leftover feed and dead organisms accumulating discharged directly into the water without treatment. One problem frequently faced by shrimp farmers is the low sources of nutrients. Furthermore, the waste leads to the eutrophication, oxygen depletion, and precipitation (Saputra et al. 2017). The aquaculture waste produced was also pollute the aquatic environment and should be addressed immediately. It can be in form of digestive remnants at the bottom of the pond, and this may cause problems such as high amount of nitrogen and phosphorus discharged into the water (Lacerda et al., 2006). These elements may be consumed and retained in fish meat at about 25%-30%, and the remaining amount released in the aquatic environment (Avnimelech, 2000).

The high stocking density used in the super-intensive shrimp culture system was expected to be followed by an increase in production, proportional to the aquaculture waste produced. According to Syah et al. (2017), the main problem in the super-intensive pond wastewater is the large number of particles of organic matter. It consists of shrimps' feces, non-inedible feed, shrimp palm oil, and dead plankton which settles at the bottom of the pond, along with high N and P content which potentially increase the fertility of waters. The wastewater with stocking density of 750-1,250 shrimps/ m^2 contains a total suspended solid (TSS) average of 798-924 mg/L, the dissolved organic matter by 81,227-88,641 mg/L, the total nitrogen (TN) of 9.8389-14.4260 mg/L, and total phosphate (TP) by 7.8770-11.8720 mg/L (Fahrur et al. 2015). These values have exceeded the permitted standards and therefore has the potential to negatively impact the quality of the water bodies. According to Preston et al. (2001) the sludge formed during the cultivation process may reach 35-60 t/ha/shrimp production cycle. Furthermore, Boyd (1992) also reported the organic materials accumulating in form of sediments increase with age. In the end, the thickness of the organic material obtained was 6.4 - 8.5 cm. According to Avnimelech and Rivto (2003), the pond sediments are rich in nutrients and organic matter. The solid waste of shrimp ponds contain 1.92% C organic materials, 0.54% N total and 1.70% P (Tungguda et al. 2015). The high content of nitrogen and phosphorus makes the sediments effective materials for organic fertilizers.

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The production of organic fertilizers from solid waste and their subsequent use for crop production as well as land rehabilitation are highly recommended. They help in reducing volumes and environmental degradation, apart from increasing the agricultural land productivity (dela Cruz et al. 2006). Further, it is environmental friendly since it is an organic source (Guardian, 2004; Fadare et al. 2009; Adeoye et al. 2005). The, organic fertilizers also improve soil texture, water retention and erosion resistance. Besides, they provide nitrogen in a usable form, which help to increase the plants growth, and cannot cause the death of the beneficial microorganisms in the soil (Sharma and Chetani, 2017). Moreover, the production of organic fertilizers provides the agronomic effectiveness, it is affordable to farmers, environmental friendly, and increases food production and security (Babalolaa et al. 2012).

The research on composting various types of organic waste shows different performances depending on the processes used. Composting is a treatment which significantly reduce the volume of the existing leftover. Besides, it can provide suitable nutrients for agriculture and fisheries as well as being used as substitutes for chemical fertilizers. Furthermore, it may be used as a land amendment, environmentally friendly, hygienic, economical and free of toxic materials (Kadir et al. 2016). For the reason, this study aimed at evaluating the potential, characteristics and utilization of solid waste of super-intensive shrimp cultures.

II. METHODS AND MATERIAL

The research was conducted at the Experimental Pond Installation, Research Institute for Coastal Aquaculture and Fisheries Extension, located in Punaga Village, Mangarabombang District in South Sulawesi. The observation of sedimentation rate, total sediment count and estimation of total nutrient load made reference to the study by Suwoyo et al. (2015). The reference study was carried out on the 3 plots of 1,000 m^2 of super-intensive ponds, the Post Larva size (PL-10) scattered with the vaname shrimp (*L. vannamei*) and a stocking density by 750-1,250 shrimp/m^2. The measurement of the sedimentation rate and the amount of sediment at the bottom of the pond was carried out by installing the sediment traps made of 4 inch paralon pipes with a length of 40 cm. The deposit collection was carried out once a week and the sedimentation rates was calculated based on the equation by Syah et al., (2004). Estimating the total nutrient load (TN, TP and C) in the sediment referred to the method developed by Ackefors & Enell (1990) in Barg (1992) and Syah et al. (2014).

The observation of solid waste characterization from the cultures was conducted on a wastewater treatment plant (WWTP), ± 7,000 m^2 by collecting the pond left-over sediments. The collection was carried out at the end of the rearing period. The variables observed include the macro-nutrient content such as C-organic. N total. P_2O_5, K_2O, pH water content and C/N ratio. The results of the nutrients' observation were tabulated and analyzed descriptively and compared to the quality requirements of organic fertilizers on the Minister of Agriculture Regulation No.70 / Permentan / SR.140 / 10/2011. To obtain more information on the potential utilization of shrimp pond solid waste, the results of several previous studies were analyzed descriptively.

III. RESULTS AND DISCUSSION

Potential of super-intensive shrimp ponds solid waste

Sedimentation in ponds occur due to the deposition process of organic particles, both stemming from the remaining feed, shrimp stool, plankton or other dead organisms, and mud particles carried to the sea. According to Hopkins et al. (1994), the sources of sediment accumulation in shrimp ponds include non-inedible feed, feces, and dead and decaying plankton/diatom. Furthermore, the erosion of pond soil and microorganisms is part of the sediment. According to Syah et al. (2006), based on the estimated sedimentation rate from the installation of sediment traps, the amount of residues during rearing period of vaname shrimp may be determined.

The total sediment analysis in solid stocking of 750, 1000 and 1.250 shrimps/m^2 were 18.2, 20.3 and 21.9 tons for 105 days rearing period (Table 1). The solid waste may potentially be used as organic fertilizer. From the results of Lacerda et al. (2006), shrimp farming in Australia estimated the number of N and P produced to 290 and 16 kg / ha / year. Other estimates include: California with N and P produced being 112 and 32 kg / ha, and Indonesia where N and P produced from the intensive and traditional ponds reached 399 and 37 kg / ha / year (Nur, 2011). According to Syah et al. (2014), at the farm productivity level of 6,376 kg / 1000 m^2, the TN, TP, and C organic waste loads amount to 50.12 gTN / kg of shrimp respectively; 15.73 gTP / kg shrimp and 126.85 g C organic / kg shrimp. Whereas in the pond productivity of 8,407kg / 1000 m^2, TN, TP and C organic waste loads are 43.09 gTN / kg shrimp respectively; 14.21 gTP / kg shrimp; and 112.85 gC organic / kg shrimp.

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Tabel.1: Nutrient load in vaname superintensive shrimp pond sediments

Variable	Stocking density (shrimp/m^2)		
	750	1.000	1.250
Shrimp production (tons)	7.86	10.70	12.16
Sedimentation rate (g/cm^2/day)	1.62	1.81	1.96
Total sediment (tons) dry weight	18.2	20.3	21.9
TN sediment (kg)	303	325	362
TP sediment (kg)	263	253	299
C organic sediment (kg)	2.082	2.042	2.334

During cultivation, TN, TP and C organic accumulated in each sediment of 303 kgTN; 263 kgTP and 2,082 kgC for solid stocking 750 shrimp / m^2 with the density of 1000 shrimp / m_2 each 325 kgTN; 253 kg and 2,042 kgC organic. The stocking densities was higher, 1,250 shrimp / m^2 each 362 kgTN; 299 kgTP and 2,334 kgC organic (Table 1). From the table, it is evident the increase in nutrients in the sediment is in line with the rise in the density of the stocked shrimp, the amount of feed given, and the total quantity of sediment produced. This is also in agreement with Lemonnier & Brizard (2001) who reported a correlation between the level of sediment accumulation and the shrimp density at 13 pond plots in New Caledonia. According to Suwoyo et al. (2009), there is a positive correlation between the amount of sediment and the final density of vaname shrimp and the total amount of feed. Shrimp density and the amount of feed contributed 58.3% in determining the amount of sediment in the intensive shrimp vaname ponds. The remaining 41.7% was attributed to other factors.

TN, TP and C are the wastes found in form of organic particles. Most of them are part of the load of waste originating from feed and organic matter formed during the cultivation process. According to Chien et al. (1989) sediment plays an important role in the balance of aquaculture systems, functioning as a buffer in the concentration of water nutrients. The sedimentation process plays an important role in the mechanism of P loss in ponds because mud is known to have a strong affinity for phosphorus (Shrestha and Lin. 1996). The high organic matter content, nitrogen and phosphorus in the sediment making are potential organic materials for fertilizer, where previously it was necessary to reduce salinity in the dredge itself.

Muendo et al. (2014) estimated the potential of organic fertilizer from 16 plots of aquaculture ponds (200 m^2) in semi-intensive tilapia to be 173 tons of sediment / ha / cycle. It has nutrients with the potential to meet the needs of nitrogenous fertilizers for 0.35-1.2 hectares and potassium fertilizers for 0.7-1.5 hectares. Besides, the accumulation of sediments containing 1.8-5 tons of organic matter with a high potential to improve soil quality. It is rich in nitrogen, potassium and organic ingredients with high potential for nitrogen and potassium fertilizers and serve as a soil conditioner. According to Rahman et al. (2002), the value of sediment fertilizers from one hectare of tilapia production ponds is equivalent to 6.26 tons of urea and 1.96 tons of TSP. Rahman et al. (2004) show the high content of organic sediment ponds may play a major role in obtaining information on soil aggregates. Therefore, it will improve the soil quality, physical and chemical conditions, and facilitate crop production.

From Syah et al. (2006), based on the estimated sedimentation rate obtained from the installation of sediment traps, the amount of deposit during the rearing period of vaname shrimp may be determined. The quantity of sediment obtained in vaname shrimp cultivation with a density of 50 / m^2 and the treatment of 2. 3 and 4 paddle wheel are 2.829 kg; 3,120 kg and 3,154 kg. Suwoyo et al. (2009) obtained a sedimentation rate in vaname shrimp farming ponds with a density of 50 shrimp / m^2 by 6.89 - 142.71 g / m^2 / day with sediment counts ranging from 676.39 - 1262 kg / plot / cycle. Preston et al. (2001) states that the increase in solid waste in the cultivation system must be prevented since it can cause a decrease in dissolved oxygen and increase ammonia levels due to the decomposition of organic matter which is toxic. Therefore, the formed disposal of the sludge needs to be carried out periodically.

Macro and micro nutrients contents of super-intensive ponds solid waste

From the results, both macro and micro nutrients from the solid waste may possibly be used as organic fertilizers. They have high for example: total N 0.58%, P_2O_5 3.33%, K_2O 0.82%, C-organic 9.94%, pH 6.73, water content 16.36%, and C/N ratio 17.14 (Table 2.). They have contents which meet the requirements of organic fertilizer on the Minister of Agriculture Regulation No.70 / Permentan / SR.140 /10/2011, and therefore the solid waste may be used as organic fertilizer.

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Table.2: Nutrient contents in solid waste from superintensive white shrimp *(Litopenaeus vannamei)* ponds

No	Parameters	Value	Quality Standard of Organic Fertilizer on the Minister of Agriculture Regulation No.70/Permentan/SR.140/10/2011
1	N-Total (%)	0.58	Hara Macro
2	P_2O_5 (%)	3.33	Min 4 % (N+ P_2O_5+ K_2O)
3	K_2O (%)	0.82	
4	pH	6.73	4-9
5	C-Organic (%)	9.94	Min 15
6	Water content (%)	16.36	15-25
7	C/N ratio	17.14	15-25
8	Fe (ppm)	7736	Maximum 9000
9	Mn (ppm)	836	Maximum 5000
10	Cu (ppm)	20.60	Maksimum 5000
11	Zn (ppm)	84.00	Maximum 9000
12	Pb (ppm)	43.81	Maximum 50
13	Cd (ppm)	2.11	Maximum 2
14	Co (ppm)	26.98	Maximum 700

The results of the analysis were not different from previous studies. For instance, according to Latt et al. (2002), shrimps' waste have high value of organic matter, total nitrogen, and phosphorus compared to normal soil. The pond waste has high biological and chemical oxygen requirements. The condition shows the high loading of nutrients which require treatment right before they are disposed. The characteristics of sludge depend on a number of factors including: the design and type of pond cultural system, farm management, and inputs used. The assertion is in line with Muendo et al. (2014) that pond solid waste is rich in nitrogen, potassium and organic matter.

The sludge compost contains the organic matter 530 g / kg. N 25.8 g / kg. P 7.3 g / kg and K 4.8 g / kg. pH 7.2, water content 23.86% (Chongrak. 1996; Rahman et al. 2004). Whereas the fish pond sediments contain C 49.81-62.0 g / kg; N 2.80 g / kg; P 0.07-0.011 g / kg and K 0.51 g / kg; pH 7.2-8.2; water content of 47-50%. (Rahman et al. 2002; Avnimelech et al. 1999). From Karabcova et al. (2015), the average macro concentration and micro elements in sludge or solid waste organic fertilizer is P 6.447 mg / kg; K 27,595 mg / kg; Ca 25,948 mg / kg; Mg 10.637 mg / kg; Cd 0.11 mg / kg; Zn 295 mg / kg; Co 2.35 mg / kg; Cu 36.3 mg / kg and C 35% and C / N in the ratio around 8.2-8.5. Meanwhile, compost organic fertilizer contains P 2,535 mg / kg; K 4.120 mg / kg; Ca 21,387 mg / kg; Mg 3.687 mg / kg; Cd 0.24 mg / kg; Zn 195 mg / kg; Co 1.19 mg / kg; Cu 20.4 mg / kg and C 52% and C / N in ratio ranging from 8.1-8.2. The results of the research conducted by Komarayati and Pasaribu (2005) showed the organic fertilizers have nutrient contents as follows: Water 29.5%; pH 6.70; CEC 31.74 meq / gr; C / N 32.00 ratio; C 23.6%; N 0.9%; P 0.4%; K 0.5%; Mg 0.6% and Ca l.9%. Texture was in form of 0.10% sand; 59.6% dust and clay 40.2%. Komarayati (2007) further reported the characteristics of organic fertilizer plus sawdust charcoal as follows: moisture content 32.90-39.40%; pH 6.70-6.90; C/N ratio of 18.70-23.70; Organic C 24.17-28.26%; N 1.19-1.29%; P_2O_5 total 0.53-0.63%; Total CaO 0.27-0.34%; MgO total is 0.26-0.27%; K_2O total 0.63-0.68% and CEC 29.34-32.44 meq / 100 g. Rina et al. (2002) showed the characteristics of the physical properties of IPAL sludge in the paper industry having a clay texture of 64% and a dust texture of only 20%.

The macro and micro nutrient content in solid waste of vaname shrimp ponds according to Tangguda et al. (2015a) ie are: C organic 30,670 g / 100 g; N total 8,842 g / 100 g; P_2O_5 12,700 g / 100 g; K_2O 7.730 g / 100 g; Fe 166.93 mg / 100 g; Cu 25.92 mg / 100 g; Zn 55.37 mg / 100 g; Mn 63.30 mg / 100 g; B 29.41 mg / 100 g; Co 22.43 mg / 100 g; and Mo 53.53 mg / 100 g.

The results of the C / N analysis of the ratio of pond waste obtained were 26. According to Rosen et al. (1993), the C / N ratio of around 15-20 is ideal for compost ready for use. Mature compost has a C / N ratio of less than or equal to 25 (Oreopoulou and Russ 2007). Nagasaki et al. (1992) proposed a C / N ratio for composting should be in the range of 16 to 21. The macro nutrients, C organic, and moisture content of some organic fertilizers compared to super-intensive pond organic fertilizer are presented in Table 3. It is evident the nutrient content of super-intensive organic fertilizer waste is not much different from other organic fertilizer.

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Tabel.3: Comparison of macro nutrient content, C-Organic and the water content of several examples of organic fertilizers with solid waste of superintensive shrimp farm

No	Type of fertilizer	N-total (%)	P_2O_5 (%)	K_2O (%)	C-organic (%)	C/N ratio	Water content (%)	References
1	Chicken manure	1.17	2.08	8.58	7.16	6.1	13.01	Suriadikarta and Setyorini. (2005)
2	Fine compost	0.68	0.77	0.06	5.04	7.4	46.43	Suriadikarta and Setyorini. (2005)
3	Bokasi	0.73	6.13	3.25	9.39	12.9	43.86	Suriadikarta and Setyorini. (2005)
4	Compost	0.37	0.09	8.95	8.95	14	62.86	Suriadikarta and Setyorini. (2005)
5	Organic fertilizer from Pulp-Mill Sludge	1.19	0.53	0.63	24.17	18.70	32.90	Komarayati. (2007)
6	Compost of succulent plant	1.22	0.32	1.70	12.2	10	12.3	Janakiram and Sridevi (2010)
7	Aquaculture sludge	0.49	0.44	1.53	21.9	25.67	-	Birch et al.. (2010)
8	Compost oil palm empty fruit bunches	2.7	-	0.03	38.5	13.8	49.3	Wan Razali *et al*.. (2012)
9	Organic Household Waste	1.9	0.95	1.2	11.7	14	2.78	Nino et al.. (2012)
10	Compost of pineapple leaves	2.3	0.46	2.67	45.8	19.8	53.3	Ch'ng et al.. (2013)
11	Municipal solid waste compost	0.85	2.52	-	15.95	18.99	23.83	Rawat et al.. (2013)
12	Solid waste compost	1.75	2.4	0.57	25.5	14.67	44	Manohara. and Belagali. (2014)
13	Food wastes compost	3.56	1.12	2.03	40	11.23	-	Okareh et al.. (2014)
14	Compost of plant residues	0.65	0.003	0.36	-	-	-	Taleb et al.. (2014)
15	Cattle manure	0.95	0.31	0.27	16.6	17.47	58.3	Khater. (2015)
16	Herbal plant resdiues	1.13	0.32	0.51	20.93	18.52	16.20	Khater. (2015)
17	Sugar cane plant residues	1.68	1.13	2.11	23.89	14.22	36.20	Khater. (2015)
18	Treated POME sludge	4.21	0.08	0.03	25.53	6.35	68.46	Khairuddin *et al*. (2016)
19	Solid waste from White Shrimp	0.54	1.70	-	1.92	3.55	-	Tangguda et al. 2015
20	Solid waste from Freshwater prawn	0.14	5	-	1.38	9.9	-	Wudtisin & Boyd (2006)
21	The compost leachate	1.72	2.60	1.75	32	18.60	-	dela Cruz et al (2006)
22	Shrimp pond solid waste	0.46	6.25	0.25	6.36	13.82	-	Rachman et al. 2015
23	Solid waste using vermicomposting	0.99	0.24	0.45	16.3	16.46	-	Londhe and Bhosale (2015)
24	Compost	0.60	1.43	0.58	7.90	13.93	-	Jigme et al..

								(2015)
25	Spent Mushroom Compost (SMC)	0.98	0.80	0.28	14.7	15	-	Kwagyan and Odamtten (2018)
26	Poultry feather waste (PFW)	4.0	0.50	0.40	20.82	5.21	-	Joardar and Rahman (2018)
27	Solid waste from Superintensive Shrimp pond	**0.58**	**3.33**	**0.8**	**9.94**	**17.14**	**16.36**	**This Research**

Potential use of waste as organic fertilizer

The use of solid waste as organic fertilizer has been analyzed in several studies. This is because the sediment accumulating in the ponds are rich in nitrogen, potassium and organic ingredients. (Rahman and Yakupitiyage. 2006; Muendo et al. 2014)

In fisheries, pond solid waste can be used for growing mangroves, for natural food growth and clumps, and for seaweed growth fertilizers. According to Latt et al. (2002), the waste has a positive effect on the growth of several mangrove species. For example, mixing soil and pond waste in a ratio of 75% of farm waste and 25% of land may increase the growth of *Rhizophora mucronata*, Rhizophora apiculata and *Bruguiera cylindrica*. From Elfrida (2012), the content of N, P and K may be used as an input for plankton growth, basing his argument on the analysis of the content of organic and inorganic compounds from solid waste floating net cage cultivation in Maninjau Lake. Zahidah. (2012) show the application of solid waste fertilizer from Floating Net Cages at a dose of 10 g / L resulted in the highest population of Daphnia sp. According to Fitri (2012), the solid sediments from Maninjau Lake can be used as fertilizer to increase the production of natural feed (*Chlorella* sp.). The use of Lake Maninjau sediment fermentation as an organic fertilizer at a dose of 5 g / L gives the best growth of *Daphnia* sp compared to other treatments (Fadlil et al 2013). According to Tangguda et al. (2015a), solid waste shrimp ponds contain macro and micro nutrients needed for the growth of *Chlorella* sp, and using them at a dose of 2 g / L showed the highest cell density (2,333 cells / ml), rapid specific growth rate (0.7677) and high chlorophyll content (89.0568 mg / m3) (Tangguda et al. (2015b). From Suwoyo et al. (2016), the use of solid waste as a single organic fertilezer at the dosage of 2,000 kg / ha and its combination with inorganic fertilizer resulted in the production of lab-lab biomass and the survival of milkfish fingerling, which were not very different from commercial organic fertilezer. Joesting et al. (2016) used solid waste from aquaculture in the production of *Spartina alterniflora* and *Juncus roemerianus* seedlings. The results of this study indicate that *J. roemerian* is a suitable plant species which can be used for remedy purpose on solid waste from marine aquaculture activities. Additionally, Saputra et al. (2017) show solid waste shrimp farms may be used as fertilizer for macro algae growth of *Caulerpa lentiilifera* type to support the development of *C. lentillifera* by 6 g / L. In addition, from Kamrunnahar et al. (2019), organic wastes from food provides higher growth of *Moina macrocopa* density than manure from chicken, pig and cattle farms. *Moina macrocopa* may be used as a larval feed for Pagrus major fish, red sea bream and as a substitute for Artemia.

According to Yeasmin (2011), the use of pond sediments to produce high number of leaf strands in maize plant showed better results than normal soil or its combination with pond sediments (ratio 1: 1). The use of pond sediments will reduce the cost of fertilizer and improve farmland conditions. Pond sediments include stable organic matter which is easily biodegradable and have a high potential to provide N, P, K. These are macro and micro elements which may be used well when combined with inorganic fertilizers. Therefore, the sediment should not be removed but analyzed and utilize its nutritional content.

The results of the study showed chili and watermelon planted in growing media with an additional 20% of pond waste (a ratio of 80% of land and 20% of pond waste) grew in a normal way (Figure a). The addition of 10% of pond waste (comparison of 90% of soil and 10% of pond waste) led to a better growth of chili plants (Treatment 100%: 0%). Interestingly, the same observation was made on watermelons which gave a relatively better growth response to the growing media when added a solid waste of about 30% (ratio of 70% soil and 30% pond waste) (Figure b).

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Fig.1: Application of solid waste super-intensive shrimp ponds on chilli (a) and watermelon plants (b)

The use of agricultural and other organic wastes has been analyzed by several studies. For instance, Dela Cruz et al. (2006) suggested the use of organic matter show enormous potential in the production of sandy vegetables such as lettuce, eggplant and tomatoes. According to Abayomi and Adebayo (2014), the use of organomineral fertilizers (organic fertilizers combined with minerals) have a significant effect on the parameters (*Amaranthus* sp) such as height, number of stalks, and higher production in spinach plant compared to the use of NPK fertilizer and compost manure (without fertilizer). The results obtained are the same as those of Akanni et al. (2011), Ayeni (2008), and Ogunlade et al. (2011), which established a combination of organic fertilizers and minerals provided better production performance in tomato plants, corn and eggplant (*Solanum macrocarpon*).

According to Jigme et al (2015), the use of a combination of compost and 200 mL of dung (CMT) provides the best manure which facilitates vegetative growth of broccoli (*Brassica oleracea*). Similarly, Naveed et al. (2018) reported that the use of organic waste from the agricultural industry may support the growth and bioavailability of Zn in corn plants. According to Kwagyan and Odamtten (2018), the use of mushroom waste compost with a ratio of 10% SMC and 90% soil was the best medium for supporting optimum vegetative growth in tomato and pepper plants. It was also (Spent Mushroom Compost. SMC) reported by Polat et al. (2009) for cucumber cultivation (*Cucumis sativus* L), Altindal, and Altindal (2015) for the growth of potatoes (*Solanum tuberosum* L.) and large chili (*Capsicum annuum*) (Roy et al. 2015). It showed the best growth performance in plant height, number of branches, production results and overall development. Besides, SMC is thought to play an important role in mobilizing phosphate from the soil through roots to the leaves.

IV. CONCLUSIONS

From the results, it was concluded that solid waste of super-intensive shrimp culture has a high potential to be used as a raw material for organic fertilizer. It has a high nutrient content, such as total N 0.58%, P_2O_5 3.33%, K_2O 0.8%, C-organic 9.94 %, pH 6.73, water content 16.36%, and C/N ratio 17.14. Therefore, it may be applied as an organic fertilizer in fisheries and agriculture.

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Invitro Regeneration and Mass-Clonal Proliferation of Wheat through embryonic formation

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Abstract**—** *The present study was conducted to determine the regeneration potential and producing mass-clonal production of wheat through embryogenesis. A total of 60 selected wheat seeds were surface sterilized and inoculated on different culture bottles under controlled condition. The seeds were emerged in MS basal media and later supplemented with different concentrations of BAP (Benzyl-amino purine) and IAA (Indole-acetic acid). The Observation on servile rate, regeneration capability, length of leaves, length regenerated seedlings, length and number roots were observed. Resultsfurther expressed that the media MS-MIII showed maximum length of leaves and roots of seedlings followed by cultured on media supplemented with 10µg/L or 20µg/L or 30µg/L each of IAA and BAP. Though, seedlings obtained under the concentration of 40µg/L of IAA and BAP were relatively similar and showed significantly moderate result followed by cultured on the medium supplemented with 30µg/L. furthermore, the rooted plants were then transplanted into the substrate and acclimatized in the laboratory greenhouse where humid cavity available. The acclimatization in the humid cavity showed optimistic effect on the number of survived plants.*

Keywords**— Wheat, Tissue Culture, BAP, IAA, explants, Media.**

I. INTRODUCTION

The regeneration and proliferation of whole plant from selected tissue is required for success application of Biotechnology in crop improvement. Wheat is one of the most important species of food crop. Therefore, it has been extensively investigated with respect toplant regeneration from *In-vitro*culture. Shoot regeneration is of crucial importance in the realization of the potential of cell and tissue culture techniques for plant improvement.

The process by which an embryo is formed and developed from the zygote is known as embryogenesis and when the egg cell is fused with a sperm cell is a prerequisite for this process. Additionally, in some plants such as Citrus and *Mangifera* may be formed by nuclear cells outside the sacs, such embryos are termed as adventives embryos, however no instance of ex-ovulo embryo development is known in the nature but it is growing in an artificial controlled media **[1].**Embryo present in the ovule, when the ovule mature it changes into seed. The foundation of agricultural industry is seed **[2].** The ability to live of seeds is a critical factor for seed quality, which is closely related to germination percentage, resistance to abiotic and biotic stresses and plant performance, which decreases with an increasing storage period **[3]**.

Wheat (*Triticumaestivum*L.) belongs to family gramineae/poaceae and it occupies 6th place in the rank of most cultivated food crops, is growing all over the world and the most important cereal crop of Pakistan. Whilst, the production of wheat crop increased by the adoption of efficient cultural practices of crops such as use of best quality of seeds become necessary for cultivation of crop **[4,5]**. Although, tissue culture practices promising the production of high-quality types of seeds.There are many beneficial and useful wheat verities has been developed through controlled *Invitro* culture for drought tolerance **[6]**, tolerant from salt **[7]**, disease resistance **[8]**, resistant from herbicide **[9]**, *Helminthosporiumsativum* resistance **[10]**, increase in the gliadin and glutenin subunits and protein content in the seed **[11]**. The *invitro* culture of wheat crop depends upon the wheat genotype **[7]**, medium culture **[13]** and growth regulators **[14]**. The seeds of wheat when cultured on MS media supplemented with hormones had low frequency of formation of the callus but have a high regeneration capacity **[7]**. The use

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of seed's mature embryo decreases greenhouse costs, save time and space associated with growing plants to post anthesis for collection of immature embryo **[15]**.

The purpose of this study wastoexamined the optimize concentration of MS medium supplemented with (BAP), (IAA) and to stabilize the method of regeneration of wheat on controlled laboratory condition. Though, new breeding method was described using *in-vitro* technique for growing plants from the seeds, get high yield of wheat crops in a tissue culture media by using the seeds of the wheat crops.

II. MATERIALS AND METHODS

2.1 Explant source

The dried seeds of wheat variety (Mehran) were purchased from the local market surrounding the area of Hyderabad district, Sindh Province, Pakistan. A total of 150 seeds were screened out with similar in size and structurally preserved.

2.2. Soaking of seeds

The samples of wheat seeds were soaked in distilled water and placed in an incubator at 20˚C. The samples were divided into five different treatment based on their soaking time. Out of 150 samples; each of 30 samples were soaked in the glass jars at 1 hr, 6 hrs, 12 hrs, 18 hrs and 24 hrs respectively, at constant temperature and pressure.

2.3 Sterilization of seeds

The sterilization of seeds was done by 5% of the common bleaching solution, the samples were shacked about 10 minutes under the laminar air flow cabinet in order to kill and remove the population of microbes, and dust particles from the seeds and creating aseptic environment for new born plantlets. After that seeds were rinsed 2-3 times with sterilized distilled water for removing the traces of bleaching solution.

2.4 Media composition

The composition of media was different in which MS medium, BAP, and IAA that were used at the different concentrations that are as follows:

- MS basalmedium +10µl/L BAP +40µl/L IAA
- MS basal medium+20µl/L BAP +30µl/L IAA
- MS basal medium+30µl/L BAP +20µl/L IAA
- MS basal medium +40µl/L BAP +10µl/L IAA

2.5 Media preparation

The preparation of media was done by adding MS basal medium 50µl/L, sterilized distilled water at constant concentrations supplemented with (BAP) benzyl-amino purine and (IAA) indole-acetic acid at different concentrations 10µg/L, 20µg/L, 30µg/L and 40µg/L as described in the above lines. We added sterilized cotton for supported the seedling during proliferations. Different concentrations of media showed different results for emerging the rooting and shooting of the wheat crop.

2.6 Culturing of seeds

The sterilized seeds were cultured in culturing bottles under the laminar air flow cabinet. Whereas, the common surgical sterilized cotton was used for supporting the germinated plantlets. About 50µl of MS media and 2ml of distilled water were used for growing the germinated plantlets. After that, cultured bottles were transferred to the incubator at constant temperature 20°C and pressure for 24 hrs. When germination was observed then two phyto-hormones (IAA) indole-acetic acid (Auxin) and (BAP) benzyl amino-purine (cytokinine) that were used in different concentrations (10µl, 20µl, 30µl, 40µl respectively) at different time duration (1hr, 6hrs, 12hrs, 18hrs, 24hrs)

2.7 Maintenance of culture

When the roots and shoots were observed, the plantlets were then shifted to the growth room. The temperature was maintained at ± 20°C in the growth room and also maintained aseptic environment for the new growing plantlets.

2.7 Phenotypic observation

The photographs were captured on daily routine basis. Phenotypic analyses was observed at two days intervals. Though the size and length of leaves were measure by using AlphaImager 2200 (Alpha Innotech).

2.9 Statistics Analysis

The obtained data were represented as the mean ± SD with three biological replicates. Although, the data of different phenotype exploration were the mean ± SD. Statistic 8.1software of analysis was used to identify differences between observations. Data were been categorized as shown * and ** point out in significant differences at $P < 0.05$ and $P< 0.01$ according to Student's *t*-test respectively.

III. RESULTS

3.1 Invitro Regeneration of wheat plantlets through embryonic formation

In the present study we have observed that he regeneration of wheat seedlings were obtained from the seeds in *in-vitro* conditions in the laboratory were disease resistance plants and were grown very well as compared to the invivo regeneration of plants in the field, because here we grown seedlings in aseptic conditions and proper constant temperature. As we know that wheat crop is Rabi season crop but we grew out of season this crop in the laboratory. When we grew these seeds by showing their regeneration capability they showed the outstanding results by adding MS medium for nutrient supplied, sterilized distilled water for imbibitions as well as enzymes activations purposed and finally added plant growth regulators or hormones such as auxins and cytokinines for proper growth and development of roots and shoots of seedlings of wheat crops.

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3.2 Initiation and Proliferation of somatic embryos

The beginning of embryos by the seeds embryogenesis process become auto-regulatory and maintained consecutive stages of the process without any or with minimal contributions from the external signals. The initiation and proliferation of somatic embryos dependedon the developmental stages of the plant material and factors of the growth medium (Figure 1). Though the initiation and proliferation of wheat seeds were observed that at 24hrs interval of soaking of seeds given surprising results as compared to others such as 18hrs, 12hrs and 6hrs intervals that showed medium results but 1hr interval of soaking of seeds showed minimum results of the seeds because soaking of seeds affected on imbibition as well as enzymes activation of the seeds of the wheat crops.

3.3 Proficiency of hormonal concentrations affects the regeneration potential of wheat

The regeneration potential of wheat on MS medium supplemented with different concentrations of IAA (Indole-Acetic Acid) and BAP (benzyl-amino purine) was observed. The results revealed that wheat seeds used as explants were cultured on MS medium without addition of these plant growth regulators showed significantly lower length of the leaves and roots of wheat seedlings compared to the explants grown on medium supplemented with various concentration of IAA and BAP. Results further expressed that the length of roots and leaves of seedlings cultured on media supplemented with 10µg/L or 20µg/L or 30µg/L each of IAA and BAP concentration was relatively similar and were significantly moderate (Figure 2)followed by cultured on the medium supplemented with 40µg/L of IAA and BAP revealed higher length of roots and leaves of the wheat seedlings.

3.4 Numbers and height of leaves highly regulated by cytokinines

It was observed that wheat seeds cultured on the medium supplemented with different concentrations of BAP (cytokinines). Results displayed that the numbers and height of leaves was remarkably higher on 40µg/L of BAP concentration as compared to explants on other concentrations 10µg/L, 20µg/L and 30µg/L. it was further noted that any how the explants cultured on in-vitro cultured medium was not died the negligible growth of the leaves appeared up to the end of the experiment and showed lower rate of growth circumstances (Figure 3)

3.5 Roots of wheat exceptionally pretentious by Auxin-Cytokinin interactions

Roots of wheat seedlings per explants cultured on medium supplemented with different concentrations if IAA was observed (Figure 4-5). Results revealed that the length of the roots was remarkably higher on 40µg/L of IAA concentration as compared to the explants on other concentrations 10µg/L, 20µg/L and 30µg/L. It was further noted that regardless the explants cultured on MS medium was not died, the growth of roots trivial appear up to the end of the experiment.

IV. DISCUSSION

In these results clearly showed that theInvitro regeneration of the wheat crop through seeds is very important because it provides great yield as well as disease free plants. In nature, the somatic embryogenesis is a very common pathway during which embryonic and post-embryonic development of the plant have been formed**[16, 17]**. Invitro regeneration through somatic embryogenesis allows production of multiple genetically identical embryos may not require aridness as well as may skip the quiescence period prior to the germination and avoiding the wait for the following reproductive season. Somatic Embryo development includes key stages of zygotic embryogenesis the globular,scutellar and coleoptilar stages of monocotyledonous species **[18,19]**. The success in invitro culture is also very important by using plant growth hormones and proper optimization of their dosage in the plants of the wheat cultivars **[20, 21]**.

The beginning of somatic embryogenesis process becomes auto-regulatory and can maintain consecutive stages of the embryogenesis process without any or with minimal contributions from the external signals. Instead of how complicated the successive steps may seem the prototype foundation and persistence of this polarity from a homogenous group of cells**[22]**. The initiation of somatic embryogenesis depends on the developmental stages of the starting plant material and factors in the growth medium kenned by the complementary sensors in the cells maintenance of the embryogenic potential during successive cultivation requires the simultaneous activity of signalling and genetic processes **[23,24]**.The present process when we applied on theembryogenesis of wheat seeds at the different concentrations of (IAA), (BAP), MS basal medium and sterilized distilled water that gives good results. These results also demonstrated that invitro regeneration culture of the seeds of wheat crop enabled to get very high plant regeneration rates than other techniques such as callus culture that is the source of genomic alteration, albinism and sterility thus creating unwanted problems in tissue culture applications due to this reason we preferred this method.

V. CONCLUSION

The results concluded that seeds were emerged on MS basal media supplemented with different concentrations of BAP (Benzyl-amino purine) and IAA (Indole-acetic acid) showed remarkably better response for embryonic formation of wheat. Additionally, the rooted plants were been successfully transplanted into the substrate and

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acclimatized in the laboratory greenhouse; remarked that through tissue culture wheat seedlings get maximum survivals

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Figures

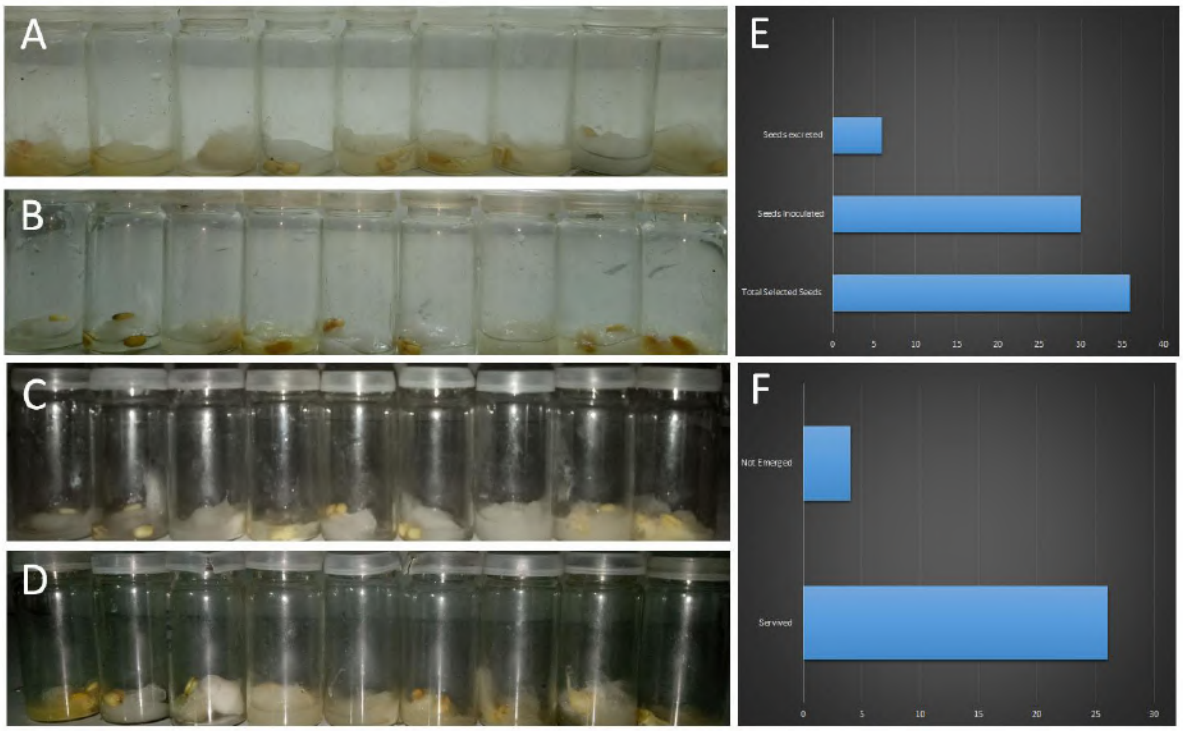

Fig.1: Invitro regeneration of Wheat through embryonic formation. (A) Seeds inoculated at MS basal medium +10µl/L BAP + 40µl/L IAA. (B) Seeds inoculated at MS basal medium + 20µl/L BAP + 30µl/L IAA. (C) Seeds inoculated at MS basal medium+30µl/L BAP +20µl/L IAA. (D) Seeds inoculated at MS basal medium + 40µl/L BAP +10µl/L IAA. (E) Percentile of seeds inoculations. (F) Emergence of seed. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. Data are the average of three different biological replications.

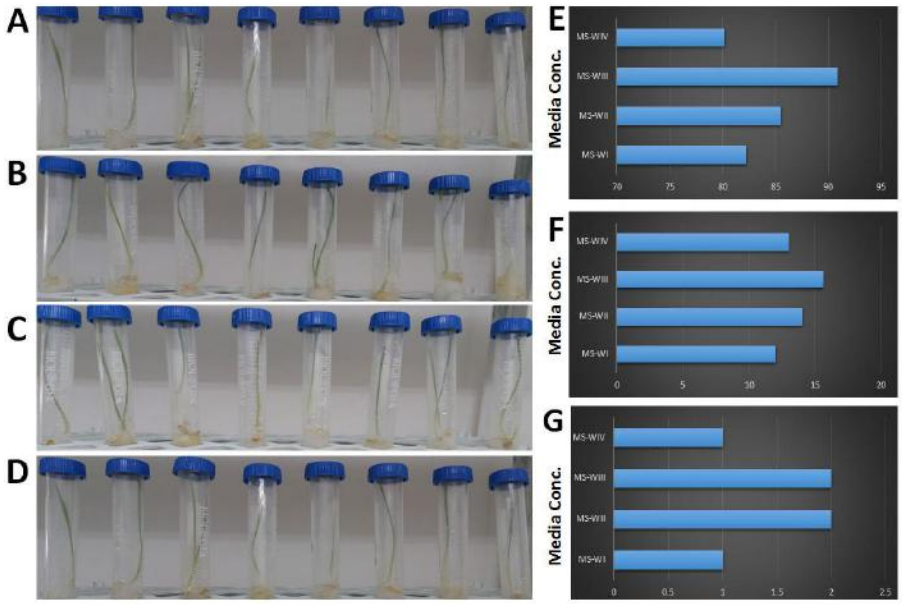

Fig.2: Proliferation of regenerated Wheat seedlings through embryonic formation. (A) Regenerated on MS basal medium +10µl/L BAP + 40µl/L IAA. (B)Regenerated on MS basal medium + 20µl/L BAP + 30µl/L IAA. (C)Regenerated on MS basal medium+30µl/L BAP +20µl/L IAA. (D)Regenerated on MS basal medium + 40µl/L BAP +10µl/L IAA. (E)Average of plants regenerated.(F) Length regenerated leaves (G) Encountering the number of leaves. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. Data are the average of three different biological replications.

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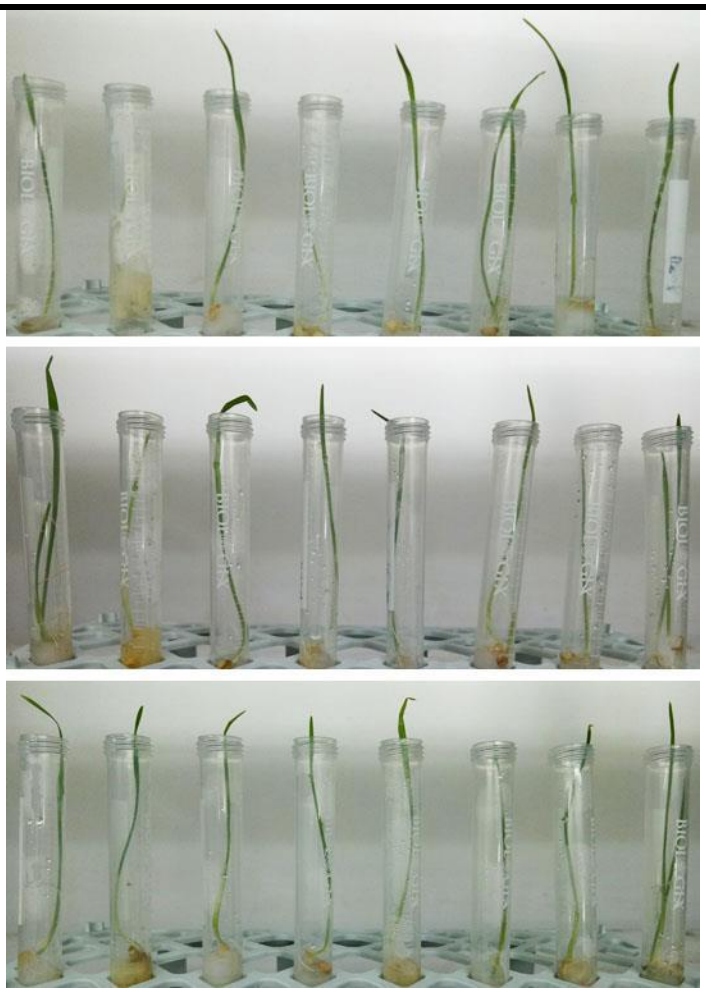

Fig.3: Proficiency of hormonal concentrations affects the regeneration potential of wheat. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. Data are the average of three different biological replications.

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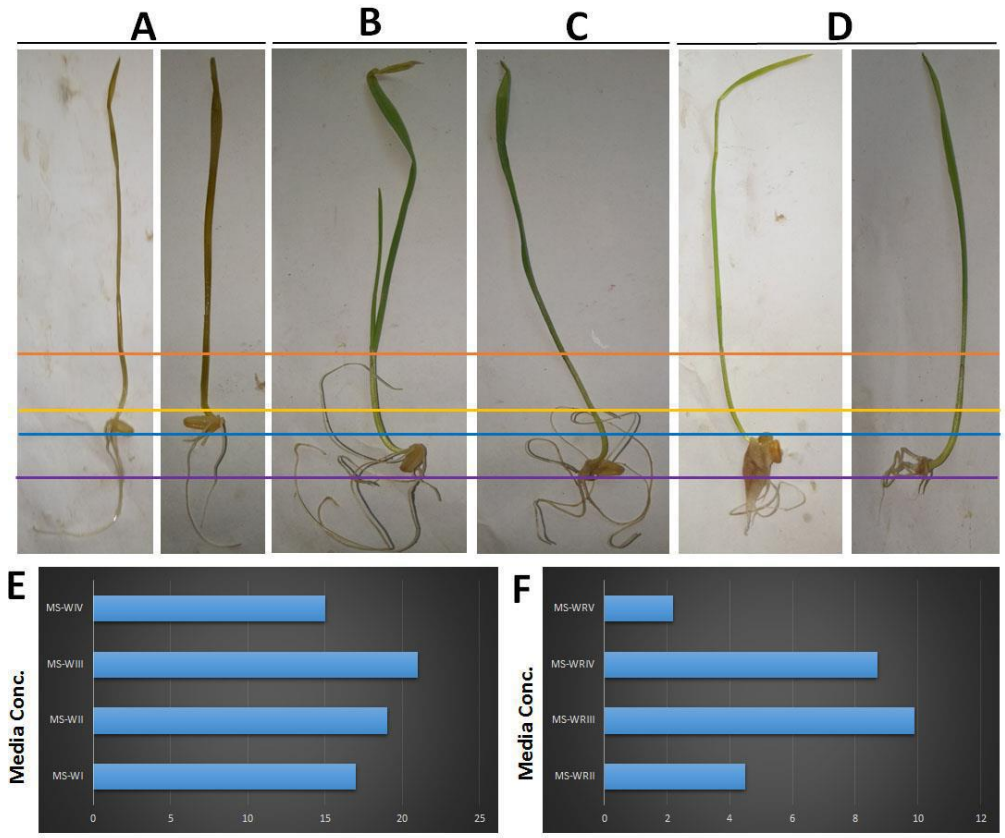

Fig.4: Roots of wheat exceptionally pretentious by Auxin-Cytokinin interactions. (A,B,C,D)Invitro regenerated seedlings having pretentions roots. (E) Length of leaves (F) Number of roots.The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. Data are the average of three different biological replications.

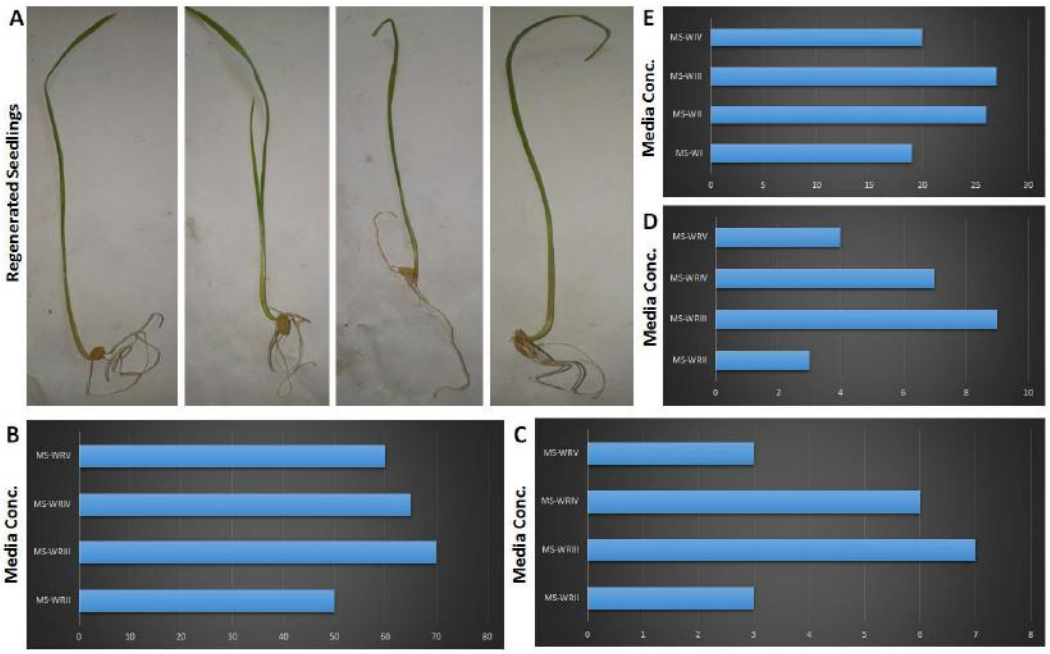

Fig.5: Mass-clonal production of wheat by embryonic formation. (A)Regeneratedseedlings at different hormonal concentrations. (B)Initiation rate of roots regeneration.(C)Length ofroots. (D)Proliferated roots. (E) Average length of regenerated seedlings. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. Data are the average of three different biological replications.

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Supplementary Table

Table.S-1 Composition of nutrient media used

	Ingredients	Conc.	ml	g
Ingredients	NH_4NO3 (Ammonium nitrate)	50X	500	41.25
	KNO_3 (Potassium Nitrate)			47.5
	CaCl2.2H2O (Calcium chloride)	100X	500	22
	MgCl2.7H2O (Magnesium sulphate)	100X	500	18.5
	KH2PO4 (Potassium Phosphate)			8.5
	Na2-EDTA (Sodium EDTA)	100X	500	1.865
	FeSO4.7H2O (Iron Sulphate)			1.39
	MnSO4.H2O (MangneseSulphate)	100X	500	0.845
	ZnSO4.7H2O (Zinc sulphate)			0.43
	H3BO3 (Boric Acid)			0.31
	KI (Potassium iodide)			0.0415
	Na2MoO4.2H2O (Sodium molybdate)			0.0125
	CuSO4.5H2O (Copper sulphate)			0.00125
	CoCl2.6H2O (Cobalt Chloride)			0.00125
	Glycine	100X	500	1
	Thiamine HCL（VB1）			0.005
	Pyridoxine HCL (VB6)			0.025
	Niacin (VB3)			0.025
	Inositol/casein acid			5

Murashige and Skooge, 1962

Table.S-2 Composition of nutrient media for shoot induction

Media	Composition
MS-WI	MS basal medium +10µl/L BAP + 40µl/L IAA
MS-WII	MS basal medium + 20µl/L BAP + 30µl/L IAA
MS-WIV	MS basal medium + 30µl/L BAP +20µl/L IAA
MS-WV	MS basal medium + 40µl/L BAP +10µl/L IAA

Table.S-3 Composition of nutrient media for root induction

Media	Composition
MS-SI	MS basal medium + 10µl/L IAA
MS-SII	MS basal medium + 20µl/L IAA
MS-SIV	MS basal medium + 30/L IAA
MS-SV	MS basal medium + 40µl/L IAA

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Assessment of Extension Agents' Perception of Mobile Phone Usage for Communication with Farmers in EDO South of EDO State, Nigeria

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Abstract**—** *The use of mobile phones as means of communication between the extension agents and local farmers in Edo South was examined to determine the perception of the extension agents on its effectiveness. Fifty-one (51) respondents were obtained through random sampling using questionnaires to obtain relevant information in the two local government areas; Ovia North East and Ovia South West selected for the study. The result obtained indicated that male extension agents were in majority (72.5%), 54.9% of the sampled population above 45 years of age and 58.8% possesed 16 – 20 years working experience. Phone calls and short message service (SMS) were found appropriate for agricultural information dissemination. Significant and positive relationship were established between the working experience of extension agents and their perception of mobile phone usage. The recommendation is that formal integration of mobile phone into extension activities be done to enhance wider coverage, reduce cost and risks associated with movement. Extension agents and farmers need more training on effective usage of SMS and phone calls for interaction on farm related issues. Enforcement of existing telecommunication laws could reduce network failure and also enhance effective usage.*

Keywords**— Perception, Communication, Mobile phone, Short Message Service.**

I. INTRODUCTION

Communication has been a veritable tool in all aspects of human endeavours such that it is an indispensable tool that links individuals, organisations and nations. The world is a global village because communication network has closed every gap such that connectivity within the globe is done within seconds using network of information communication technology.

Asemah *el al* (2017), described communication as the process through which individuals or group of individuals exchange ideas, information, messages, feelings and notions through previously agreed symbols, in order to influence one another. In agricultural extension, communication using various devices and channels have become the effective means of technology transfer. The cycle of research – extension – farmer linkage is only feasible as the innovations are communicated in understandable manner. Communication in agriculture is peculiar and audience specified because it basically involves technical and economic information relating to agriculture.

Agricultural communication is a specialised process of information sharing relating to agricultural production processing and marketing. It is a professionalised information exchange through which stakeholders in agricultural value chain interact. The policy makers, researchers, extension professionals, producers and marketers creatively interact to solve agricultural problem using communication skills and methodologies.

According to Yahaya (2003), it is tempting to use mass media product advertising techniques to sell new agricultural practises as if they were soft drinks or soap. In agriculture it is much different, complex and highly interrelated as it involves innovations to many segments of the population.

The agricultural communicator must present issues and problems from the farmer's orientation using specialised vocabulary to ensure effective communication.

Technology is in every aspect of development because communication is relevant in every human action as a basic tool for interaction. It is currently used to interlink information technology devices like personal computers

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with the use of communication gadgets like telephones and other telecommunication networks.

The role of technology in development globally is a great asset as a result of ever increasing population the emergence needs of human which is due to various challenges of war, insurgence, famine, disease outbreak and various effects of climatic change.

In agriculture technology has been a booster because it replaces the manual labour with technology driven gadgets which ease operations, reduces cost and increases land area under cultivation. Lynch (2018) opined that technology has been acclaimed as the answer to agricultural transformation with its potential as an aid to increase farming efficiency, resilience and yields while maximising costs. Several initiatives are springing up to introduce various information communication devices to facilitate agribusiness

Dookie (2018) reported Revofarm activities. It is a Jamaican Short Message Service and web-based application leading the way in providing weather and agronomic data to farmers on their phones and empowering them to make smart field-level decisions. It was observed that over 200,000 farmers registered in Jamaica lived in poverty and were unable to break even. In 2014, annual agricultural production declined by 30% after months of extreme drought and bush fires and this propelled Ricardo Cowdie the initiator of Revofarm, such that the objective of his organisation focused on reducing the devastating effect of the disaster. The focus was majorly to reduce the impacts of climate change on crop production thus reducing poverty.

In Ethiopia blueMoon is a youth agribusiness incubator that guides start-ups towards sustainable growth. African Agribusiness Incubators Network, the Entrepreneurship Program for Innovation in the Caribbean and Technical Centre for Agricultural and Rural Cooperation (CTA) Pitch Agrittack are also involved in information communication technology promotion for young entrepreneurs.

Globally, 92% of internet users access the web via mobile devices and 81% of Africa's population have subscriptions while 29% are active internet users (Hootsuite 2018)

The use of mobile phones is widespread in Nigeria such that it has become indispensable in all categories of business and interactions both in the rural and urban settings. The introduction of Electronic Wallet System in Nigeria in 2011 led to a breakthrough in farm input distribution to farmers. The system basically depended on the use of mobile phones, according to Adesina (2015) vouchers for subsidised inputs; seeds and fertilizers were programmed to be received on farmers' mobile phones to buy inputs directly from the agro-dealers such that 20 million smallholder farmers were reached.

Chatel (2018), asserted that mobile phones will be essential in agriculture because future farmers will not necessarily be born into farming families. They will be more likely to become farmers with greater opportunities to learn the trade and adapt their knowledge and skills to address new challenges. It was further reported that (GSMA) Global System for Mobile Association projected that the number of mobile phone subscribers in sub-Sahara Africa should grow from 420 million to 535 million between 2016 and 2020 with 40% of them having internet access.

Information communication technology is becoming trail blazer in every aspect of economy globally because it enhances information exchange such that information transfer is done faster and cheaper than it has ever been.

Communication Channels in Extension

The various existing communication channels have been employed in agricultural extension as a means of transmitting information between the sender and the receiver. According to Torimiro and Alfred (2008), information is transmitted over a channel that links the sender and the receiver. Messages may be oral, written or verbal and they could be transferred through electronic, print, surface mail and information communication technology including mobile phones.

The Global System of Mobile, Internet, computers, digital radios etc are information communication technology devices that are currently being employed all over the world to promote agricultural development by all stakeholders, farmers, agro input and output marketers, extension professionals, researchers, policy makers etc. The mobile based technology have the potential to deliver to every farmer who has a phone, farm innovation that will be relevant to help adopt the new technology anytime (Ajala 2015)

II. METHODOLOGY

The study was carried out in Ovia North East and Ovia South West Local Government areas (Edo South agro ecological zone) of Edo State, Nigeria

The area of study is located between longitude $05^{0}04$' North and $06^{0}43$' East and Latitude $05^{0}44$' latitude. It is bounded in the North by Kogi State and Delta State by the South; Ondo State by the West and Anambra by the East. The State is made up of eighteen Local Government Areas and has a

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land mass of 19819km^2 with a population of 3,233,366 persons and population density of 163.14 (NNPC 2006)

The study area (Ovia North East and Ovia South West Local Government areas) are made up of twelve and nine communities respectively and the study covered the Extension Agents working with farmers in the area. sixty extension workers were randomly selected for sampling but only fifty-one respondents were surveyed and used for the analysis.

Purposive sampling technique was used to select two local government areas from the seven local government areas constituting Edo South Agro-ecological zone because of their peculiarity as predominantly agrarian communities.

2.1 Measurement of Variables

The measurement of variables was done taking socioeconomic characteristics of the respondents, frequency of contact with farmers, Global System Mobile features considered appropriate for information dissemination and respondents' perception of the efficiency of mobile phones usage in dissemination of agricultural information using 5-point rating scale. Pearson Product Moment Correlation was used to test the hypothesis.

III. RESULTS AND DISCUSSION

Table 1; Respondents' Socioeconomic characteristics

The socio-economic survey indicated that majority (72.5%) of the respondents were male extension agents and 54.5% were above 45 years old while 90.2% were married. This indicated that they were adults with family and dependants as shown in Table 1 below. The majority possessed Ordinary and Higher Diploma (84.3%) while 2% are holders of Bachelor degree.

Gender	Frequency	%	
Male	37	72.5	
Female	14	27.5	
Total	51	100.0	
Age (Years)			Mean(Yrs)
25-35	4	7.8	
36-45	19	37.3	44.7
>45	28	54.9	
Total	51	100.0	
Education			
Secondary	5	9.8	
OND/HND	43	84.3	
Bachelor Degree	1	2.0	
MSc/PhD	2	3.9	
Total	51	100.0	

Source: Field Survey 2017

Table 2: Working Experience

Most of the extension agents have 16 years working experience and above thus putting them in the right condition to share agricultural knowledge.

Working Experience (yrs)	Frequency	%	Mean
6	1	2.0	
6-10	5	9.8	
11-16	5	9.8	14.61
16-20	30	58.8	
>20	10	19.6	
Total	51	100.0	

Source: Field Survey 2017

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Table 3: Respondents' Frequency of Visit to Farmer

Table 3 shows that 92.2% of the respondents visited the farmers fortnightly and the practise conform with the peculiarity of Training and Visit extension system used by Agricultural Development Projects as designed by Benor et al (1984). Those whose visits were monthly and occasional constituted 5.9% and 2% respectively.

Variables	Frequency	%
Once a month	3	5.9
Fortnightly	47	92.2
Occasionally	1	2.0
Total	51	100.0

Source: Field Survey 2017

Table 4 Respondents" perception of GSM:

The Mobile Phone features were found to be appropriate for usage based on the survey as shown in Table 4 below was phone calls (X = 4.80) and Short Message Service (X = 4.47).This situation confirms the reality in Nigeria where majority of users are familiar with the use of these two features particularly in the suburban where farmers dwell. The peculiar feature of handsets used and the level of farmers' education may also account for this.

This finding agrees with the activities of Sygenta under the program eShamba which disseminated training and advice on good farming practises using text messages to reach 230,000 young farmers in Kenya and Tanzania with results of farmers increase in crop yields of 50% and 125% return on investment (Chatel, 2018).

Variables	Mean	Std Deviation
Phone calls	4.80*	0.40
SMS	4.47	0.61
MMS Message	1.98	0.81
Voice Message	1.59	0.96

Source: Field Survey 2017

Table 5: Respondents' perception of the efficiency of mobile phone usage for agricultural information dissemination.

Respondents indicated that mobile phones facilitate the dissemination of agricultural information to farmers (X = 4.25) thus reducing personal contact with farmers (X = 4.10) with the associating risks and costs of frequent visits. The extension agents also revealed the need for training in use of mobile phone to package agricultural information (X = 3.37) such that farmers will be able to understand the messages.

Perception statement	Mean	Std Deviation
Facilitate agricultural information dissemination	4.25*	0.74
Reduces personal contact with farmers	4.10*	0.92
Misinformation may occur	3.67*	1.01
Extension agents are trained on mobile phone usage	3.45*	1.38
Farmers understand the messages	3.37*	0.97
Network problem will not limit usage for extension	3.24*	1.21
Mobile phone can be used to transfer technical and economic agricultural information	3.14*	1.11
Erratic power supply will not limit usage	3.08*	1.16
Information can be sent easily through SMS. MMS, Voice Message	2.78	1.22
Govt policy exist concerning mobile phone usage for information dissemination to farmers	2.44	1.22
Many farmers can operate mobile phone	2.33	1.03
Many farmers lack personal mobile phones	2.27	0.98

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Mobile phone network exist in many rural areas	2.24	1.03
Mobile phones can be misplaced easily by farmers	2.08	1.18
Mobile phone is not an expensive information dissemination medium	1.25	0.44

Agreed(Mean > 3.00)
Source: Field Survey 2017

Table 6: Relationship between respondents' socioeconomic characteristics and perception of mobile phone usage
The Table 6 below shows a positive correlation between respondents' work experience (r=0.38; p<0.05) and their perception of mobile phone usage.

Variable	Correlation coefficient (r)	P level
Age	-0.28	0.846
Gender	0.194	0.173
Marital status	0.159	-
Family size	0.145	0.312
Education	0.049	0.734
Working experience	0.384*	0.005
Contact with farmers	0.047	0.743

Significant at 5% level
Source: Field Survey, 2017

IV. CONCLUSION

This study shows that Mobile phone as a communication device facilitates agricultural information transfer in the area of study as perceived by the extension agents, majority of whom were well experienced in agricultural extension work, had experience of over sixteen years. Majority of the extension agents (90%) visited farmers fortnightly as expected. The use of Mobile phones was perceived as appropriate for transfer of agricultural information using the phone call and Short Message Service (SMS) and this reduced frequency of visits and associated costs and risks involved in movement.

Training and retraining of extension workers to ensure proper coding of messages without distortion become necessary as revealed in the study. It was also discovered that farmers were able to understand the messages relayed through mobile phones since the extension agents were trained professionals and were able to communicate with the farmers in an understandable dialect or language.

V. RECOMMENDATIONS

The use of Mobile phones should be formally integrated as a core communication device in agricultural extension work by all the stakeholders involved in extension farmer's linkage to ensure wider outreach per time and cost reduction.

The major telecommunication service providers should be mobilized to install infrastructure in rural settlements with effective control to ensure regularity of network flow.

There should be introduction of training opportunities for farmers and extension agents on the technicalities of using Mobile phones in extension work so as to understand the peculiarities of coding agricultural information.

Software applications suitable for use in emergency cases to meet current needs in agriculture such as climate change and pests/diseases outbreak should be developed.

Though the challenge of network failure may pose a problem, maximum advantage should be taken when the network is good.

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The main Removal Mechanism of Organic micropollutants and Organisms in an Irrigation System using Untreated Wastewater

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***Abstract*—** *The presence of organic micropollutants on residual water used for irrigation is common in development countries, and their human impacts and the ecological consequences are still completely unknown. The aim of this study was to determine the main mechanisms involved during removal of three organic compounds (carbamazepine, ibuporfen, and 4-nonylphenol), and three usually pathogenic organisms (Escherichia coli, Giardia lamblia and Ascaris lumbricoides) contained in untreated wastewater dumped to agricultural soil, based on laboratory studies with inoculated soil, as well as the corroboration with field measurements. The results suggest adsorption and biodegradation as the predominant processes responsible for the removal of all contaminants analyzed, reporting efficiencies greater than 95% during the first 30 cm of the soil depth. Nevertheless, the efficiency depends on the type of pollutant, and also the physicochemical characteristics of the soil. For ibuprofen and 4-nonylphenol occurs mostly by biodegradation, and the adsorption of carbamazepine is associated with the organic matter content, while E. coli is adsorbed to the clay fraction of the soil. Finally, G. lambia and A. lumbricoides removal is due to different processes from adsorption such as colloidal filtration.*

***Keywords*—Endocrine disrupting chemicals, pharmaceuticals, removal mechanisms, soil, unintentional wastewater reuse.**

I. INTRODUCTION

The maintenance of the cities implies the supply of a large volume of water, which implies a model of complicated sustainability. The reuse of raw or treated wastewater for specific cases is an ancient and common practice around the world, especially in countries with emerging economies. One of the main uses is for agricultural irrigation which is estimated to amount to 29 183 897 m^3/d (Jiménez and Asano, 2008). Most of developed countries practice the irrigation with wastewater in a controlled way, contrasting the situation in developing countries, owing to a lack of appropriate infrastructure, irrigation is seen as an economic and feasible way of disposing of raw wastewater. Each of these two ways of management has advantages and disadvantages. For the former, risks to health are minimized as the concentration of pollutants and nutrient content like nitrogen, phosphorus, and organic material is controlled, although, for the firsts, depending on its subsequent uses can be added. In the latter case, crop production is favoured as a result of the abundance of nutrients present in the irrigation water, but there is a greater risk to the health of the farm workers as well as consumers, nevertheless in most cases these menaces have not been quantified. The pollutants that represent the greatest danger to the population include heavy metals, pathogenic organisms, organic compounds and other not regulated with metabolic implications. However, the content not removed during the pre-treatment process ends in the environment, where their impacts and environmental implications in the medium and long term are still unknown

In Mexico, irrigation of crops with raw wastewater is a common practice estimated in 40% of the 208 m^3/s of wastewater collected in the whole country, although the goal is to achieve 60% by 2012, although there is still no updated official data available. The most well-known area where this practice occurs is the Tula Valley, where the irrigation uses raw wastewater from Mexico City. Information on the irrigation is available from 1912 (the rate was 2 m^3/s over 14 000 ha of land), and records how the increasing availability of wastewater as the population of the city has grown has involved in use of greater volumes of wastewater (such that today 45 m^3/s is used for irrigation of 85 450 ha of land). Previous studies

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Gibson *et al*., (2007), Jimenez and Asano, (2008), Gibson *et al*., (2010), and Chávez *et al*., (2011) have shown that the infiltration of raw wastewater through the soil has functioned as a natural purification system on the organic, inorganic, and biological pollutants presents in wastewater. The rates of removal of diverse contaminants are due to a complex series of natural processes that can act in a synergistic manner, as mention Yamamoto *et al*., (2009). The mentioned author emphasizes that, in the case of organic micropollutants; the natural mechanisms involved in the degradation and removal of the water soluble material are adsorption, biodegradation, and to a lesser extent photodegradation.

On the other hand, regarding to the pathogenic organisms, adhesion and depredation are the dominant removal processes, particularly in agricultural systems using wastewater for irrigation (Stevik *et al*., 2004). In this sense, it is important to consider the dependence of removal of pollutants, as much on the physicochemical properties of the compounds, as on the particular characteristics of the area (solar radiation, humidity, temperature, properties of the receiving soil, and the wastewater itself). The Tula Valley is well known as the oldest and largest example in the world of an irrigation system that uses raw wastewater (more than 100 years). Despite this, there are few studies at a regional level that have explored the processes of removal and/or degradation of pollutants involved. In addition, little is known about the capacity of this system, or the strategies that might be necessary in order to manage it efficiently, and whether could be applied to other similar areas.

The first step is to recognize in a global manner the processes by which this reduction in the concentration of pollutants occurs. The objective of this work is to understand the natural mechanisms of reduction in infiltrated water by comparison to the irrigation water, in particular the adsorption and biodegradation of pollutants in the agricultural soils of the area, and that cause the drop in concentration of the pollutants (the anti-inflammatory ibuprofen, the anti-epileptic carbamazepine, and the surfactant metabolite 4-nonylphenol), and three groups of organisms, including bacteria (*Escherichia coli*), protozoa (*Giardia lamblia*), and helminth (*Ascaris lumbricoides*) eggs. This was carried out by means of a field study complimented by laboratory studies for the different groups.

II. METHODS

Studies are focused on the determination of adsorption and biodegradation of organic micropollutants and pathogenic organisms were carried out in the laboratory while a field monitoring study was used to add weight to the laboratory data. Methodology used is described in the following sections.

2.1 Sampling and characterization of the soil

A soil from the Phaeozem class was studied. For the laboratory tests, samples from 0 to 30 cm depth were collected by triplicate. For the field monitoring study, samples were taken from three soil horizons of each soil profile. All of the samples were stored in plastic bags at 4º C. In the laboratory, the soils were gently broken up and sieved to 2 mm and then characterized in terms of pH, humidity, cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}), cationic exchange capacity, organic and total carbon, and the composition by its particle size, determining particularly the sand, silt, and clay contents). The details and techniques are listed below.

2.2 Batch adsorption assays

Organic compounds evaluated: 2 g of dried soil sterilized with a dose of gamma rays of 25 kGy was weighed into amber vials, then 10 mL of $CaCl_2$ [10 mM] were added (the final ratio of soil to solution was 1:5). Then carbamazepine, ibuprofen, and 4-nonylphenols were added to the vials at nine different concentrations (10, 50, 75, 100, 200, 400, 500, 1.000 and 5.000 µg/L); blanks without the micropollutants, and without soil were included to evaluate liberation of the contaminants from the soil, and the possibility of sorption to the walls of the vials were made, respectively. The flasks were shaken at 150 rpm for 24 hours at 25 °C in absence of light as possible (to avoid photodegradation). After shaking, the vials were centrifuged (3000 rpm for 10 min), and the supernatant was transferred to a clean vial and stored at -18 °C until chromatographic analysis.

Organisms evaluated: These assays were carried out by varying the quantity of soil instead of the concentration of organisms under test due to the difficulty of establishing and maintaining exact their concentrations. Samples of soil (5 masses) were tested for E. coli ATCC 700078 WG5 (0.5, 1, 1.5, 2 y 2.5 g), while for *G. lamblia* and *A. lumbricoides* eggs a mass of 0.1, 0.5, 1, 1.5, 2 y 2.5 g of soil were used. Then the soils were weighed into Teflon centrifuge tubes and the organisms were added using 20 mL of a solution of 0.85% NaCl.

The concentrations tried were $8x10^8$ CFU/mL for *E. coli*, 2.5 cysts/mL for *G. lamblia* and 2.5 eggs/mL for *A. lumbricoides*. The tubes were shaken at a speed of 110 rpm for 30 min for *E. coli*, and during 2.5 hours and during the same time for the other two species, then each the suspension was centrifuged (3000 rpm for 15 min), quantifying organisms from supernatant. All assays were carried out in triplicate; blanks (without soil and with no organisms) were made in parallel.

2.3 Biodegradation and adsorption column experiments for the organic micropollutants

PVC columns (5.08 cm internal diameter by 30 cm

height) were packed with dry soil. Layers of sand and then silica (mean 5 mm and 1 mm diameter) were added to the bottom of the columns above a mesh of steel (pore size 0.2 mm). The soil was packed manually 1 cm at a time to a depth of 10 cm; the total amount packed was 197 g, and the soil bulk density reached was 0.97 g/cm^3. The columns were covered with aluminum foil to avoid possible photodegradation of the analytes. For both, the biodegradation and adsorption experiments were used four columns (three replicates and a blank), making a total of eight columns in total; for the adsorption tests the columns were packed using sterile soil. Six irrigation events were simulated in each column, the mixture of organic micropollutants being only 4-nonylphenols (36.4 µg/L) and ibuprofen (6.5 µg/L). Each irrigation was carried out manually with 13.6 cm^3 of water (275 mL) poured in a single event, similar to rates used for irrigation of maize in the valley. For the biodegradation experiments a sterile solution of 29 mg/L of nitrogen as NH_4Cl, and 39 mg/L of phosphorus as KH_2PO_4 were added, similar to concentrations found in the local wastewater. The columns were saturated with a solution of [10 mM] of $CaCl_2$ before the first irrigation. For each one, the water was allowed to pass through the packed soil without the application of a vacuum. The leachates were collected in glass vessels and stored at -18 °C until their analysis. The irrigation events were applied every 21 days, simulating the time periods between irrigations in the field. At the end of the tests, the soil columns were taken apart under antiseptic conditions and the concentrations of the organic micropollutants determined.

2.4 Chemical reagents

All the analytes, internal and the recovery standards, as well as the derivatising agents N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA), and N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) were obtained from Sigma-Aldrich (St. Louis MO, USA). The solvents used were HPLC grade, bought from Burdick and Jackson (Morristown, NJ, USA); the OASIS HLB extraction cartridges (200 mg) were supplied by Waters (Milford, MA, USA).

2.5 Analytical methods

Organic compounds evaluated: The determination of organic micropollutants in wastewater and leachates was done according to the method validated by Gibson *et al.*, (2007). Briefly, the leachates and aqueous solutions were acidified to pH = 2 with concentrated H_2SO_4. After passage through the Oasis HLB cartridges (conditioned with 2 x 5 mL of $CH_3(CO)CH_3$ followed by 5 mL of 5% CH_3COOH. The acidic pharmaceuticals were recovered by elution with 5.5 mL of a solution of $CH_3(CO)CH_3$:$NaHCO_3$ (40:60 at pH = 10), and the phenolic compounds were eluted with 5 mL of $CH_3(CO)CH_3$. The derivatives of the acidic pharmaceuticals were produced using MTBSTFA as the derivatising agent while derivatives of the phenols were obtained by reaction with BSTFA. For soils, the analytes were extracted by accelerated solvent extraction and details of the procedure can be found in Durán-Álvarez *et al.*, (2009). The extracts (20 mL) were evaporated to approximately 3 mL, diluted to 15 mL with water, and then passed through the Oasis cartridges. From this point the procedure was the same as described for the aqueous samples. The final analysis and quantification of the analytes was carried out using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass selective detector. 2,3-dichlorophenoxyacetic acid (2,3-D), [2H4] 4-n-nonylphenol, and [2H16] bisphenol-A were used as internal standards and 3,4-dichlorophenoxyacetic acid (3,4-D), 4-n-nonylphenol, and 10-11, dihydrocarbamazepine were used as recovery standards. In addition, solvent blanks were analyzed with each batch of samples.

Organisms evaluated. Bacteria were detected by the membrane filtration method (using 0.45 µm pore size membrane filters Millipore Corp., Berdform, MA selective agar), and most probable number with selective broths), methods according to Standard Methods for the examination of water and wastewater mentioned in APHA (1998). For the case of *G. lamblia*, the sample was concentrated through filtration in a polycarbonate membrane (5 µm pore and 20 mm diameter) incubated with polyclonal antibodies (rabbit sera and anti-rabbit immunoglobulin G) at 37 °C for 30 minutes, for each equipped with epifluorescence and phase contrast, according to APHA (1998). The concentration and further identification of helminth eggs in wastewater and water supply was performed using continual washing, combined with diverse filtration stages (sieve pore 150 and 20 mm) and flotation (utilizing a saturated solution of $ZnSO_4$; density 1.3 mg/cm^3) and continual centrifugation concentration.

III. RESULTS AND DISCUSSION

3.1 Sampling and soil characterization

The physicochemical properties of the soil are recorded in Table 1. There is an elevated concentration of organic material in the first 30 cm of the soil profile (in comparison with others) as a result of the intensive use of wastewater for irrigation. In addition, there is a prevalence of expandable clays in the soils dominated by the smectite type (Friedel *et al.*, 2000). The soil moisture was well below the field capacity (34%) as the soils were sampled in the dry period between irrigations (which occur every three weeks approximately), a time when microbial activity is also reduced.

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Table.1: Physicochemical characterization of the soil

Horizon (cm)	pH (pH units)	Exchangeable Cations (mg/L)				CEC	OC	TC	TN	Particle size		
		Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	(cmol/kg)	(mg/g)	(%)	(%)	Sand (%)	silt (%)	Clay (%)
0-10	6.63	37	6.6	1.8	1.2	47	40.8	4.6	0.4	39	35	26
10-20	6.73	38	6.6	1.8	1.2	48	41.5	4.6	0.4	40	35	25
20-30	6.61	39	6.5	1.8	1.3	49	42.2	4.5	0.4	38	36	26
>30	7.33	38	6.0	1.6	1.3	47	13.9	1.6	0.1	45	32	23

CEC = cationic exchange capacity; OC = organic carbon, TC = total carbon; TN = total nitrogen

3.2 Batch adsorption experiments

Organic compounds evaluated: Adsorption isotherms for the pollutants studied are shown in Fig. 1, and adsorption parameters for the three compounds are evidenced in Table 2. Ibuprofen was adsorbed less than either carbamazepine or 4-nonylphenol, possibly because the pH of the soil would encourage dissociation of the anti-inflammatory drug (pKa = 4.15) leading to greater aqueous solubility and also less adsorption due to repulsive forces as the organic material, additionally because the clays have negative charges. Carbamazepine is present in the soil in its non-ionized form (pKa = 14), and adsorption is favored because of its hydrophobicity. Chefetz *et al.*, (2008) suggested that the aromatic rings present in carbamazepine can form π-π interactions with the aromatic rings of the highly humified organic material of the soil, resulting in rapid irreversible adsorption. Therefore, a high degree of adsorption of carbamazepine can be expected in soils deeper than the first 30 cm of the soil profile. A similar pattern could be expected for ibuprofen, but with less adsorption overall due to its ionized state.

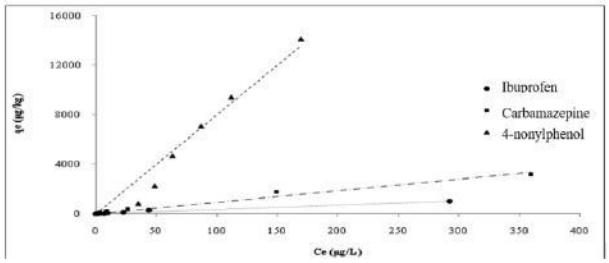

Fig. 1: Adsorption isotherms obtained for the organic micropollutants in the tested soils

Bi *et al.*, (2006) found a removal of carbamazepine when studying its adsorption in smectic clays similar to those found abundantly in the Tula Valley. In case of 4-nonylphenol showed the strongest affinity for the soil studied consistent with literature reports such as Düring *et al.*, (2002) who reported high distribution constants for 4-nonylphenol in soils with diverse characteristics (8.5-321 L/kg). The high value of the adsorption constant (normalized for organic carbon content) suggests that this adsorption occurs principally by means of non-specific hydrophobic interactions. The analysis of blanks without soil and blanks without the presence of the contaminants showed that adsorption to the walls of the vials and their liberation into the aqueous solution was not significant.

Table.2: Adsorption parameters for the compounds studied in tested soil

Compound	K_d (L/kg)	R^2	K_d literature (L/kg)	K_{oc} (L/kg)	R (theoretical)
Ibuprofen	3.6	0.9789	3.7[a]	84	12
Carbamazepine	9.4	0.9812	12.3[b]	218	30
4-nonylphenols	80	0.9538	8.5-321[c]	1862	250

K_d = Distribution coefficient; K_{oc} = Organic carbon adsorption coefficient; R^2 = correlation coefficient; R = Delay factor

[a]Xu *et al.*, 2009; [b] Stein *et al.*, 2008; [c] Düring *et al.*, 2002

Organisms evaluated: The adsorption isotherm for *E. coli* is presented in Fig. 2 adjusted to the Freundlich model. It can be seen that its concentration fits the model used, and the value of the factor "n" suggests that adsorption of this microorganism can be considered linear. The value of constant of Freundlich (KF) obtained was 1.8×10^3, less than that reported by other researchers who have worked with soils with similar organic carbon contents but different amounts and types of clays as Smith and Badawy, (2007). It could be inferred that the adsorption of this microorganism is determined by the clay fraction of the soil, and it has been suggested that the production of extracellular polymeric substances which contain polyvalent cations help to bind bacteria with soil minerals, notably clays (Stevik *et al.*, 2004). In this sense, Guber *et al.*, (2005) found that the presence of natural organic material in the soil was not favourable for the process of adsorption of microorganisms.

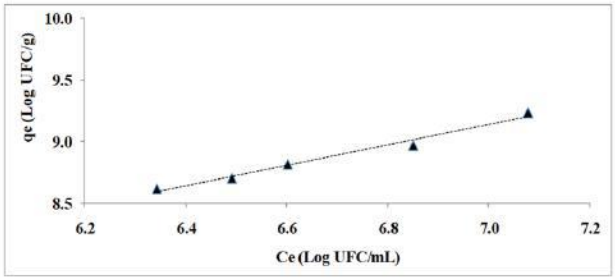

Fig.2: Adsorption isotherm for E. coli, adjusted to the Freundlich model

Data generated for *G. lamblia* and *A. lumbricoides* eggs could not be adjusted to either Freundlich or Langmuir models (R^2<0.4) and so the isotherms are not shown. This could be due to reasons such as (a) the size of the organisms, (b) the complexity and heterogeneity of the soil which resulted in a multifarious adsorption process that could not be described by these models, or (c) the existence of other dominant processes such as colloidal filtration. In fact, last process has been reported by Brusseau *et al.*, (2005), but only for *G. lamblia*. Searcy *et al.*, (2005) found that cysts could adhere to different substrate, however, Dai and Boll, (2003) showed no

interaction between cysts and soil particles. For helminth eggs there is a paucity of information about their removal and inactivation in soil, although Capizzi and Schwartzbrod, (2001) have reported its capacity to adhere to silica particles present in clays and sand in soil, notably in clays of the smectite type abundant in the soil studied. In general terms, through this assay it was possible to determine removal rates in the liquid phase of 1 log unit for *E. coli*, 65% for *G. lamblia*, and 97% for *A. lumbricoides* eggs.

3.3 Biodegradation and adsorption of organic micropollutants in columns

Fig. 3 shows the concentration of the micropollutants in the leachates from the six irrigations. In the non-sterile columns less 4-nonylphenol was leached during the irrigations. The concentration of 4-nonylphenol recovered in the leachates was less after the third irrigation compared with previous, indicating that biodegradation of the compound was less after 60 days of residence. In the sterile soil columns, a large amount of leaching of 4-nonylphenol was observed until the third irrigation, after which the concentration in the leachate dropped by 55%. This could be due to greater adsorption of the soil or intra-particle diffusion where the compound enters into the mesopores and micropores of the soil, making it progressively more difficult for the compound to move through the soil. This last hypothesis is backed up by the fact that for the sixth irrigation the concentration of 4-nonylphenol leaching from the column was less than that found in the previous three irrigations, which were approximately constant.

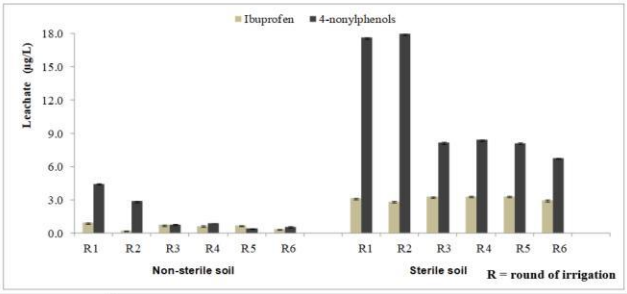

Fig.3: Concentration of the organic micropollutants in the leachates from columns packed with sterile and non-sterile soils

The leaching of ibuprofen from the sterile columns was constant over the six irrigations, indicating a low affinity for the soil; this was similar to the results for adsorption obtained from the batch tests. It is important to consider that ibuprofen and 4-nonylphenol could be adsorbed to the dissolved organic material and migrate through the soil column associated with this dissolved material and so arrive in aquifers (Hollrigl-Rosta *et al.*, 2003). In the non-sterile columns only a small amount of ibuprofen was seen in the leachates, indicating that biodegradation is the process that defines the fate of this compound in the irrigation system. Ibuprofen has been reported to degrade rapidly in water and solid matrices (Xu *et al.*, 2009; Yamamoto *et al.*, 2009). However, it is necessary to evaluate the biodegradation of compounds that are poorly adsorbed into soil in irrigation systems where periods of dryness occur; causing large drops in the microbial activity of the soil, and this follows irrigation events that cause anaerobic conditions in the soil for short periods.

In general terms the removal of ibuprofen and 4-nonylphenol was between 66% and 89% in sterile columns, and between 85% and 93%, respectively in non-sterile columns. This indicates that biodegradation is the most important mechanism that determines the fate of both compounds in the irrigation system studied.

3.4 Field study

Organic compounds evaluated: Fig. 4 shows the concentration of organic micropollutants in the upper horizons of the soils studied. It could be observed an accumulation of ibuprofen in the three of them, indicating the occurrence of degradation together with a poor affinity for the soil, and data that reinforces the findings from the batch and column studies. Carbamazepine was retained in the first 30 cm of the soil profile, which suggests that it has a greater affinity for the labile organic material present in the upper profile. In order to clarify this, it is necessary to explore deeply about the quality of the organic material in the soil, and its qualitative and quantitative properties. The concentrations of carbamazepine in the deeper horizons studied could be attributed to the lower content (17 mg/g) or different quality of the organic material present, or to the presence of preferential flows caused by "alfalfa" (*Medicago sativa* L.) and roots of maize (the most common crops in the zone). but not to the biodegradation of this compound that although not evaluated in this study, nevertheless is known to be slow in soil compared with other cultivated species. Similar results have been reported by Kinney *et al.*, (2006) (although for soils irrigated with treated wastewater), where acidic pharmaceuticals similar to ibuprofen were degraded before migration to lower soil layers, while carbamazepine was retained in the first centimeters of the soil. In the light of these results, it appears contradictory that concentrations of tens of ng/L of carbamazepine are found in underground water; it suggests the necessity to study in the field the movement of this recalcitrant compound.

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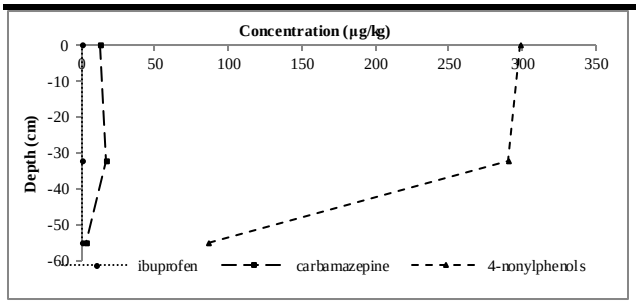

Fig.4: Concentration of organic micropollutants in the first three horizons of the soil profile.

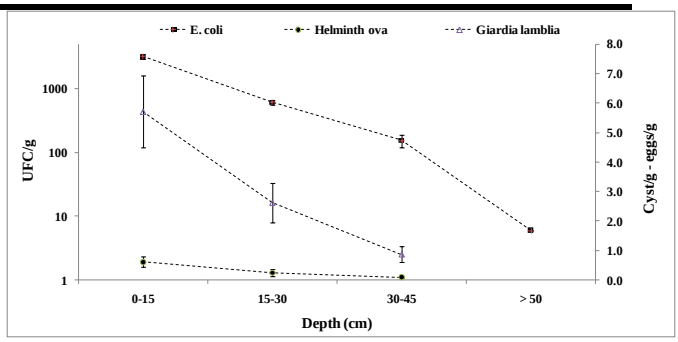

Fig. 5: Concentration of organisms in the first three horizons of the soil profile studied.

Elevated concentrations of 4-nonylphenol suggest that it is not so rapidly degraded in the soil (as was seen in the column study). However, consideration should be given to the fact that the mass of 4-nonylphenol entering the soil in the wastewater (58.2 g/ha year) is greater than that of ibuprofen or carbamazepine (11.4 y 0.53 g/ha year). The decrease in concentration of 4-nonylphenol below 50 cm depth indicates a fall in the adsorption of this compound that explains its presence at trace levels in the local aquifer (Gibson *et al.*, 2007).

Organisms evaluated: Similar to the concentration of the organic pollutants, the organisms showed a decrease in concentration down the profile of the soil studied. The eggs of *A. lumbricoides* seemed to be completely retained in the first 30 cm of the soil horizon. Other studies have found that the removal of helminth eggs from water by filtration through soil occurs in the first 15 cm of the soil profile (O'Lorcain, 1994). However, this still entails a risk to health as these eggs can remain viable for several years. The same process appears to occur with the cysts of *G. lamblia*, whose concentrations tended towards zero with increasing depth. These results are greater than those reported by Mawdsley *et al.*, (1996), who found removal rates of 73% of oocysts of *Cryptosporidium parvum* applied to the soil, mostly by retention in the first 2 cm of the soil profile. The mechanisms that govern the transport of protozoa through the soil have not been completely understood, although Brusseau *et al.*, (2005) suggested, that this could be controlled by processes of colloidal filtration, although studies in this area are still scarce. The removal of *E. coli* reaches 1.5 log units, and this result is in line with the presence of total coliforms (up to 1 x 10^3 CFU/100 mL) in the water of the local aquifer, as well as with observations seen in the adsorption tests (removal of 1 log unit) (Fig. 5).

Previously mentioned authors have suggested that bacteria (such as *E. coli*) are retained in the top 8 cm of the soil during infiltration, although others comment that could be seen at much greater depths, but both are agree with their removal, In contrast, cysts of *G. lamblia* and eggs of *A. lumbricoides* can be removed from the water by simple filtration compared with *E. coli* that cannot be eliminated by that process, another difference is its survival time of the species (2 to 3 months), which is function of soil moisture and temperature as well the effect of the particular microfauna (Gerba and Lance, 1978). Soil clay content is a determining factor in the removal of bacteria from wastewater, so it is important to define which soils are suitable for wastewater irrigation and which pollutants should be eliminated as a priority before the agricultural irrigation.

IV. CONCLUSIONS

The physicochemical properties of the soil (significantly the content of clay and organic matter) promote organic micropollutants and pathogens removal by adsorption and biodegradation. Laboratory tests indicate for ibuprofen and 4-nonylphenol the biodegradation as the dominant removal process when wastewater is used for irrigation. On the other hand, carbamazepine is adsorbed in the soil, presumably in the organic matter content. For the case of organisms, the results show that *E. coli* is removed through adsorption to clay particles, while the removal of *G. lambia* and *A. lumbricoides* cannot be described by typical means of adsorption, implying other mechanisms such as filtration colloidal occur in the system.

Field studies have established that the removal of both organic pollutants and organisms occur in the first 30 cm depth of the soil, which is probably related to strong adsorption of these contaminants to soil organic matter, except for ibuprofen where the main factor for its removal is biodegradation. *E. coli* removal is associated with the adsorption capacity of soils, which is correlated mainly to clay content.

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Competency of *alpha-D-glucopyranosyl* Regulates Cells Death and Functions in Senescence Inhibition

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Abstract— *Senescence is a key process determining the life interval of several cut flowers and sugars added to the nutrient are known to increase vase life in several flowers, very little is known about endogenous variations in* ***glucopyranosyl*** *levels in several floral tissues, predominantly beyond petals, during natural flower development. In present study, we had evaluated the* ***glucopyranosyl*** *efficiency at different concentrations and different time intervals. We had found that the efficiency of* ***glucopyranosyl*** *slightly affect the senescence approaches and played a significant role in inhibition of senescence. Furthermore, it also played a key role on increasing the cells size and enlargement of cells at growing stages of petals. Further we had randomly investigated the content of sucrose in treated petals and control. Interestingly we had found increasing amount of sucrose in treated petals compare to that of untreated. Sucrose contents increased with flower development reaching 50% to 74% higher levels at later stage compared to that of early stagein excised petals.*

Keywords: Senescence, Glucopyranosyl, Petals, Cells, Competency, Sucrose.

I. INTRODUCTION

Beauty is the main reason why cut flowers are sold, much effort has been put on lengthening flower lifespan, one of the major problems in the floricultural commercial sector. In this regards, various studies have been conducted to unravel the pathways that lead floral organs to death so that the resulting scientific and technological advances can be used to extend flower longevity **[1,2]**. Although, senescence is a programmed cells process that does not occur in all floral organs at the same time. According to its specific biological function petals are the first tissues showing signs of senescence, while the gynoecium, particularly the ovary, remains functional throughout all phases of flower development to ensure seed development. In this way, since tepal senescenceis the limiting factor for flower longevity, the main objective off loral senescence studies relies on the maintenance of a viable and visually healthy corolla for a long time **[3].**

Many studies have shown how exogenous sugar added to the vase solution delays the onset of visible signs of flower senescence in several cut flowers, although its role in petal senescence seems to be indirect **[4]**. However, petals can also act as a source during flower senescence **[5]**. Furthermore, cutting the stems leads to a complete reduction of sucrose import to sink tissues of cut flowers and an altered carbohydrate metabolism. Therefore, depending on the floral tissue and stage of development sucrose on the vase solution may have a complex effect on endogenous levels of sucrose and the physiological status of different floral tissues. Exogenous sugar effects on flower longevity may also differ between ethylene sensitive and

insensitive species. In the former, sugars seem to delay petal senescence by reducing ethylene sensitivity **[6,7,8,9]**. In contrast, it has been suggested that in ethylene-insensitive flowers sugars might prevent a decline in osmotic pressure and delay celldeath **[10]**.

In view of the facts stated above, the present study was carried out to evaluate the effect of *Glucopyranosyl* on vase life of rose on controlled conditions with the objectives; to examine the effect of *Glucopyranosyl* at various concentrations on the growth attributes ofthe rose and to inhabit the senescence approaches.

II. MATERIALS AND METHODS

2.1 Plant materials and growth conditions

For infiltration of rose petals, about 1.8-stage cut roses flowers (Samantha) were collected from a greenhouse and brought into the Genomic laboratory. For the treatment of rose flowers, 1.8 stage flowers were collected and stem being cut about 20 to 25 cm under water, and placed in deionized water, more the flowers were placed into solution containing different concentration of *Glucopyranosyl*, and observed the phenotype.

2.2 Infiltration of flowers and petals

The petals were immersed in different concentration of *Glucopyranosyl* infiltrated at vacuum of 0.8 atm. After the release of the vacuum, the petals were washed by deionized water and kept vertically in deionized water for 3 days at 8^{o}C, then transferred to 23^{o}C for 5 days. Photos were taken for the petals every day, and petals were picked for RNA isolation on the 8thday after infiltration. Petal size was measured according to the photos by using the ImageJ software. Statistical testing was performed by using the SPSS software.

2.3 Preparations of different concentration *glucopyranosyl*

The each solution was prepared with supplementation of *glucopyranosyl* at different concentrations i.e. 1%, 3% 5% and 7% and (control) without any *Glucopyranosyl* concentration. Solution was stirred on magnetic stirrer and remaining sterilized distilled water was added to final volume. After the next day, petals were then infiltrated with different concentrations. With treatment of these concentrations, the samples of petals were also been infiltrated with normal water as negative control.

2.4 Measurement of cell size by Scanning Electron Microscopy

Measurement of cell size and encountering the number of cells was performed as described by (Ma et al., 2008). In short petal samples were taken as 0.5-cm 3 0.4-cm slices from regions of petal length from the top. For Scanning Electron Microscopy (SEM), slices of the petal middle region (50% of the length) of fully opened flowers were selected and then fixed and processed according to a standard protocol **[11]**. The slices were flat mounted on either their adaxial or abaxial surface, or fractured to reveal internal anatomy transverse to the petal longitudinal axis. Scanning electron microscopy was performed using a Philip S3400N apparatus. AbsE cell photography and cell counting were performed as described by **[12]**. Petal samples were taken as 0.5-cm 3 0.4-cm slices at 25%, 50%, and 75% of the petal length from the petal top. The slices were fixed in formaldehyde and then cleared in ethanol. AbsE cells of the slices were photographed using a Nikon IX-71 camera. The traces were drawn using Photoshop 7.0 software. Fifteen flowers were used in each treatment. Numbers of AbsE cells were counted using Image J software in a visual field of 1,360 3 1,024 mm

2.5 Experimental procedure

Experiment was performed by according the method reported **[13]**. The plant materials were prepared, about total of 30 petals were used on each treatment supplemented with concentrations of *glucopyranosyl* i.e. 1%,3%,5% and 7% and petals were also immersed in water as a control. The observations were recorded at two days intervals. Further, about 15 whole flowers were collected and placed into solution containing different concentration of *glucopyranosyl*, as described above. For tissue culture plant about 12 plants were used for each treatment applied 3% of *glucopyranosyl* solution at 1:1 interval with water. Experiment was replicated in three biological repeats.

2.6 Statistical analysis

Data representing the mean±standard error (SE) of three replicates were analyzed by one-way ANOVA procedures using the SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Significant differences between the treatment means were evaluated by Duncan's multiple range test at $p<0.05$.

III. RESULTS

3.1 Competency of *glucopyranosyl* on inhibition of excised petal senescence

As we all know and it is well reported that flower opening and senescence are key processes determining the vase life of several cut flowers and sugars added to the vase solution are known to increase vase life in several cut flowers, little is known about endogenous variations in sugar levels in

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several floral tissues, particularly beyond petals, during natural flower development. In uncut flowers, endogenous sucrose contents varied throughout flower development in all floral organs. However in present study, we had evaluated the *glucopyranosyl* efficiency at different concentrations and at different time intervals. We had found that the efficiency of *Glucopyranosyl*s lightly affect the senescence approaches and played a significant role in inhibition of senescence. Furthermore, it also played a key role on increasing the cells sized and enlargement of cells at growing stages of petals. However, further we had randomly investigated the content of sucrose in treated petals and control(Figure 1).Interestingly we had found increasing amount of sucrose in treated petals compare to that of untreated. Sucrose contents increased with flower development reaching 50% to 74% higher levels at stage5 compared to that of stage1 in excised petals(Figure 2).

3.2 Efficiency of *glucopyranosyl* accelerates flower opening

Further we also had investigate the efficiency of sucrose in whole tissue culture plants, it was interestingly to observed that the flower were opened very quickly in *glucopyranosyl* treated plant compare that of control which took normally more time to open the flower.It is of interesting to note that, *glucopyranosyl* levels increase with flower development in petal from different cut rose flower, reaching higher amounts from second stageto fifth stage of flower opening(Figure 3). On the other hand, it seems to be an association between petals sucrose content and flower longevity, where in normal flower it has less sugar content and have a shorter lifespan. Thus, exogenous *glucopyranosyl* might expand vase life of rose cut flowers by counteracting that possible lack of carbohydrates due to stem cutting. In present study, flower durability from early stage to later stage was not affected by sucrose addition to the vase solution. However, results showed an acceleration of flower opening days.

3.3 Effectiveness of *glucopyranosyl* involved in delaying senescence

It is well known reports that, the process of senescence is the final stage of flower development that significantly precedes the termination of the floral organ of any plant. However, in present work we had investigated that *Glucopyranosyl* drastically delayed the senescence of flowers(Figure 5). However during our first parameter we found the specific efficiency of sucrose on excised petals. In present study we had applied about 3% of *Glucopyranosyl* concentration on each of plants, further we applied the water about 1:1 intervals with *Glucopyranosyl* (Figure 6). Interestingly we found significant effect of *Glucopyranosyl* concentration on delaying durability of rose flowers compare to that of control (plant grow without *Glucopyranosyl* treatment).

3.4 *Glucopyranosyl* involved in increasing of cells enlargement

Amusingly further we found that the efficiency of *Glucopyranosyl* drastically increased the cell density and as well as the number of cells. However after treatment at two days intervals we had investigated the microscopic examination of sucrose treated and without addition of *Glucopyranosyl*. Surprisingly from the early stage to later stage, the sizes of cells were outstandingly increased compare to that of normal grow plants and excised petals as well(Figure 7). The results showed that the rose treatedwith *Glucopyranosyl* concentration produced flowers with maximum diameter (7.61 cm);while, the lowest average flower diameter (2.23 cm) was observed in control without addition of any *Glucopyranosyl* treatment.

IV. DISCUSSIONS

Flower opening and senescence are key processes of determining the vase life of several cut flowers and sugars added to the vase solution are known to increase vase life in several cut flowers, little is known about endogenous variations in sugar levels in several floral tissues, particularly beyond petals, during natural flower development. In uncut flowers, endogenous sucrose contents varied throughout flower development in all floral organs. The treatment comprised of sucrose at 3%concentration prolonged the time period required for opening the flower; these results are in line with those of **[14]** who reported that treatment of cut flowers with sucrose potentially affected the physiological factors of roses and with application of these solutions at higher rates the days to open flower were increased.

Although it was found that, the *Glucopyranosyl* efficiency at different concentrations and at different time intervals affect the petals of flowers in many aspects. Further had found the efficiency of *Glucopyranosyl* slightly affects the senescence approaches and played a significant role in inhibition of senescence, these results remarkably supported with the evidence of **[15]** who reported the role of sugar in senescence of cut flowers. Furthermore, it also played a key role on increasing the cells sized and enlargement of cells at growing stages of petals. However,

further we had randomly investigated the content of sucrose in treated petals and control. Interestingly we had found increasing amount of sucrose in treated petals compare to that of untreated. Sucrose contents increased with flower development reaching 50% to 74% higher levels at stage-5 compared to that of stage-1 in excised petals. Furthermore, it is worthy to note that the major decreases in sugar levels were observed between stages IV and V in normal petals compare to that or treated petals. In any case, cytokinins are known to delay flower senescence in several species irrespective of their sensitivity to ethylene **[16]**, therefore, sucrose-mediated increases in cytokinins may be responsible for the delay of senescence**.**

***Glucopyranosyl* accelerates flower opening and delaying of senescence**

It was noticing to observed that the efficiency of sucrose in whole tissue culture plants, was interestingly showed significant effect on flower opening as it was opened very quickly in *Glucopyranosyl* treated plant compare that of control which took normally more time to open the flower. Morover, *Glucopyranosyl* levels increase with flower development in petal from different cut rose flower, reaching higher amounts from stage 2 to stage 5 of flower opening stage. On the other hand, it seems to be an association between petals sucrose content and flower longevity, where in normal flower it has less sugar content and have a shorter lifespan. However, results showed an acceleration of flower opening days**[16]**. While, the process of senescence is the final stage of flower development that significantly precedes the termination of the floral organ of any plant**[17]**. However, in present work it as investigated that *Glucopyranosyl* drastically involved in senescence of flowers. Further found that about 3% of *Glucopyranosyl* concentration on each of plants showed significant effect, compare to that of control without *Glucopyranosyl* treatment. Interestingly we found significant effect *Glucopyranosyl* concentration on delaying durability of rose flowers compare to that of control, these work have been supported with the work of **[18]**. Further, it was amusingly found that the efficiency of *Glucopyranosyl* drastically increased the cell density and as well as the number of cells. However, microscopic examination was done at two days intervals and revealed cells enlargement from the stage 2 to stage5 of treated rose petals, the sizes of cells were outstandingly increased compare to that of normal grow plants and excised petals as well. The flower opening is one of the key factors that possess economically consequence that influences the common performance of a flowering prickle**[19]**. In rose plants treated with *Glucopyranosyl* at 3% concentration, the number of dropped flowers maximally reduced to 1.32;although, the highest number of dropped petals (5.32) was recorded under controlled plants where only distilled water was supplied without addition of *Glucopyranosyl* **[20]**.The results showed that the rose treated with *Glucopyranosyl* at 3% concentration produced flowers with maximum cells size.

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CONFLICT OF INTEREST

All authors disclosed that they have no any conflict of interest.

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Figures

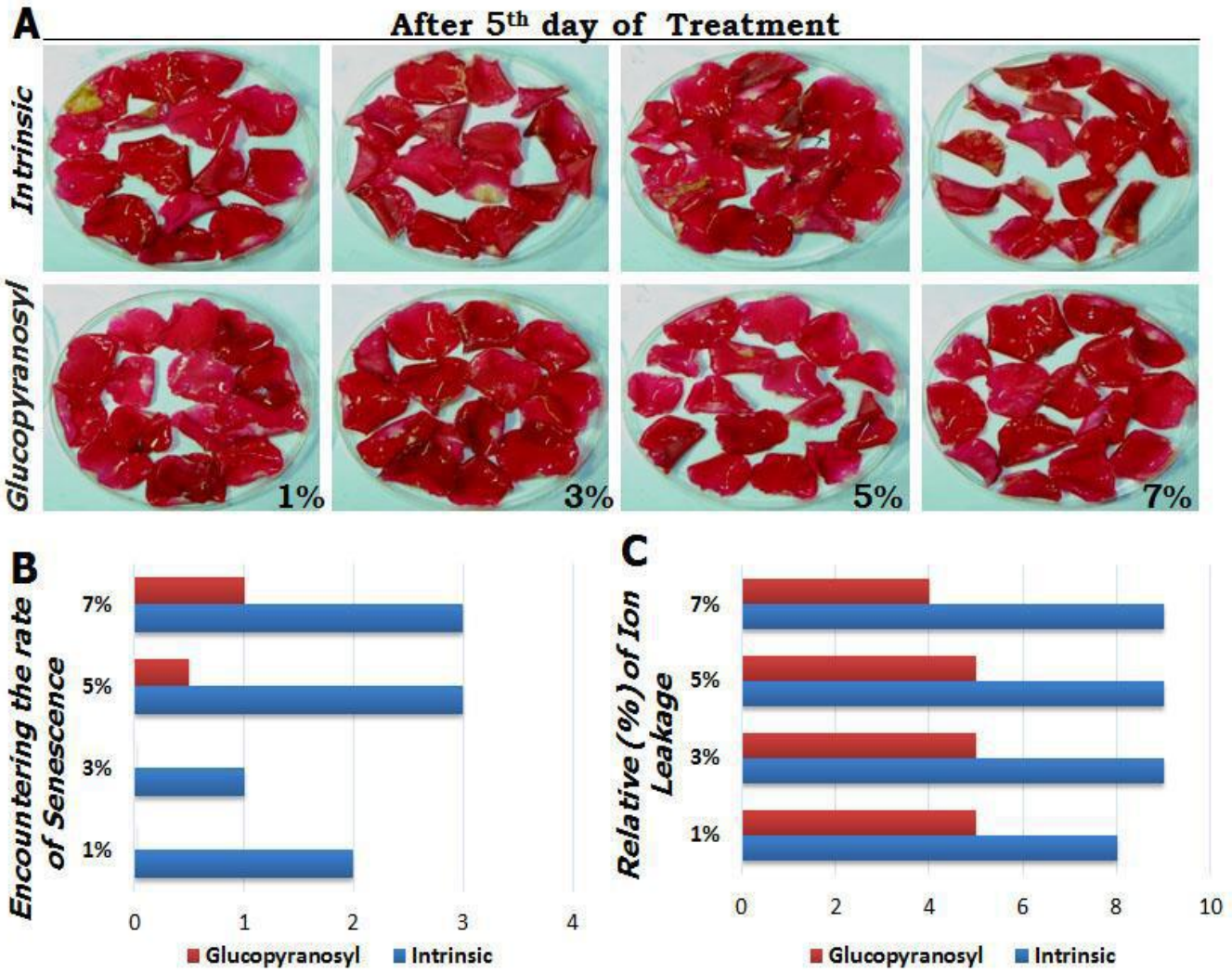

Fig.1: Efficiency of glucopyranosyl on inhibition of excised petal senescence. (A) Phenotypic analysis of after 5 days of glucopyranosyltreatment. (B) Encountering the rate of Senescence. (C) Relative percentage of Ion Leakage. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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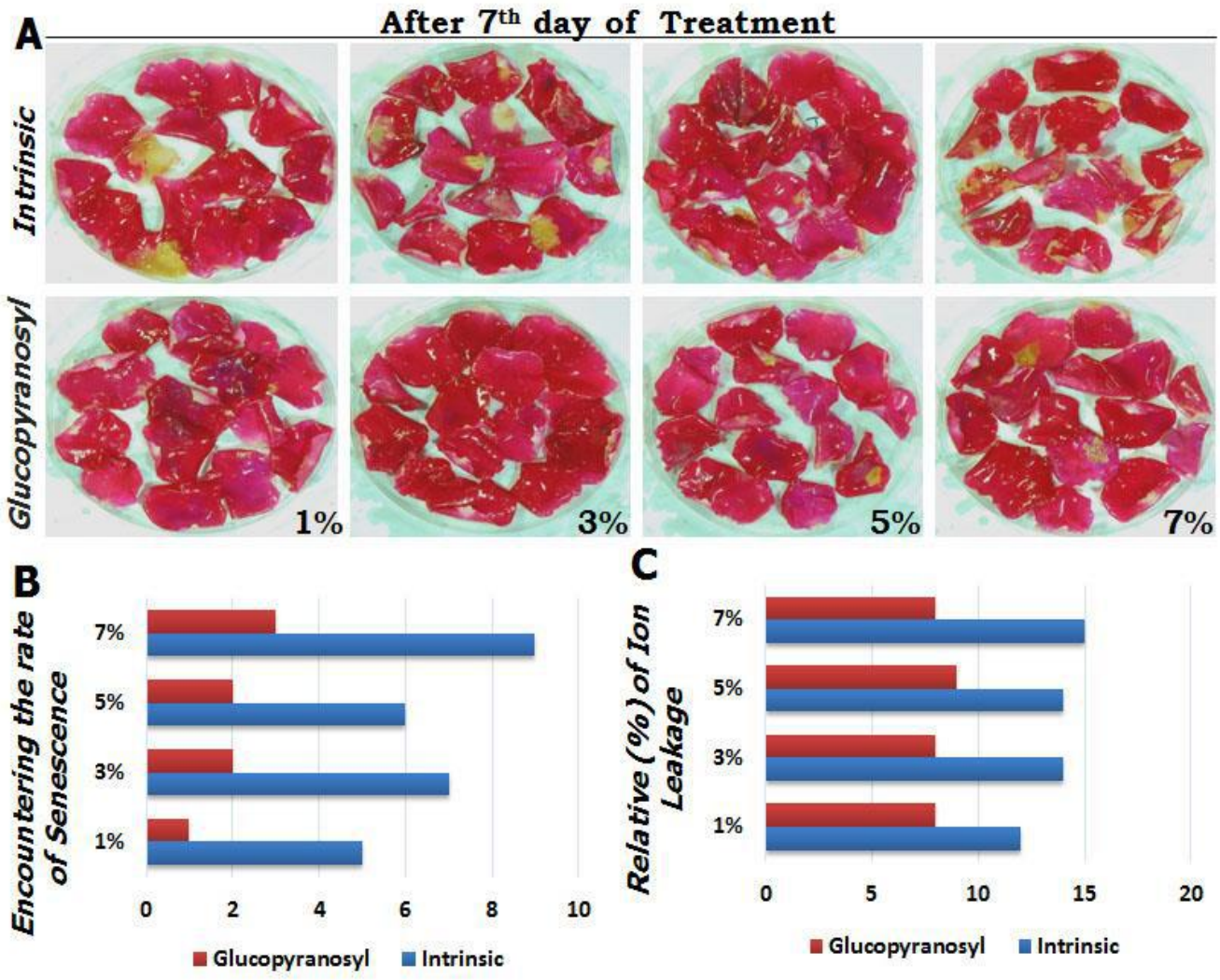

Fig.2: Glucopyranosyl inhibited the excised petal senescence. (A) Phenotypic analysis of after 7 days of glucopyranosyl treatment. (B) Encountering the rate of Senescence. (C) Relative percentage of Ion Leakage. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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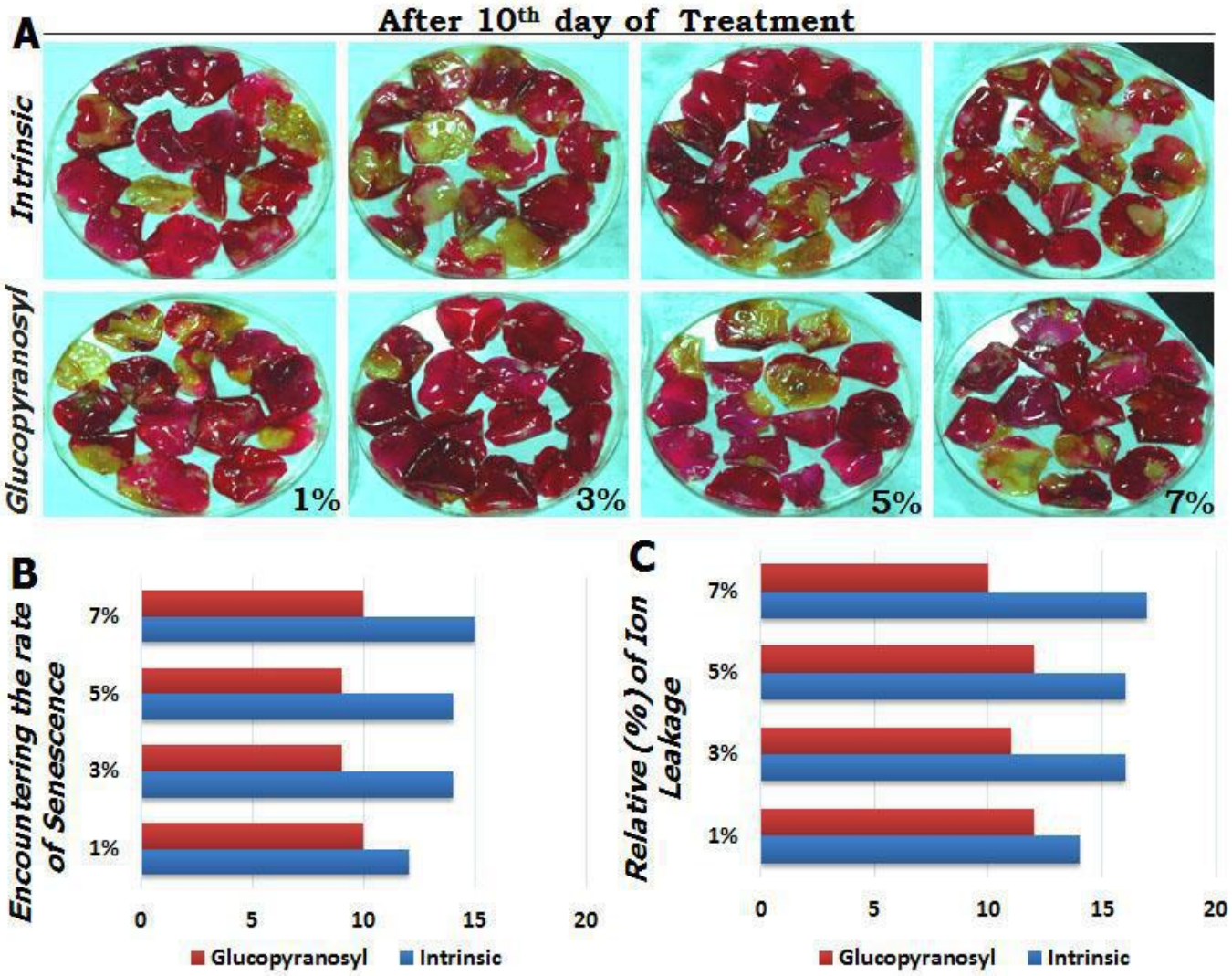

Fig.3: Glucopyranosyl inhibited the excised petal senescence. (A) Phenotypic analysis of after 10 days of glucopyranosyltreatment. (B) Encountering the rate of Senescence. (C) Relative percentage of Ion Leakage. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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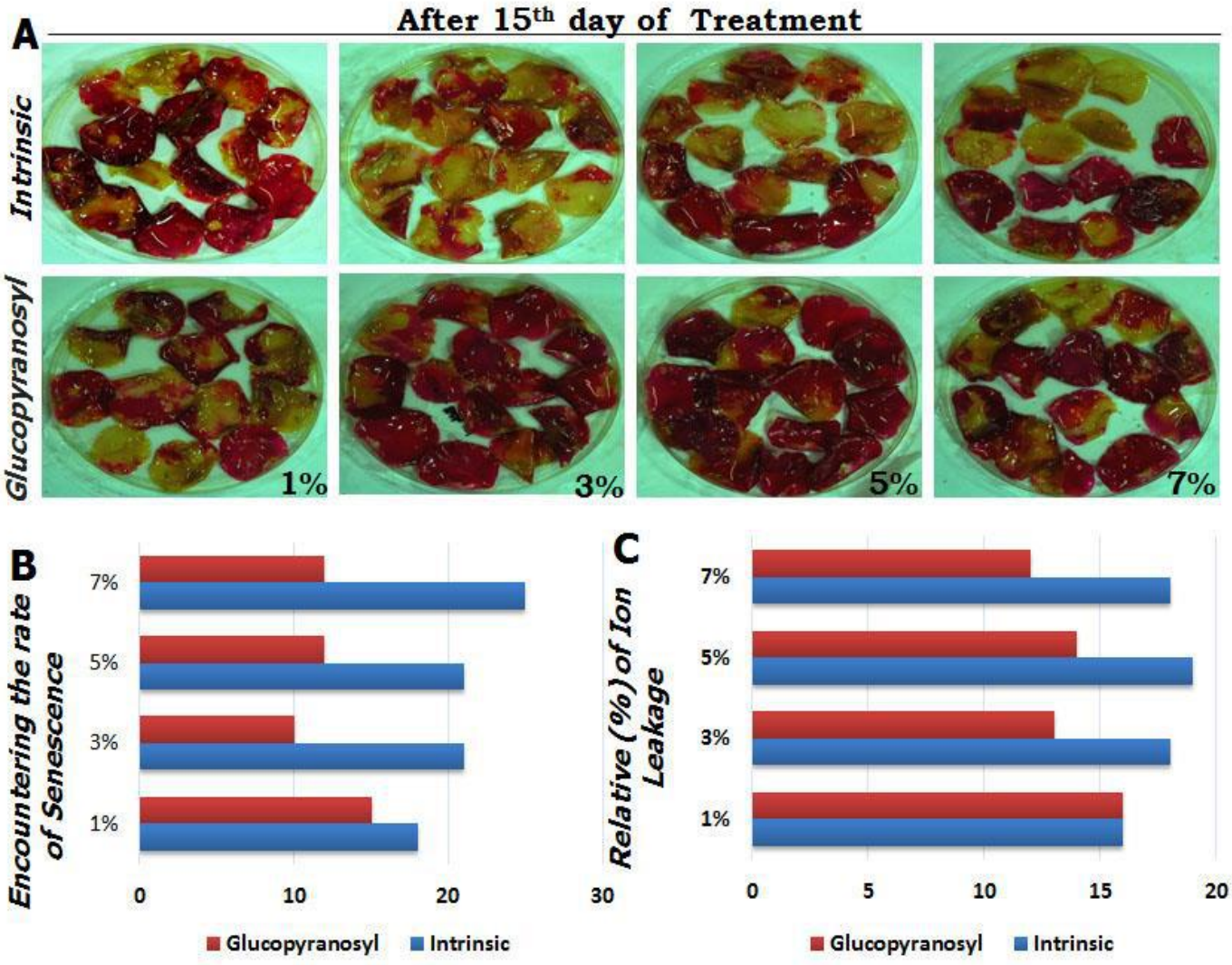

Fig.4: Effectiveness of Glucopyranosyl involved delaying of senescence. (A) Phenotypic analysis of after 15 days of glucopyranosyl treatment. (B) Encountering the rate of Senescence. (C) Relative percentage of Ion Leakage. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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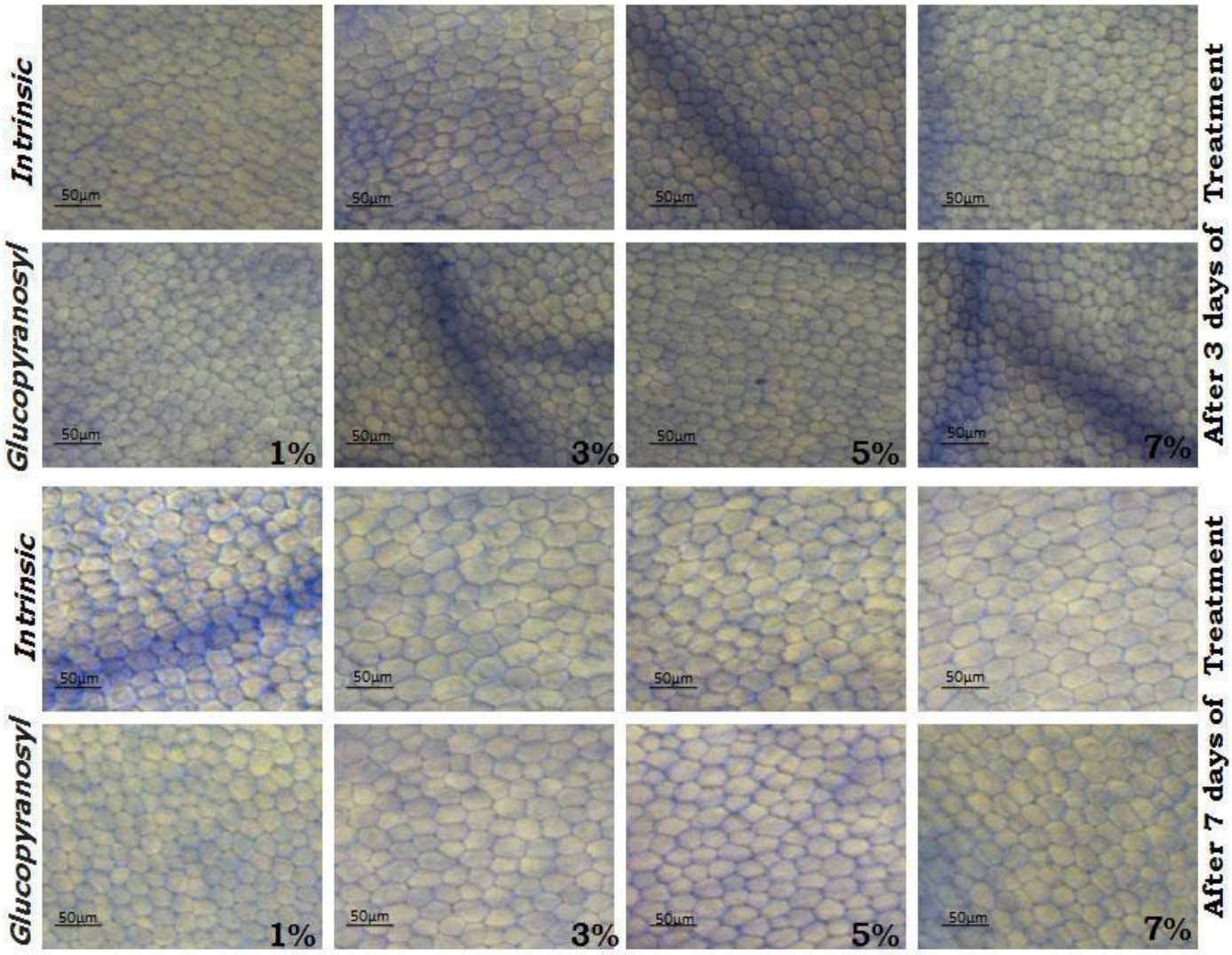

Fig.5: Histological examination of glucopyranosyl treated petals of rose after a week interval. Scanning Electron Microscopy (SEM) was performed using a Philip S3400N apparatus. AbsE cell photography and cell counting were performed as described by (Dewitteet al., 2007). Photograph was performed using a Nikon IX-71 camera. Numbers of AbsE cells were counted using Image J software in a visual field of 1,360 3 1,024 mm. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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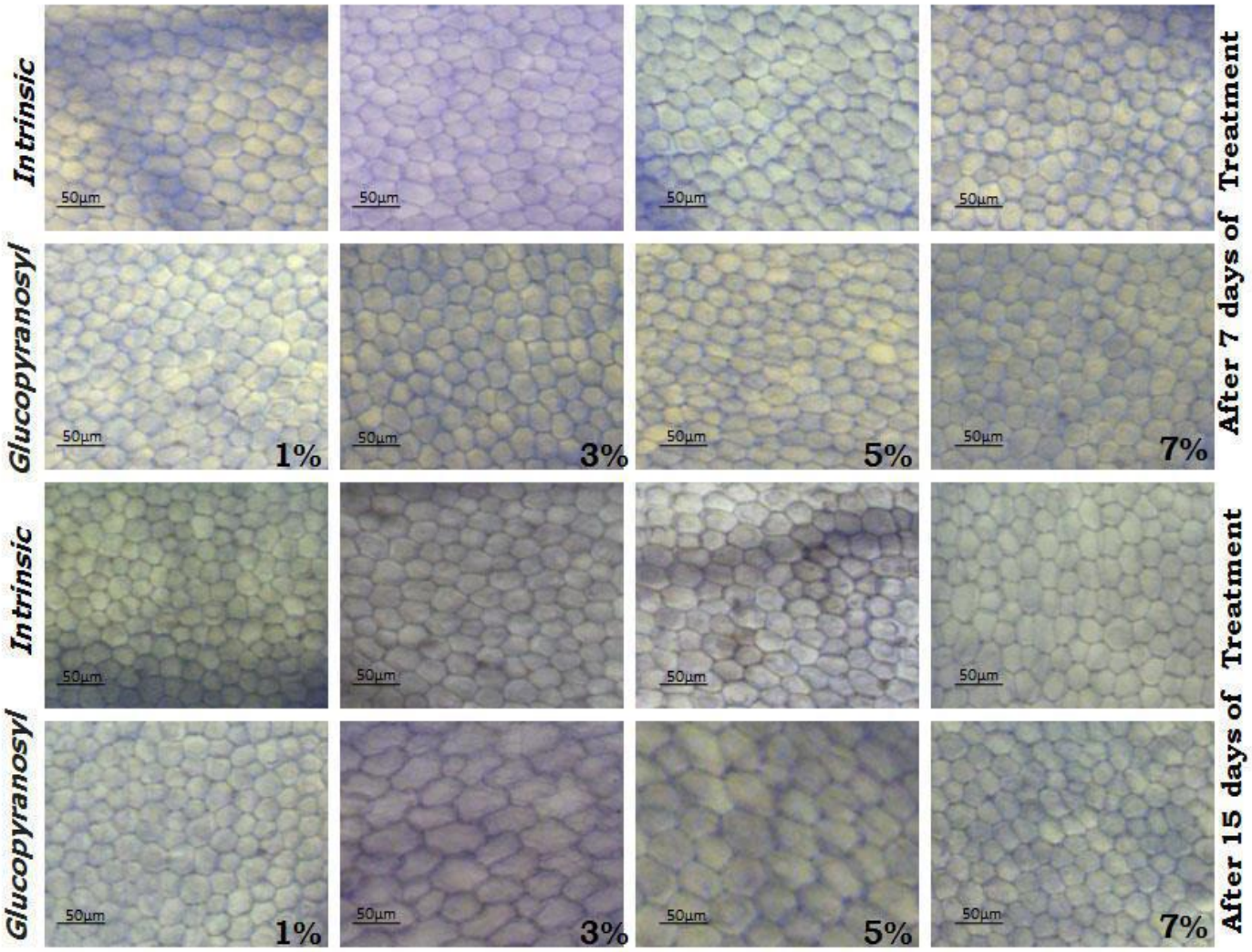

Fig.6: Histological examination of glucopyranosyl treated petals of rose after two weeks. Scanning Electron Microscopy (SEM) was performed using a Philip S3400N apparatus. AbsE cell photography and cell counting were performed as described by (Dewitteet al., 2007). Photograph was performed using a Nikon IX-71 camera. Numbers of AbsE cells were counted using ImageJ software in a visual field of 1,360 3 1,024 mm. Various concentrations of glucopyranosyl at different time intervals applied. Observation is the concise with three biological repeats.

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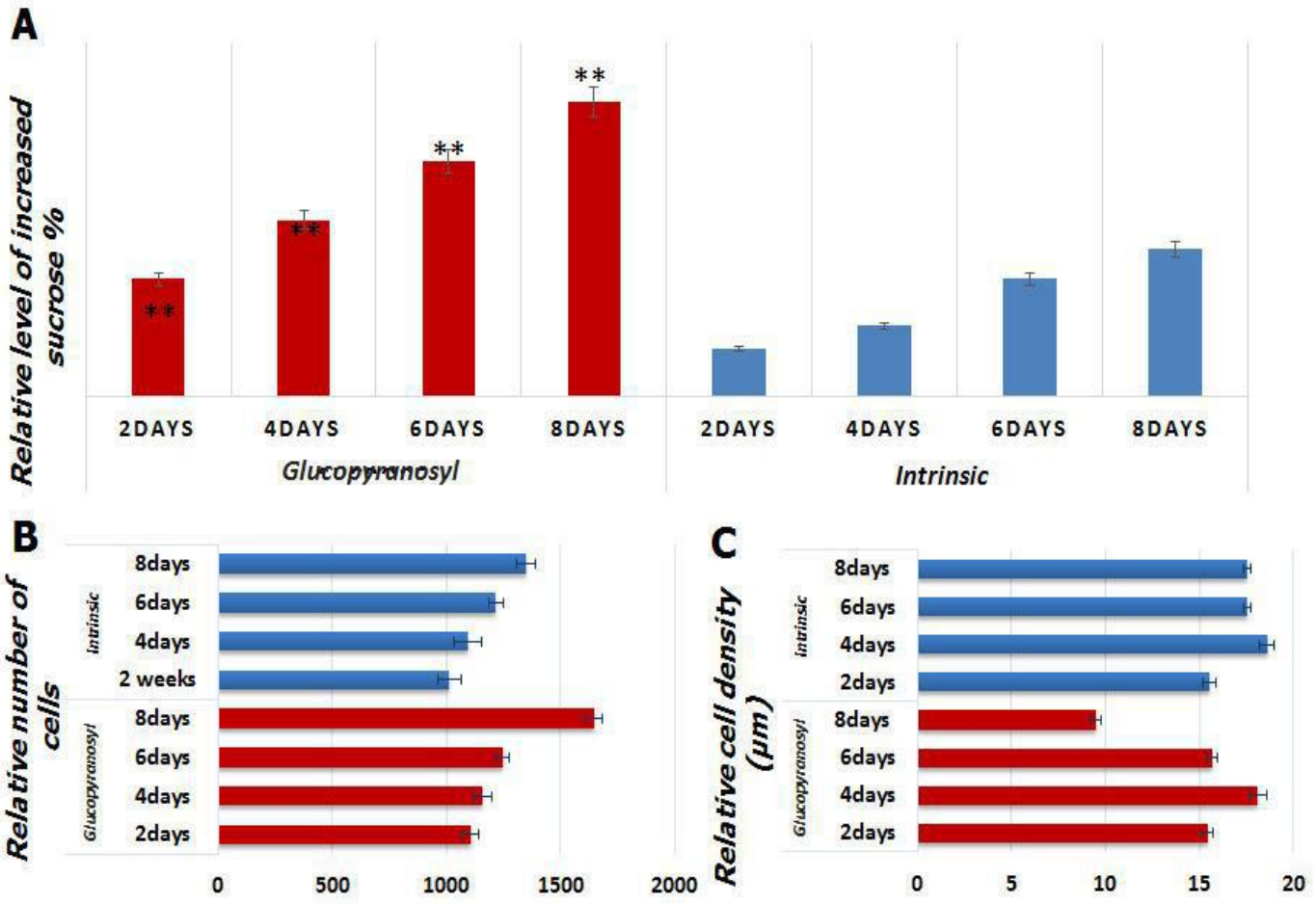

Fig.7: Glucopyranosyl elaborate to increase the cells size. (A) Increase the level of sucrose content. (B) Relative number of cells. (C) Relative cells density. Observation is the concise with three biological repeats.

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Studies on Vermifiltration of Restaurant Effluent and Reuse in Benue State, North Central, Nigeria

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***Abstract*—** *Vermifiltration was used for the treatment of restaurant effluents in Benue state. Four vermi-bio-filters were used; V_1 (Lumbricusterritris), V_2 (Eudriluseugeniae), V_3 (Eiseniafetida), V_4 (mixture of Lumbricusterritris, Eudriluseugeniae and Eiseniafetida) and V_5 (Control without earthworm-geofilter). The effluent from vermifilters and control were analysed for microbial load. The Earthworms' body worked as a 'biofilter' and as a bioreactor with the mechanism of 'ingestion' and biodegradation of organic wastes. Bacteria analysed in the six effluents (Staphylococcus spp, Streptococcus spp, Escherichia coli, Salmonella spp, Enterobacterspp, Proteus spp and Pseudomonas spp) and fungi (Aspergillus, Penicillium Rhizopus and Mucor. Saccharomyces cerevisae) were reduced in the range of 70.0-97.7% and 68.3-97.4% respectively. Analysis of Variance (ANOVA) to determine the differences in microbial load concentration between the influent and effluent were considered significant at 5% level ($p \leq 0.05$). The synergistic action of enzymes, microorganisms and earthworms significantly reduced the amount of microbial load compared to a geo-filter, without earthworms' presence (control). Vermifiltration is a low cost technology recommended for treatment of effluent before discharge into the environment. This is an odor-free process and no sludge formed in vermin-filtration. Vermifiltration technology can be applied as an environmentally friendly technique as the treated effluents met the set standards for irrigation purpose, parks, gardens and hydroponics.*

***Keywords*— Environment, Effluent, Microbial load, Treatment, Re-use, Vermifiltration.**

I. INTRODUCTION

Wastewater treatment is the removal of contaminants from any form of wastewater and it includes physical, chemical and biological processes so that the water can be re-used (Kharwade and Khedikar, 2011). As water pollution critically affects water availability, it needs to be properly managed in order to mitigate the impacts of increasing water scarcity (UN WWAP 2017). Access to safe water is a human right (UNDP, 2006). Over (90%) of diarrhoea diseases are caused by unsafe water supply and unhygienic sanitation (WHO, 2004).Vermifiltration of wastewater using waste eater earthworms is a newly conceived novel technology with several advantages over the conventional systems. It is eco-friendly and sustainable for the treatment of wastewater, and appeared to have high treatment efficiencies without the formation of sludge (Damodhar *et al*., 2014). Earthworms feed readily upon the sludge components; rapidly convert them into vermicompost without odor, reduction in pathogens to safe levels. Treated bio-clean water can be reused for secondary purpose like floor washing, toilet-flushing, gardening, hydroponics and irrigation etc. except for direct human consumption (Aguoru*et al*., 2015).

II. MATERIALS AND METHODS

Area of the Study

The area of study covered restaurant effluents produced within the three geopolitical zones (A, B, and C) of Benue State. Benue state coordinates are: Latitude 8^0 08' 00"N, 6^0 26' 00"S and Longitude 9^0 54' 00" E, 7^0 30' 00"W

Collection of Specimens (Earthworms) and Samples

The species of earthworms native to each of the three geopolitical zones of Benue state were collected from their habitats by Hand sorting technique as described by Glenn (2006) and identified using the method described by Ansari and Saywack (2010).Earthworms' species used were *Lumbricusterrestris* (Night Crawler), *Eudriluseugeniae* and *Eiseniafetida*. A total of fifteen restaurant water samples were collected; five samples from each zone.

Vermifilter System

The effluent was fed by gravity flow with the help of sprinkler to avoid direct hydraulic influence on the earthworms. An infusion set was used to control the volumetric flow rate of wastewater (Bhise, 2015). The body of the reactor is made of PVC drum. The depth of 40cm was divided into 4 parts in which gravel, sand and soil bed for earthworm (weight of 75g). The soil was compacted using a

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piston. The garden soil and compositing material (sawdust and cow dung) were mixed at a volume ratio of 3:1 (Lakshmi *et al*., 2014). Pieces of baked clay pot were used to sandwich PVC drum and vermifilter to regulate temperature of the system. The system has provisions to collect the filtered water at the bottom which opens out through a pipe fitted with tap. The topmost layer of about 10 cm consists of soil bed in which the earthworms were added. The worms were given one week settling time to acclimatize in the vermifilter. A plastic mesh covered the top to prevent escape of worms and from predators. A layer of plastic mesh was placed below the layer of soil bed to allow only water to trickle down while holding the earthworms in the soil bed (Bhise, 2015). There are five vermilfter units labeled V1, V2, and V3 containing 1 kg of *Lumbricusterrestris*, *Eudriluseugeniae* and *Eiseniafetida* respectively. The fourth (V4) was a mixture of *Lumbricusterrestris*, *Eudriluseugeniae* and *Eiseniafetida*while the control was (V5) without earthworms.

Dissection of Earthworms

The peripheral surfaces of earthworms were disinfected with sodium hypochlorite (25 ppm) for 10 min before dissection. The gut contents of each segment were squeezed into sterile test tubes using a sterile scalpel, forceps, and sterile knife under aseptic conditionRavindran*et al*., (2014). The collected gut content (l g) was diluted in saline solution (NaCl, 0.9% (w/v)) and microbial diversities were determined (Prakash and Karmegam, 2010). The serial dilutions were made up to 10^{-6} dilution and an aliquot of 0.1 mL was spread onto plates of the agar media. The plates were then incubated at 30°C for 24 h for bacteria and 28°C for 5 days for fungi. The average per dilution will be determined and multiplied by the reciprocal of the dilution ratio and expressed as colony-forming units per milliliter (CFU/ml) of the sample (Amadi and Ayogu, 2005).Identification of bacteria will be based on Bergey's Manual of Determinative Bacteriology (Krieg and Holt, 1984).

Data analysis

Percentages were used to determine the reduction in microbial load after vermifiltration. One-way Analysis of variance (ANOVA) was carried out with a software statistical package (SPSS version 21) to test the existence of statistical significant differences and significant differences were detected the Duncan's New Range Multiple Test (DNRMT) to be significant at ($p<0.05$). The mean values compared with National Environmental Standards and Regulations Enforcement Agency (NESREA) standards for surface water (Aguoru*et al*., 2015).

III. RESULTS AND DISCUSSION

Reduction of Bacteria in Restaurant Effluent

Table 1 shows the percentage reduction of restaurant wastewater for 360 days. The mean bacteria count in Restaurant effluent in this study ranged from 3.66 x 10^{-4}CFU/ml to 5.04 x 10^{-4}CFU/ml representing 70.5 % in effluent treated by *Eudriluseugenia* (V_2) within (60) days to 93.6% in effluent treated by treatment mixture of *Lumbricusterritris*, *Eudriluseugeniae*and *Eiseniafetida*) within 180-240 dayswas lower than the findings of 5.5 x 10^{-7} to 7.4 x 10^{-7}CFU/ml before treatment by Ogidi and Oyetayo, (2012). Percentage reduction in Control (V_5) ranged from 50.9 % -55.8%. Figure 1 shows the highest result of bacteria reduction in restaurant wastewater after vermifiltartion between 121–180 days. Analysis of Variance (ANOVA) shows significance difference F (calculated) =3.36<F (tabulated) 231.6 at $P < 0.05$. The bacteria isolates from restaurant include *Escherichia coli*; *Pseudomonas* spp.; *Staphylococcus* spp., *Shigelladysenteriae*, *Proteus vulgaris*, *Klebsiella*spp and *Salmonella typhi*. These pathogens can cause human health hazards. The high occurrence of *Staphylococcus aureus*may be due to shedding of resident *Staphylococcus aureus*in human skin which may contaminate food and water during handling, processing and washing of hands after eating.

The high amount of bacteria in the restaurant effluents may be as a result of drained wastewater having contact with the soil already contaminated with decomposed waste foods dumped nearby which may be source of nutrient for the organisms along the drainage. Since most bacteria live under starvation conditions or water stress in the soil, they have adapted to quickly reproduce when water, food, and the environmental conditions are abundant. These organisms are versatile in utilizing the limited nutrient and have the ability to adapt to the toxic condition of soap and detergent contained in restaurant wastewater. Bacteria populations can easily double in 30 minutes (Nester *et al*., 2007).Foul odor during processing was trmoved (Hughes *et al*. 2011; Sinha*et al*. 2008). They create aerobic conditions in the waste materials by their burrowing actions, inhibiting the action of anaerobic microorganisms which release foul-smelling hydrogen sulfide and mercaptans (Sinha*et al*., 2009).

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Table.1: Percentage Reduction of Bacteria in Restaurant Effluent (10^{-6} CFU/ml)

No of days	I	V_1	%	V_2	%	V_3	%	V_4	%	V_5	%	NESREA(2011) <400 CFU/ml
0-60	4.14	1.14	72.5	1.22	70.5	1.02	75.4	0.84	79.7	1.50	63.8	
61-120	3.84.	0.86	77.6	0.94	75.5	0.74	80.7	0,58	84.9	1.32	65.6	
121-180	4.60	0.98	78.7	1.06	76.9	0.78	83.0	0.64	86.1	1.68	63.5	
241-300	5.04	0.87	82.7	1.00	80.2	0.66	86.9	0.50	90.1	1.72	65.3	
181-240	3.66	0.54	85.2	0.69	81.1	0.38	89.6	0.27	92.6	1.47	59.8	
301-360	4.82	0.65	86.5	0.72	85.1	0.47	90.2	0.31	93.6	1.81	62.4	
Mean ±S.D	**4.35± 0.51**	**0.84± 0.19**		**0.94 ± 0.2**		**0.68± 0.19**		**0.52 ± 0.2**		**1.58± 0.17**		

F=3.3 D.F. = 4 ($p<0.05$)

I= influent (Before treatment)
%= percentage reduction
V1=effluent (treatment with *Lumbricusterritris*)
V2= effluent (treatment with *Eudriluseugeniae*)
V3 = effluent (treatment with *Eiseniafetida*)
V4= effluent (mixture of *Lumbricusterritris, Eudriluseugeniae* and *Eiseniafetida*)
V5= Control (without earthworm)

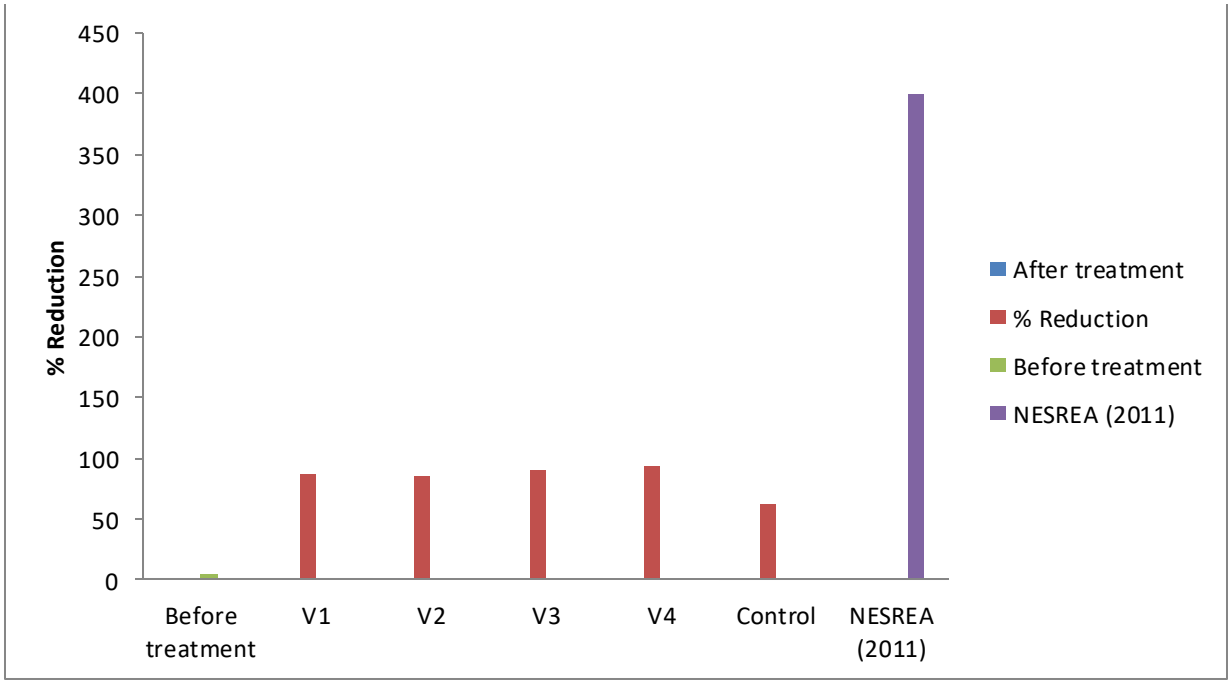

Fig.1: Vermifiltration of bacteria (10^{-6} CFU/ml) in restaurant effluent

Reduction of Fungi in Restaurant Effluent

Table 2 shows the mean Fungi count in Restaurant wastewater in this study ranged from 3.76 x 10^{-4}CFU/ml 5.22 x 10^{-4}CFU/ml shows the percentage reduction of fungi for 360 days in restaurant wastewater representing 85.8 % in effluent treated by *Eudriluseugeniae* (V_2) within (60)

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days to 96.7% in effluent treated by treatment mixture of *Lumbricusterritris, Eudriluseugeniae*and *Eiseniafetida*) within 120-180 days which corroborates with the result of 4.1×10^{-4}sfu/ml to 5.2×10^{-4} CFU/ml before vermifiltration by Ogidi and Oyetayo (2012) Percentage reduction in Control (V_5) ranged from 68.4 % -74.8%.. Figure 2 shows the highest result of fungi reduction in restaurant wastewater after vermifiltartion between 121–180 days. ANOVA shows F (calculated) =2.72<F (tabulated) 231.6 at $P < 0.05$. There is significant difference between the mean values.

The fungi isolates in restaurant wastewater includes *Saccharomyces cerevisae, Mucor spp. Aspergillus fumigates* and *Penicillium spp*. The presence of *Rhizopusstoloniferw*hich is commonly found growing on bread and soft fruits such as bananas and grapes are capable of causing opportunistic infections of humans (zygomycosis) could be ascribed to the presence of its spores (Aslankoohi, 2013). *Aspergillusniger*is known to causes a disease called black mold on certain fruits and vegetables. Inhalation of *Aspergillus*sp. can result in Asthma with difficulty in breathing. A large Aspergilloma in the lungs can block respiratory gas exchange and cause death due to asphyxiation (Ronald, 2003). *Rhizobium* sp. on the other hand can infect leguminous plant roots, where they cause formation of tumorous growth root nodules with which they live in a mutually beneficial relationship with leguminous plant thus enhancing their growth.The microbial removal can be attributed to the presence of earthworms, which is known to reduce organic matter content of wastewater thus making the environment unsuitable for pathogens. This is because under favorable conditions, there is a symbiotic interaction of earthworms and microorganisms to enhance the decomposition of the organic matter that causes the release of coelomic fluids from their body cavity (Sinha *et al.,* 2008). This fluid has antibacterial properties, which destroy all the pathogens from the media in which it inhabits (Kumar *et al.,* 2014). Bacteria and fungi results in the restaurant effluent are within the permissible limit of 400CFU/ml set by National Environmental Standards and Regulations Enforcement Agency (NESREA, 2011).

Table.2: Percentage Reduction of Fungi Load in Restaurant Effluent

No of days	**I**	**V_1**	**%**	**V_2**	**%**	**V_3**	**%**	**V_4**	**%**	**V_5**	**%**	**NESREA (2011) <400CFU/ml**
0-60	4.43	0.36	91.9	0.32	92.8	0.26	94.1	0.22	95.0	1.40	68.4	
61-120	5.22	0.56	89.3	0.46	91.2	0.33	93.7	0.18	96.7	1.48	71.6	
121-180	4.60	0.40	91.3	0.46	90.0	0.39	91.5	0.34	92.6	1.32	71.3	
241-300	3.76	0.24	93.6	0.30	92.0	0.28	92.6	0.20	94.7	1.10	70.7	
181-240	4.20	0.54	87.1	0.61	85.5	0.44	89.5	0.32	92.3	1.06	74.8	
301-360	5.12	0.60	88.3	0.52	89.8	0.48	90.6	0.30	94.1	1.40	72.7	
	4.55± 0.3	**0.45± 0.1**		**0.44± 4.9**		**0.36 ±0.1**		**0.26 ±0.06**		**1.29 ±0.2**		

F=2.72 D.F. = 4 ($p<0.05$)

I= influent (Before treatment)
%= percentage reduction
V1=effluent (treatment with *Lumbricusterritris*)
V2= effluent (treatment with *Eudriluseugeniae*)
V3 = effluent (treatment with *Eiseniafetida*)
V4= effluent (mixture of *Lumbricusterritris, Eudriluseugeniae*and *Eiseniafetida*)
V5= Control (without earthworm)

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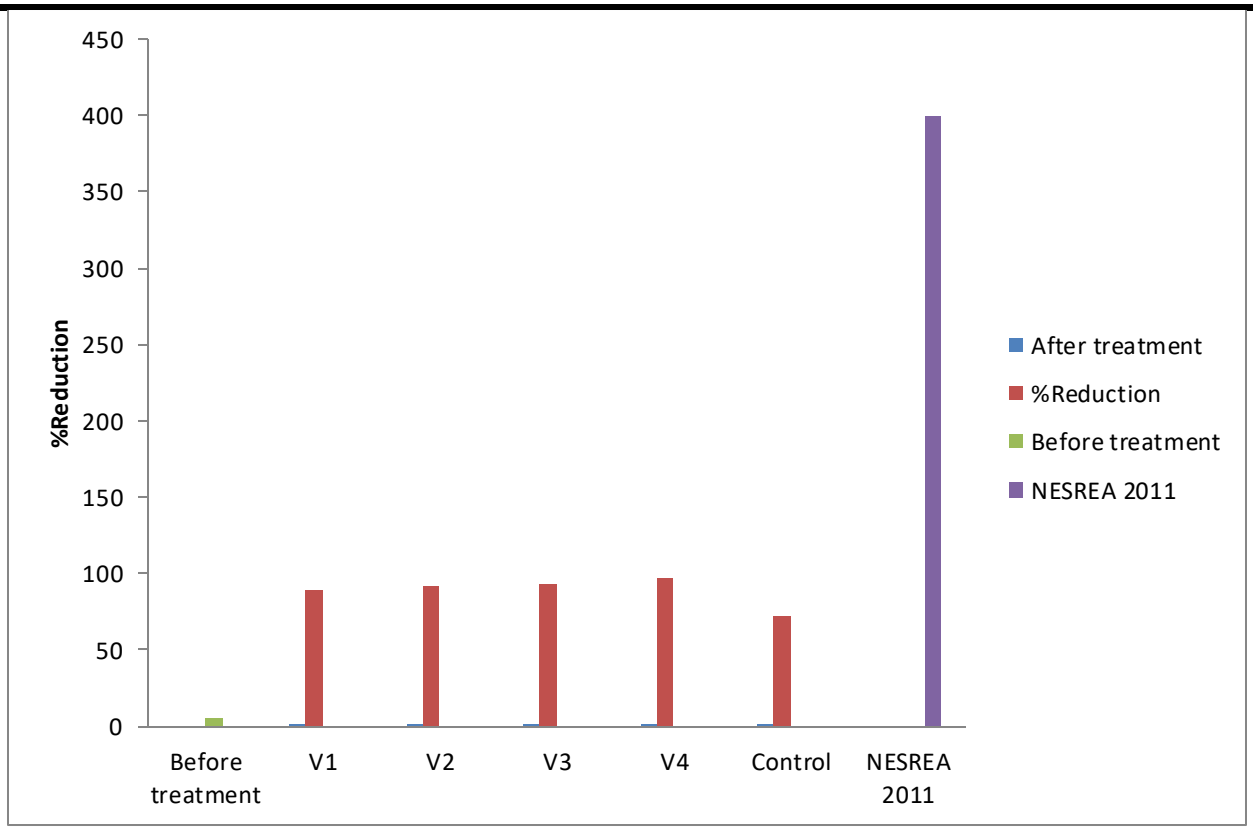

Fig.2: Vermifiltration of fungi (10^{-6} CFU/ml) load in restaurant effluent

IV. CONCLUSION

Wastewater treated with earthworms (vermifiltration) has proven that earthworms are capable of reducing microbial load in wastewater, thus reducing the high rate of pathogenic organism using ecofreindly method that is sustainable. Among the three earthworm species, *EiseniaFetida*is best suited for treatment of waste water, followed by *Lumbricusterritris* and lastly, *Eudriluseugeniae*. There is no foul odor as the earthworms arrest rotting and decay of all putrescible matters in the wastewater and the sludge. They also create aerobic conditions in the soil bed and the waste materials by their burrowing actions, inhibiting the action of anaerobic microorganisms which release foul-smelling hydrogen sulfide and mercaptans.No sludge formed in the vermin-filtration unit during the wastewater treatment. The waste water treated through this method was clear and good enough for irrigation purpose or parks and gardens.

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Mycorrhizal Colonization, Growth and Yield of Several Promising Lines of Black Rice between Sterilized and Non-Sterilized Soil

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***Abstract*—** *Sterilization of plant growing media was reported to have different effects on different crops. This research was aimed to examine the effects of autoclave sterilization of soil (sterilized vs non-sterilized) on root colonization by indigenous arbuscular mycorrhizal fungi (AMF), growth and yield of various genotypes of promising lines of black rice in pot culture. The pot culture experiment was conducted in a glasshouse by arranging the treatments in a factorial Completely Randomized Design with three replications. The results indicated that autoclave sterilization of soil for growing black rice significantly reduced AMF colonization, productive tiller number, average panicle length, and grain yield per clump but increased weight of 100 grains. However, there were significant interactions between sterilization and rice genotypes on plant height, AMF colonization levels, average panicle length, grain yield per clump, and weight of 100 seeds, which means different genotypes showed different responses to soil sterilization. Among seven genotypes of black rice tested, G12 showed the highest grain yield (50.93 g/pot) and G8 showed the lowest yield (37.29 g/pot) due to the highest reduction in grain yield on the sterilized soil (46.00 g/pot vs 28.58 g/pot).*

***Keywords*— arbuscular, black rice, mycorrhiza, soil sterilization**

I. INTRODUCTION

Ideally, growing media for use to grow crops of high economic value should be free of soilborne pathogens and should contain sufficient amount of required nutrients. Therefore, before growing those crops, the soil or growing media are usually disinfested or sterilized to avoid infection by soilborne pathogens. According to Lazarovits and Subbarao [3], Verticillium wilt, caused by the fungus *Verticillium dahliae* Kleb, is still the most important soilborne disease of crops worldwide. This pathogen is capable of producing microsclerotia as resting structures in the soil, which can rest in soil and plant debris for decades, and the most successful way of eradicating these structures has been the application of soil fumigation using broad spectrum fumigants [3].

Among the chemicals used for fumigation, methyl bromide was the most popular and effective fumigant for use in preplant fumigation of soil in the US agriculture, until its use was scheduled to be phased out in the US by 2001 due to the evidence that this chemical can result in depletion of the Ozone layer in the atmosphere [5]. Due to the environmental threats of methyl bromide, growers then used non-chemical techniques for sterilizing soil or growing media to eradicate or suppress soilborne pathogens to avoid soilborne diseases, especially on crops of high economic values but high risk of losses due to soilborne diseases. These involve heating of soils either using steam or solarization. The later involves heating of the soil in daily cycles for a period of several weeks [2].

Different techniques of sterilizing soils were reported to have different effects on the conditions of the soil following sterilization processes, which also affected growth and yield of crops grown on the sterilized soils. Yamamoto *et al*. [11] reported different effects among methyl bromide fumigation (MeBr), chloropicrin fumigation (CP), and steam sterilization (SS) on nitrogen dynamics and microbial properties of growing media in pot culture. In terms of nitrogen dynamics, all the methods of soil sterilization were reported to increase ammonium-Nitrogen over non-sterilized control, but the magnitude of increase was highest on steam sterilization followed by chloropicrin fumigation, and the least on methyl bromide fumigation. So did the severity of sterilization effects on the microbial properties of the media following sterilization decreased in the order SS ≥ CP > MeBr [11]. Crop growth was also reported to be different between sterilized and non-sterilized soil. Williams-Linera and Ewel [10] reported that among eight plant species, two native species grew faster on autoclave-sterilized than on non-sterilized soil but six

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species, including soybean and radish, grew better on non-sterilized than sterilized soil. In relation to inoculation of arbuscular mycorrhizal fungi (AMF), data reported by Miransari *et al*. [4] show that AMF inoculation on 12 time compacted non-sterilized soil tend to decrease uptake of N, P and K by wheat seedlings, but the same treatments tend to increase uptake of N, P and K on autoclave-sterilized soil. This could mean that the indigenous AMF natively present in the non-sterilized soil were more effective than the introduced AMF isolates, but on the sterilized soil, the indigenous AMF were kill during autoclave sterilization process so that the introduced AMF isolates had no competitors in infecting roots of the wheat seedlings.

This research project was aimed to examine the effects of autoclave-sterilization of mungbean rhizosphere soil on arbuscular mycorrhiza colonization, growth and yield of various promising lines of upland black rice on pot culture in a glasshouse.

II. MATERIALS AND METHOD

The experiment in this study was carried out in the glasshouse of the Faculty of Agriculture, University of Mataram, Mataram, Lombok, Indonesia, by growing upland black rice in polybag pots. The growing media were taken from dry land with Entisol soil in North Lombok, which was frequently used to grow mungbean and the soil used as the growing media in this research was mungbean rhizosphere, which roots were colonized by arbuscular mycorrhizal fungi (AMF) with an average colonization level of more than 80%.

The treatments:

This mungbean rhizosphere soil was used to grow 7 promising lines of upland black rice with two treatments of growing media, i.e. S0 = without sterilization (containing indigenous AMF) and S1 = sterilized using autoclave for 2 hours at 121 °C. Thus there were two treatment factors in this research, i.e. Sterilization (S) of growing media, and various genotypes of the promising lines of black rice, which are the results of hybridization & selection by rice breeding research team. Of the 15 genotypes selected, 7 promising lines with relatively high grain yield were tested in this research. Therefore, there were 14 treatment combinations, namely the genotypes G4, G7, G8, G9, G12, G13 and G15 planted on non-sterilized growing media (S0), and G4, G7, G8, G9, G12, G13 and G15 planted on the sterilized growing media (S1), and each treatment combination was made in 3 replications, so that there were 42 polybag pots.

Preparation of growing media and planting:

The mungbean rhizosphere soil from North Lombok, after being air-dried, was sieved with a 2 mm opening sieve and mixed evenly, and then its moisture content was measured to determine its moisture content at field capacity and air-dried condition. For the treatment of non-sterilized media (S0), 21 pieces of polybag were prepared, and then were filled with 9 kg of the air-dried growing media. For the treatment of sterilized growing media, 21 autoclave bags were prepared, each of which was filled with 9 kg of the air-dried growing media, then each was sterilized in the autoclave for 2 hours at 121 °C, and after being cooled, each was used to fill a polybag pot. On the day before planting rice seeds, the growing media in the 42 polybag pots were watered with aquadest until its field capacity. Before being planted, the black rice seeds were pre-germinated, and then 4-5 germinated seeds were planted per pot, but after 7 days after seeding (DAS) thinning was done by allowing only 2 seedlings to grow per pot. When finished, soil in the pots was fertilized using Phonska (15-15-15) fertilizer at a dose of 1.2 g / pot (equivalent to 300 kg / ha), which was dibbled next to the young rice plants.

Plant maintenance and harvest:

Watering was done every 2 days when the plants were 2-20 days old, then every day in the morning when the plants were 21-40 days old, and after 40 DAS, watering was done 1-2 times per day depending on the soil conditions in the pots. Watering was done using tap water with the same volume per pot until the condition of field capacity at each watering. Follow-up fertilization was done using Urea (45% N) fertilizer at a dose of 0.5 g / pot (equivalent to 125 kg / ha) at the age of 30 and 50 DAS, by dibbling the fertilizer beside the base of the rice stems. Harvesting was done after the rice panicles reach harvest state, with a range of days to harvest of 95-117 days.

Variable measurement and data analysis:

Observation variables included plant height at harvest, days to harvest, degree of AMF colonization in rice roots, number of productive tillers, average panicle length at harvest, weight of dry straw, dry grain yield, and weight of 100 dry grains. To measure the degree of colonization levels, samples of rice roots were taken at anthesis using soil sampler by taking 2 soil cores per pot. The root samples after being washed were then cut into 1 cm pieces, then they were stained using Trypan blue followed the method used by Wangiyana [9]. Data were analyzed with analysis of variance (ANOVA) and Tukey's HSD test at 5% level of significance, using the statistical program CoSta
t for Windows ver. 6.303.

III. RESULTS AND DISCUSSION

Based on the results of data analysis, the ANOVA results indicate that there were significant interaction effects

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between sterilization and genotypes on plant height, AMF colonization levels, average panicle length, grain yield per clump, and weight of 100 filled grains, which means that mean values of those variables on a genotype depend on whether the growing media were sterilized or not. However, unlike the effects of genotypes, which were significant on all measurement variables except for the weight of 100 grains, the sterilization treatment only affected AMF colonization levels, and the weight of 100 filled grains (Table 1).

Table.1: Summary of ANOVA Results for All Observation Variables

Observation variables	Steril-ization	Geno-type	Inter-action
Plant height	ns	***	**
Days to harvest	ns	***	ns
Straw dry weight	ns	*	ns
AMF colonization levels	***	***	***
Productive tiller number	***	***	ns
Average panicle length	*	***	*
Grain yield per clump	***	***	***
Weight of 100 grains	*	ns	*

Remarks: ns = nonsignificant; *, **, *** = significant at $p<0.05$, $p<0.01$, and $p<0.001$ respectively

Among the growth variable, only plant height showing interaction effect of both treatment factors, which means differences in plant height between genotypes depend on whether the growing media were sterilized or not, although there was no significant effect of soil sterilization on plant height. The patterns of interaction between the two factors on plant height are as in Fig. 1, in which plant height was higher on sterilized than on non-sterilized soil, but only on the genotypes G9 and G12. The pattern of differences in these genotypes was also similar between plant height (Fig. 1) and weight of 100 grains (Fig. 2).

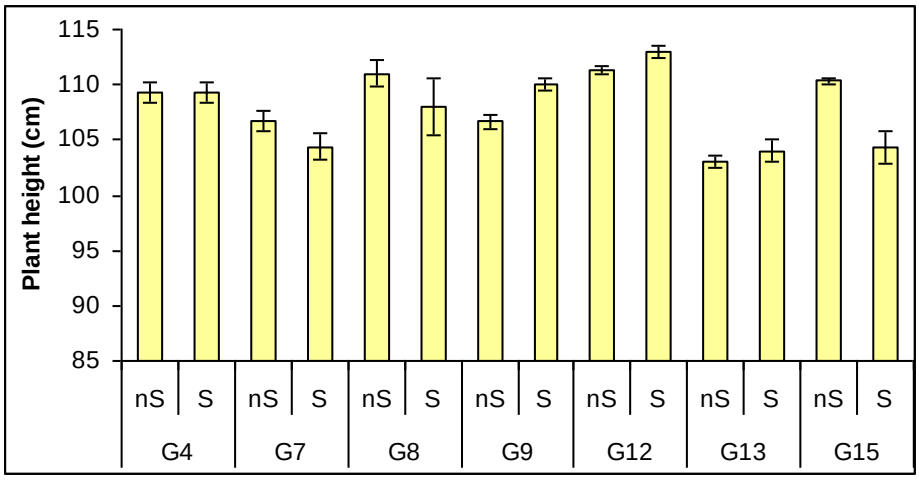

Fig.1: Average (Mean±SE) plant height (cm) of various promising lines of black rice between sterilized (S) and non-sterilized (nS) soil

In addition, average weight of 100 grains on G8 was also higher on sterilized than on non-sterilized soil (Fig. 2). On the contrary, grain yield of G8 was higher on non-sterilized than on sterilized soil (Fig. 5). This was probably due to the higher number of productive tillers per pot on the non-sterilized than sterilized soil, and this trend was similar almost on all genotypes tested (Fig. 6), although there was no interaction effect of both treatment factors on number of productive tillers per pot (Table 1).

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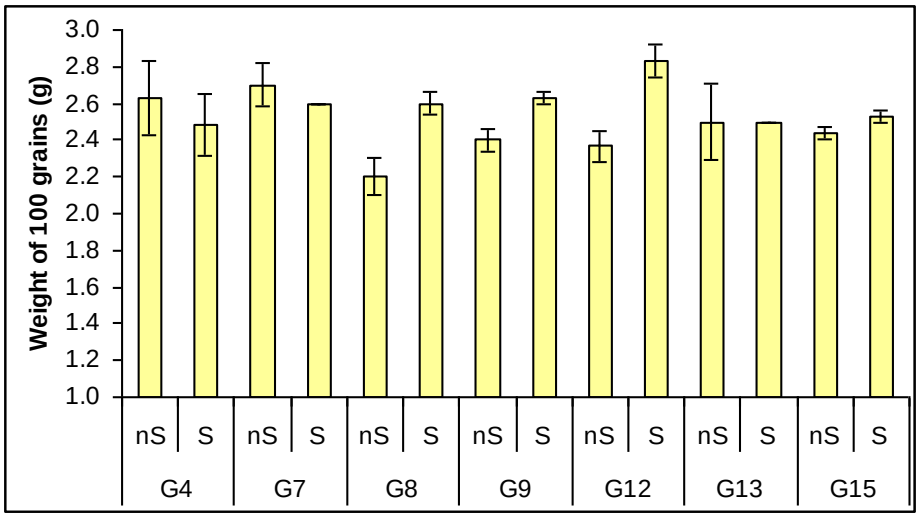

Fig.2: Average (Mean±SE) weight of 100 filled grains (g) of various promising lines of black rice between sterilized (S) and non-sterilized (nS) soil

Among the variables showing significant interaction effects of both treatment factors, there were three response variables showing both significant interaction and main effects, namely AMF colonization levels in the black rice roots, average panicle length, and grain yield per clump. For the levels of root colonization by the indigenous AMF, the significant interaction seems to be due to the highly significant differences in colonization levels between genotypes of the black rice plants grown on the non-sterilized soil, while AMF colonization on roots of those grown on the sterilized soil was not found. The patterns of interaction effects of both treatment factors are as in Fig. 3.

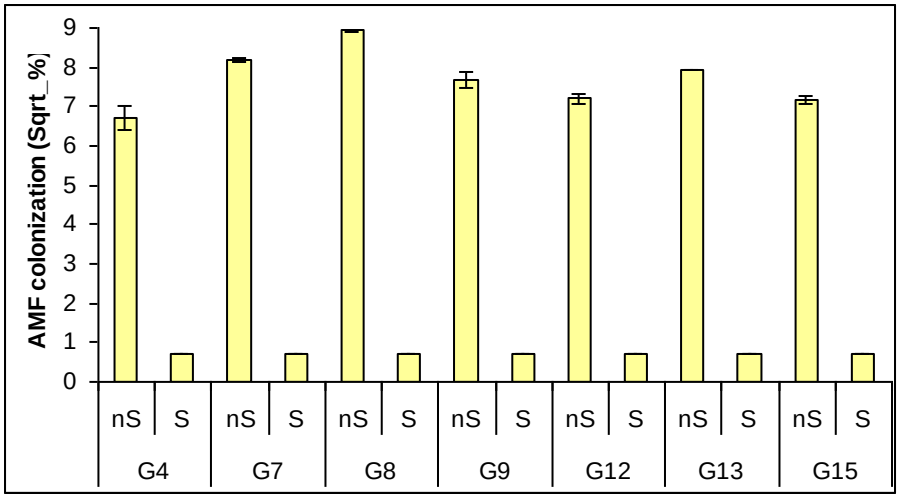

Fig.3: Average (Mean±SE) AMF colonization levels (data were transformed into √(x%+0.5)) on roots of various promising lines of black rice between sterilized (S) and non-sterilized (nS) soil

For the average panicle length, the patterns of interactions between both treatment factors are as in Fig. 4, in which it can be seen that different genotypes responded differently, i.e. the genotypes G4, G7 and G12 showed higher panicle length on non-sterilized than on sterilized soil, while G13 showed higher panicle length on sterilized than on non-sterilized soil.

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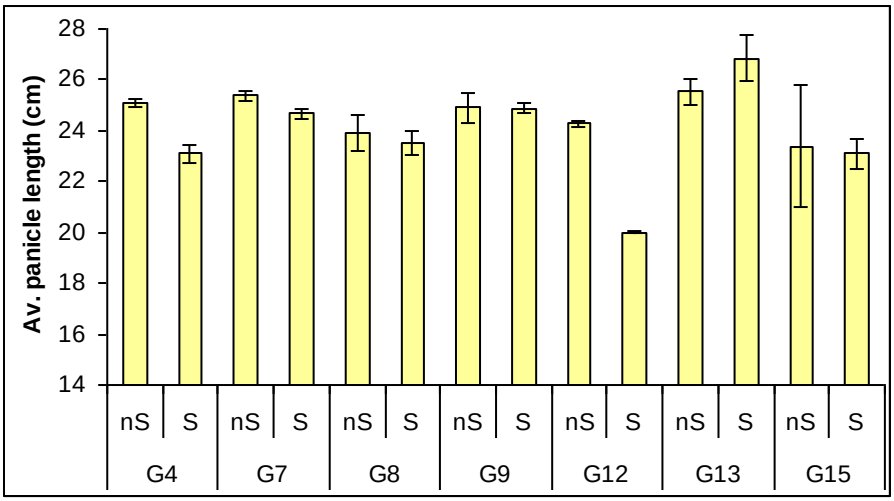

Fig.4: Average (Mean±SE) panicle length (cm) of various promising lines of black rice between sterilized (S) and non-sterilized (nS) soil

A similar patterns of interactions also occurred on grain yield per pot, except for the genotypes G7 and G8 (Fig. 5). In relation to grain yield, the genotype G7 showed higher yield on sterilized than on non-sterilized soil, while G8 showed significantly higher grain yield on non-sterilized than on sterilized soil. Thus, among the seven genotypes tested, three showing no differences in grain yield between sterilized and non-sterilized soil, three showing higher grain yield on non-sterilized soil and only one showing higher grain yield on sterilized soil, i.e. G7 (Fig. 5).

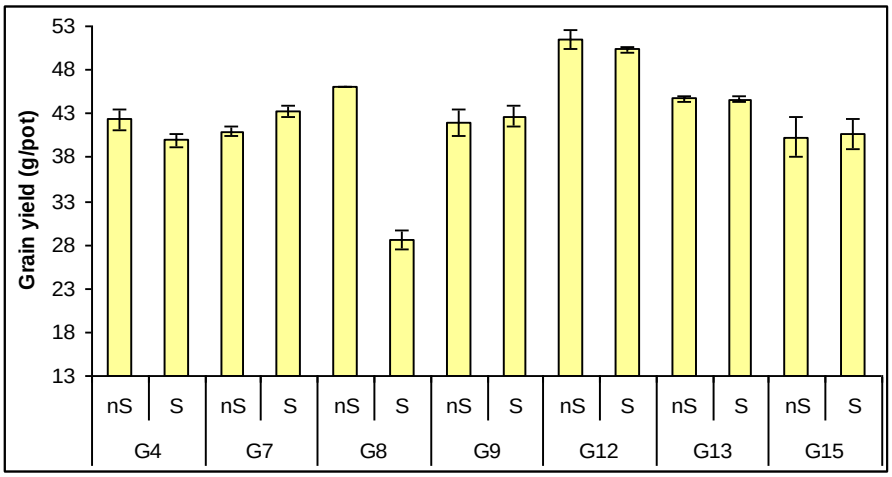

Fig.5: Average (Mean±SE) grain yield (g/pot) of various promising lines of black rice between sterilized (S) and non-sterilized (nS) soil

In the case of grain yield of G7, which was higher on the sterilized than on the non-sterilized soil (Fig. 5), it is clearly unrelated to productive tiller number (Fig. 6), average panicle length (Fig. 4), AMF colonization levels (Fig. 3), or weight of 100 grains (Fig. 2), because they were higher on non-sterilized than on the sterilized soil. However, dry straw weight on the genotype G7 was higher on sterilized than on non-sterilized soil (Fig. 7), although there was no significant interaction affect on dry straw weight (Table 1). The genotype G7 was harvested on the same day both for the plants on the sterilized and on non-sterilized soil, so that the duration of their seed filling period was the same between the two growing media. According to Wang *et al*. [8], carbon reserve in the stem and leaves of rice plants can be remobilized to developing seeds during the grain filling period, to increase grain yield. Since the grain filling duration of G7 on both growing media was the same, it was possible that the proportion of the carbon reserve remobilized to seeds was probably the same between the two growing media. Since straw dry weight of G7 was higher on sterilized than on non-sterilized soil, it could be possible that the higher grain yield of the genotype G7 growing on the sterilized than non-sterilized soil was due to the higher

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dry straw weight of G7 on the sterilized than non-sterilized soil. In addition, the higher number of productive tillers of G7 on non-sterilized than on sterilized soil might have contributed only to a higher proportion of unfilled grains on G7 growing on the non-sterilized soil.

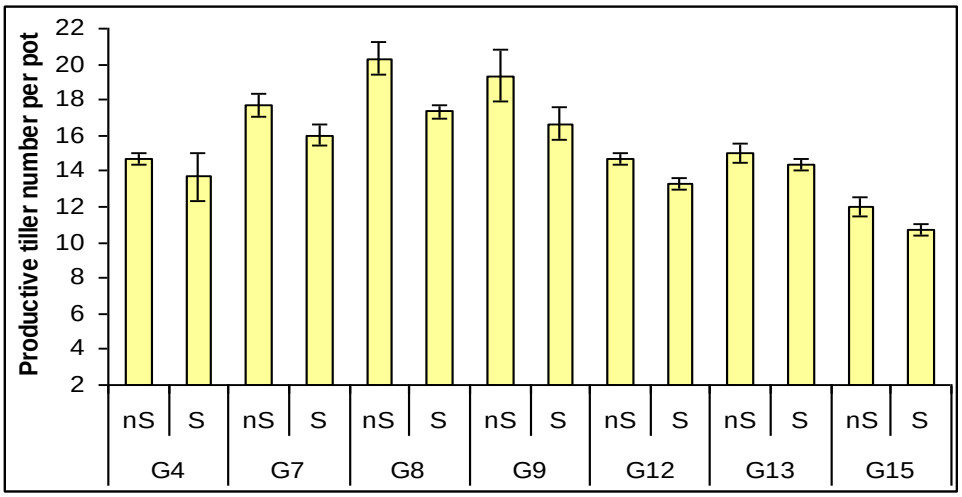

Fig.6: Average (Mean±SE) productive tiller number per pot of various promising lines of black rice between sterilized (S) and non-sterilized (nS) soil

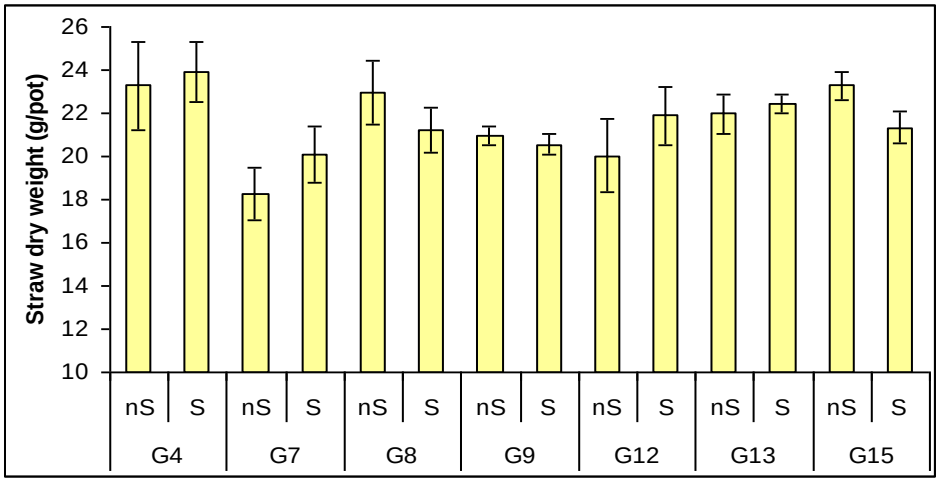

Fig.7: Average (Mean±SE) straw dry weight (g/pot) of various promising lines of black rice between sterilized (S) and non-sterilized (nS) soil

However, for other genotypes showing higher grain yield per pot on the non-sterilized than on sterilized soil, such as G4, G8 and G12, the most possible causes were higher productive tillers and AMF colonization in roots of black rice plants growing on the non-sterilized than on sterilized soil. The contribution of AMF colonization is well-known to help their host plants to take up more nutrients from their growing media, including N, P, K, Ca, Fe, Cu, Na, B, Zn, Al, Mg and S [1, 6, 7]. According to the data published by Miransari *et al*. [4], symbiosis was more effective with indigenous AMF than with the introduced species, and many researchers have proven that rice is a good host for AMF symbiosis [1, 6, 7]. Therefore, better nutrition due to indigenous AMF colonization coupled with higher productive tiller numbers (Fig. 6) of the black rice plants grown on the non-sterilized soil would have contributed to higher grain yield per pot (Fig. 5).

Based on the main effects on yield components of various promising lines of black rice, it can be seen from Table 2 that soil sterilization reduced productive tiller number per pot, average panicle length, and grain yield per pot, but increased weight of 100 filled grains. The lower weight of 100 filled grains on black rice plants grown on non-sterilized soil could be due to increased productive tiller number and average panicle length, which both can result in higher number of spikelet per pot, which in turn could reduce weight of 100 grains.

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Table.2: Average Grain Yield and Yield Components of Various Promising Lines of Black Rice Grown on Autoclave Sterilized and Non-sterilized Soil

Treatments	Productive tiller number per clump	Average panicle length (cm)	Weight of 100 filled grains (g)	Dry grain yield (g/pot)
Sterilization:				
Non-sterilized	16.24 a	24.64 a	2.46 b	43.99 a[1)]
Sterilized	14.57 b	23.74 b	2.60 a	41.45 b
HSD 0.05	0.80	0.85	0.12	1.24
Genotypes:				
G4	14.17 c	24.09 abc	2.56 a	41.16 bc
G7	16.83 ab	25.02 ab	2.65 a	42.12 bc
G8	18.83 a	23.74 abc	2.40 a	37.29 d
G9	18.00 a	24.90 ab	2.52 a	42.34 bc
G12	14.00 c	22.14 c	2.60 a	50.93 a
G13	14.67 bc	26.20 a	2.50 a	44.69 b
G15	11.33 d	23.24 bc	2.48 a	40.50 cd
HSD 0.05	2.33	2.47	0.34	3.60

[1)] Mean values in each column followed by the same letters are not significantly different based on the Tukey's HSD test at 5% level of significance

IV. CONCLUSION

Based on the overall results, it is concluded that sterilization of soil for use as growing media could reduce growth and yield of black rice probably due to eradication of AMF by the sterilization process.

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A Survey on Premature Death in Early Chicks and the Assessment of the Responsible determinants: A Case Study in the Afigya Sekyere South District of the Ashanti Region of Ghana

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***Abstract*— *Objective**: An investigation was carried out in the Afigya-Sekyere South District to determine the rate of early chick mortality in the first four weeks and the factors that may have caused this.*
***Methods**: Structured questionnaires and personal observations were used to obtain information from twenty-eight (28) farm managers.*
***Results:** The study revealed that mortality was higher in the first and second week with an average mean mortality of 11.39 and 6.43 respectively. According to the farm managers, all the mortality reported was a result of disease infection. Statistical analysis also depicted that the source of the day-old chicks (local or imported), experience and educational level of the managers and the type of breed were the factors that affected disease occurrence on the farm which consequently caused early chick mortality.*
***Conclusion:** Diseases such as Gumboro and Coccidiosis were reported with a percentage of 35.7% and 39.3% respectively and these caused a mortality percentage of 33.86% and 36.99%. The studies further revealed that mortality increased with a decrease in the inefficiency of the managers. Statistical analysis also showed that local chicks were associated with higher mortality recording 0.80% as compared to 0.31% of the imported chicks in the first week. Mortality was also associated with the type of breed, with the Bovane black recording the highest percentage in all the four weeks recording 2.34% and 0.92% in the first and second week respectively.*
Keywords*— *Early, Mortality, Diseases, Day-old chick, infection.

I. INTRODUCTION

Chick mortality during the early weeks poses a great economic challenge to the poultry farmer and is a matter of great concern. Early chick mortality, in general, can be attributed to a number of factors of which some is traced to the hatchery where the chicks are hatched before they are transported to their designated farms. Diseases play a dominant cause of mortality in the early chick's life, and mostly these diseases are associated with the source of the day-old chicks. So it is always advisable to tackle the source of where the chicks are coming from since it is going to determine whether the day-old chicks are quality enough to minimize mortality.

The yolk sometimes becomes infected with bacteria during the hatching process which possibly results in mortality in day-old chicks. Mortality in the early chicks' can be attributed to a number of factors of which yolk sac infection causes a greater percentage of 31.45% as reported by Ghodasara et al (1992) and also causative agents are known to influence infection in chicks are mainly isolates of staphylococci and Escherichia coli as also reported by Bains, 1979. Some diseases such as salmonellosis, aspergillosis, and colibacillosis are known to increase mortality in the initial few weeks of the life of chicks (Shane, 1999).

Records of mortality during the first seven days of brooding have been used to assess the quality of chicks in the broiler industry according to Chou *et al*., (2004) and North (1984) and Kitsopanidis and Manos (1991) also reported a reduction in net profitability with increased mortality levels in chicks. Apart from diseases, mortality in day-old chicks can also be associated with poor management, inadequate brooding temperatures and heat stress in hot climates (Chou *et al*., 2004).

OBJECTIVES

The objectives of this study were to;

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1. To consider factors that may cause chick mortality from the day- old through the first four weeks.
2. To consider the management practices to adapt to minimize mortality on the farms.

II. MATERIALS AND METHODS

Sample area for the survey

The study area chosen for the collection of data was the Afiya Sekyere South district in the Ashanti region of Ghana **(Fig 13)** where the majority of the poultry farmers and poultry businesses are located. Also according to the (**Ghana statistical service 2010),** Chicken rearing dominates with 67,995 birds in the district and 2,697 keepers followed by goats with 19,756 animals and 1,826 keepers and the keepers of Sheep are 1108, that of are duck 150, that of guinea fowl is 133, and cattle 108 with 13,868 sheep, 2,093 ducks, 1,436 guinea fowls, and 1,198 cattle respectively **(Table 5).**

Type	Total	Number of keepers	Average animals per keeper
All livestock	110,231	6,263	18
Beehives	3	2	2
Cattle	1,198	108	11
Chicken	67,995	2,697	25
Dove	997	17	59
Duck	2,093	150	14
Goat	17,756	1,826	11
Grass-cutter	211	19	11
Guinea fowl	1,436	133	11
Ostrich	108	5	22
Pig	909	55	17
Rabbit	321	29	11
Sheep	13,868	1,108	13
Silk worm	262	17	15
Snail	27	3	9
Turkey	486	55	9
Other	331	36	9
Fish farming	230	3	77
Inland farming	0	0	0
Marine farming	0	0	0
Other	331	36	9
Marine fishing	0	0	0

Source: Ghana Statistical Service, 2010 Population and Housing Census

Data collection

Data were collected with well-structured questionnaires. The questionnaires incorporated both closed and open-ended questions and were designed to obtain data to meet the objectives of the study. A total of twenty-eight (28) poultry managers/owners were considered and the technique involved in the collection of data was through direct interrogation with the farm managers and where possible a review of the farmers' farm records. Observations were used to verify the data collected.

Data analysis

Parameters or questions included in the survey were the sex of the respondents (farmers), experienced group of the farmers in the poultry industry, the level of education of the farmers, source of the day-old chicks, breed of the day-old chicks, percentage distribution of the number of chicks by each farmer, source of feed for day-old chicks, factors responsible for mortality in the first four weeks, diseases causing early chick mortality and management practices to minimize mortality.

Statistical Package for Social Sciences (SPSS, 2007) version 16 was used to analyze all the data solicited from the farms and the results were presented in tables and charts.

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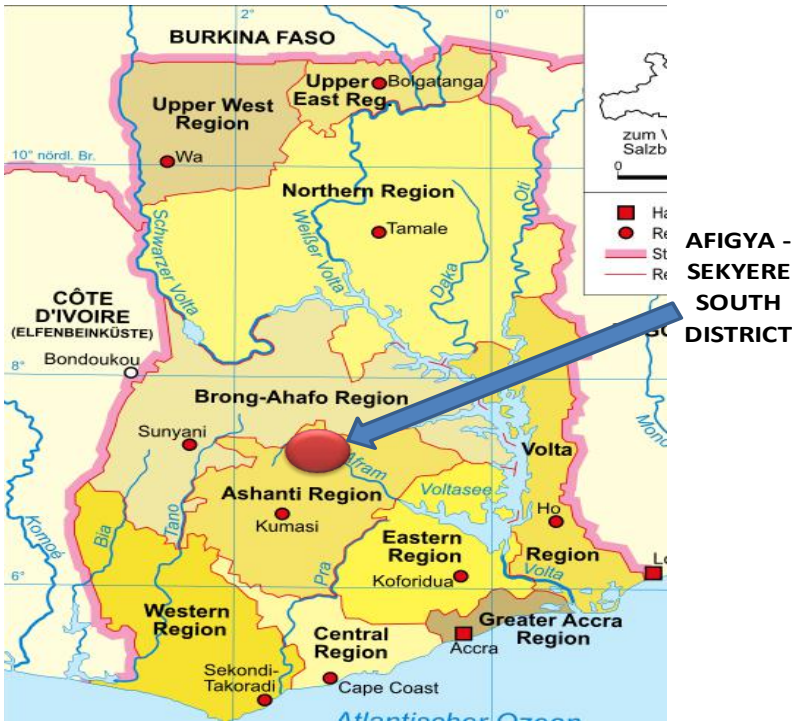

Fig.13: A map of Ghana depicting the various regions. Note the study site is shown with a blue arrow

III. RESULTS AND DISCUSSIONS

DEMOGRAPHIC/BACKGROUND OF RESPONDENTS

Gender of respondents and their corresponding percentage of the bird population

During the survey, it was revealed that out of the 28 respondents, only 3 were females representing 11% of the entire managers, and this indicated how males dominated the poultry industry in the Afigya-Sekyere south district.

From the survey, it was also revealed that the total number of birds by the 3 female farmers' was

26% while that of the male poultry farmer's was 74% which indicated that the male farmers had the largest number of birds.

LEVEL OF EDUCATION OF RESPONDENTS AND THEIR CORRESPONDING NUMBER OF BIRDS

All the respondents had some form of formal education with the majority attaining SHS/ A level / O level representing 57.14% of the entire farmer's and also with the largest number of birds (80.63%). This was followed by those in the middle/ JSS group which represented 25% of the entire farmer's with 8.36% of the population of the birds (**Fig 2**). The managers in the tertiary education group were 14.29% of the respondents and these had 10.19% number of birds. Finally, those in primary school group were 3.57% and had the least number of birds, 0.82%.

Personal observation depicted that the respondents in the SHS/ A level / O level group were managers of their own small-scale farms. However, the number of respondents who completed tertiary institutions was very low and these were employed to manage medium to large scale farms with a high number of birds. These stands to suggest that people with tertiary education will most probably like to sit in offices or manage farms owned by others whiles those with relatively low-level education are into the poultry business with a mostly small number of birds. Their level of education or curriculum vitae may not give them the opportunity to be managers of other people's farms or get the position of their choice in a poultry firm hence they enter into the poultry business with their own available resources.

MORTALITY REPORTED AND POSSIBLE CAUSES OF THIS MORTALITY

Mortality of the first four weeks

Mortality in Afigya-Sekyere District as can be inferred from (**Fig 3)** was highest in the first week and decreased throughout to the fourth week. The percentage distribution was 51.95% for the first week, 29.32%, 13.19% and 5.54% for the second, third and fourth week respectively. This showed that a farmer is more likely to record high mortality in the early days of the bird's life than the later days. Amin *et al*. (1995) and Singh *et al*. (1994) reported that higher

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mortalities of 26.23% were recorded during brooding (1-2 weeks) and 24.56% during growing (3-4 weeks).

DISEASES CAUSING THE EARLY CHICK MORTALITY

As can be seen from (**Table 1)**, coccidiosis was reported to have caused the highest mortality in the farms of the respondents throughout the week 1 to week 4. It recorded mortality percentages of 36.99%, 46.66%, 51.85% and 47.06% for week 1, 2, 3 and 4 respectively. This indicates that coccidiosis is the major disease that affected the chicks in the district and could occur at any point within the first four weeks in the life of the chicks. This supports what Lillehoj *et al*., 2004 found that avian coccidiosis is the major parasitic disease of poultry causing mortality, inefficient feed utilization, the impaired growth rate in broilers chicks, and reduced production in layer chicks. Boado *et al*. (1991) also reported that coccidiosis could occur at any stage of the chick's life and during any season of the year; however, it was found to be more prevalent in the summer season. From personal observation, it was seen that some farms had poor litter management practices. For example, wet bedding/litter was observed on some of the farms visited and the outside environment of most of the farms was unclean and lacked footpath at the entrance. All these could directly help to transfer diseases to the farm. However, wet litter can harbor and serve as a favorable medium or environment for coccidian growth *(Eimeria species)*. Also to buttress this point and also to support Hofstad et al (1978) findings, it can be said that a lot of conditions favors or triggers the sporulation of the oocyst such as a conducive house temperature in the range of 20-28^{o}C, wet bedding material and most importantly an unclean environment. Also, contaminated feed and water can also lead to coccidian infection causing mortality in day-old chicks.

This was followed by Gumboro which recorded its highest mortality in week 1. Mortalities caused by gumboro decreased with increased age of the chicks. From (**Table 1)** above, 33.86% of day-old chicks died in week 1 from Gumboro infection but the mortality minimized to 20.59% as the bird gets to the fourth week. Gumboro disease could cause massive damage in the early stage in the chick's life ranging from 10-75% and 80-100% according to the findings by Sah *et al*. (1995) and Chowdhury et al. (1996) respectively. It was also reported that birds of all ages were susceptible to gumboro but losses were greater (20-76%) between the ages of 1-12 weeks than at any other stage of life (Rao *et al*., 1990; Philip and Moitra, 1993; Prabhakaran *et al*., 1997). As stated above under coccidiosis, gumboro is also a disease that is prevalent in the filthy and unhygienic environment. They are therefore mostly referred to as the AIDS of chicks and breaks down the immune system of the birds creating the platform for the incidence of coccidiosis, and most factors that can cause coccidiosis. The vulnerability and the relative incidence of gumboro in chicks as reported by (Anjum et al., 1993; Farooq et al 2000) can be traced probably to an unhygienic environment, wrong way of vaccination and subjecting factors like coinciding infections with E.coli, Coccidiosis and other bacterial infections according to the findings by (Singh *et al*., 1994).

Therefore, care must be taken to administer vaccines at stipulated times and successfully overcoming predisposing factors working as contusive media for the outbreak of gumboro. Massive damage or losses as a result of gumboro diseases in chicken can be averted by following the standard hygienic plan and also averting simultaneous infections like coccidiosis and E. coli.

Chronic Respiratory Disease (CRD) caused high mortality after gumboro. This disease decreased with the age of the chicks. About 13.48% chicks died from this disease in week 1, 11.11%, 8.64% and 5.88 also died in week 2, 3 and 4 respectively. The early stage of the chick's life can become vulnerable to the disease at any time. This supports the findings by Javed *et al*. (1991) who explicitly said that, the chickens life is not immune to the disease and the disease can attack at any stage in the chicken's life in respective of the season however during the winter season, the rate or incidence of the disease is very high (66.6%) and also found to be more rife in 7days to 5 weeks with a percentage score of 35.7%. The chick becomes more fragile during the first few weeks of its life because of its poor resistance and poor growth. In like manner, the high rate of Infectious Bursal disease could also be triggered more during the winter condition because, at that time, the weather becomes uncomfortable and unfriendly to the chick. Thus, the high incidence of this incidence can be averted through the continual upkeep of a good environment and the sheltering of the bird from the extremely harsh weather. It is important to note that as coccidiosis was increasing CRD was decreasing. The reason is that coccidiosis increases in the summer seasons while the CRD increases in the winter seasons, therefore these two are inversely related in their occurrence.

Bacterial and yolk sac infections followed the CRD. Whiles the bacterial infection decreased with the age of the chicks the yolk sac infection increases with the age of the chicks. The Newcastle caused the least mortality in the chicks.

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EXPERIENCE GROUP OF RESPONDENTS IN THE POULTRY INDUSTRY

From the survey, as can be seen from (**Fig 4**), it was revealed that 89.3% of the managers were 1-20 years experienced in the poultry business and the percentage of birds they had was 67.13%. This was followed by those who were 20-40 years' experience in the business and they had 29.58% of the total birds. Those with above 40 years were the least with the minimum number of birds (3.29).

The above results depicted that more of the managers were in the 1-20 years' experience range and they also had the largest number of birds than the other experience groups.

EXPERIENCE AND EDUCATION AS FACTORS THAT MAY HAVE INFLUENCED MORTALITY

Experience and the level of education of managers are some of the factors that influenced management practices. However, it is known from the literature that disease occurrence, and for that matter, mortality is associated with management, it, therefore, implies that experience and education can influence disease occurrence and mortality on poultry farms. According to Chou *et al*., (2004) mortality in day-old chicks can also be associated with poor management, inadequate brooding temperatures and heat stress in hot climates.

Sometimes, experience in the poultry business can be synonymous with the education level of the manager. For example, one manager with 20 years' experience in poultry and Junior High School education may be as efficient as another manager with tertiary education in poultry management but only 1-year experience in the field. Sometimes too, some managers with a high level of education are very inefficient in spite of their educational background. It is therefore imperative to find how managers with the same experience in the poultry business but different level of education performed to reduce disease incidence and mortality on their farms or it is necessary to find how managers with the same level of education but different experience in the poultry business performed to reduce disease incidence on their farm.

It can clearly be seen from (**Table 2)** above that for a specific experience group mortality decreased with increased level of education. For example in 1-20 years experienced group, mortality was 2% for those with primary education, which decreased to 1.75% for those with Mid/Junior High School (JHS) Education, 0.33% of mortality for Senior High School(SHS) or A level and finally decreased to 0.23% for those with tertiary education. These results showed that mortality in day-old chicks is associated with the level of education of the respondents. In other words, the level of education of a manager is a factor that can affect the incidence of mortality on his/her farm. The reason is that management will be different for managers with different educational background and according to Chou *et al*. (2004) mortality in day-old chicks can be associated with poor management and inadequate brooding temperatures. That means a person with a low educational background is more likely to poorly manage his farm than a person with a high level of education.

BREEDS OF DAY-OLD CHICKS USED BY RESPONDENTS

It was observed from the survey that the breed commonly raised by the managers/owners was the Lohmann brown chicks representing 76.5% of the bird population and the percentage of managers raising this breed was 67.9%. The reason according to the managers/owners is because the Lohmann brown breed is well adapted to harsh and tropical conditions and also they have high productivity and produced relatively large egg sizes in these harsh environmental conditions. This is also in agreement with findings by Hendrix, (2005) that the Lohmann brown breeds are able to lay for longer periods than the other exotic breeds.

From **Table 3** it can be seen that in week 1, the Bovane black, recorded the highest mortality (2.34%) followed by Isa brown (0.75%), Arbor acres (0.75%), Lohmann brown (0.43%) and White Cobb (0.10%). This pattern repeated itself for week 2, 3 and 4.

The above results showed that mortality in day-old chicks is associated with the breed of the chicks. It can be inferred from the results above that a manager rearing Bovane black chicks is more likely to record high mortality than a manager raising Lohmann brown chicks. This is because different breeds have different genetic make-up and show different responses to different conditions like diseases, hot temperatures. Therefore the breed which has been genetically engineered to withstand diseases and harsh environmental conditions will record low mortality.

SOURCE OF DAY-OLD CHICKS BY THE RESPONDENTS

About 64.3% of the managers/owners indicated that they took their day-old chicks from the local hatcheries in Kumasi and Accra namely; Sydal farm, Akate, Asamoah Yamoah, Mfum Afariwaa, Darko and Topmann farms. The total number of birds from these local hatcheries was 27,360 representing 44.95% **(Fig 6)**. Other managers/owners who imported their day-old chicks were 17.9%. These had 11,100 birds representing 18.24%.

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Finally, the managers/owners who took their day-old chicks from both the local hatchery and the imported were 17.9% and had 22,400 birds representing 36.81% of the bird population.

From the above results, it can be observed that most of the managers (64.3%) took their chicks from the local hatchery. The reason is probably that of availability and proximity of the hatchery to the manager. The reason, most importantly, would be the cost and ease of transportation associated with the local hatcheries. This is also in agreement with findings made by Bundy and Diggin (1968) who stated that birds suffer lighter stress over shorter distances than over longer distances.

From (**Table 4)** below, it is clearly seen that chicks from the local hatchery died more than the imported chicks in week 1. This pattern was repeated throughout the other three weeks. This result indicated that the source of the chicks, whether local or imported, influenced the rate of mortality. In other words, a manager raising chicks from local hatcheries is more likely to record higher mortality than a manager raising imported chicks.

The explanation to these high levels of mortality in the chicks from local hatcheries is based on the management practices at these hatcheries. In an interview, most of the managers reported that most of these local hatchery men do not do proper sorting out. They fail to separate the weak, deformed and diseased chicks from the healthy ones. This, therefore, results in the transfer of disease from the diseased chicks to the healthy ones causing mortalities.

SOURCE OF FEED FOR THE DAY-OLD CHICKS

The survey also revealed that 46.4% of the managers/owners preferred the already compounded feed or the feed from the outside source and the number of birds being fed on this source was 28.75% (**Fig 7**). The managers/owners who use both their own feed and also feed on outside amounted to 35.7% and their number of birds was 38.96% and also 17.9% of the managers/owners formulated their own feed with 32.29% number of birds.

From the above results it can be observed that most of the managers (46.4%) preferred the already compounded feed, Personal observation showed that those managers who used the already compounded feed had less number of birds and in order to save time and energy preferred to use already compounded feed instead of formulating their own feed which would take time and energy. Also from personal observations, due to the high feed ingredient cost, most of the managers tend to buy already compounded diet to feed their day-old chicks in the early stage of production when feed intake is low but as time goes on they tend to formulate their own feed due to an increase in the feed intake of birds. The managers, therefore, buy the ingredients in bulk/large quantities to reduce the cost of the diet in what the economist called 'economy of scale'.

MEANS OF TRANSPORTATION OF THE DAY-OLD CHICKS

From (**Fig 8)** below, it is seen that majority of the respondents, about 64% transported their day-old chicks using well-aerated vehicles and the rest using a truck. This indicates that mortality could be higher by the managers/owners using the truck which is not designed for such purpose. This poor means of transportation results in vibrations that can cause injury to the chicks and also chicks can be exposed to too much air which can affect them as compared to those using the well-aerated vehicles. With the well-aerated vehicles, there will be the low impact of air/ vibrations on the day-old chicks and because the vehicle is well enclosed, the required amount of ventilation will be ensured for the survival of the day-old chicks.

TIME FOR TRANSPORTING THE DAY-OLD CHICKS

From the survey, 71% of the managers/owners took their day-old chicks from the hatchery very early in the morning while the remaining 29% transported their day-old chicks in the evening (**Fig.9**)

This indicates that mortality could be higher in the day-old chicks by the managers who transported their chicks in the evening due perhaps to road traffic at that time keeping the bird in long hours of traveling. Again, during the evening temperatures are usually high in the tropics (especially when there was very high sunshine in the afternoon) due to the emission of long infrared waves from the earth/land that warms the atmosphere. This warmth can cause stress to the birds and in extreme cases may lead to death. However, when chicks are transported early in the morning, they experience cool and favorable weather which most may not lead to stress.

TEMPERATURE ADJUSTMENT IN BROODER HOUSE

From the survey (**Fig 10**), it was revealed that 67.9% of the managers/owners said that they adjust the temperature in the brooder house to suit the right temperature for the day-old chicks to avoid overheating which can possibly lead to mortality in chicks and the number of birds by this category of the managers/owners is 81.7%. This is in agreement with the findings by Gillespie (1992) who reported that the right temperature required for day-old chicks should be 32-35 ^{0}C

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each week until it reaches 21-23.9 ^{0}C. Also, the remaining 32.1% of the managers said they do not adjust the temperature in the brooder house and the number of birds in this category is 18.3%. These managers/owners complained of frequent overheating in the brooder house and recorded high mortality than those who regulated the temperature of the brooder house. This is also in agreement with the findings by Osbaldiston and Sainsbury (1963) who they stated that incorrect brooding temperatures from the first day of brooding could result in poor growth of chicks with increased mortality rate. Another factor that could also influence mortality among the managers who do not adjust the temperature in the brooder house is poor ventilation which will deprive the chicks of oxygen which could make them also very weak and can even predispose them to respiratory diseases. This also supports findings by Corkish (1974) who reported that important concern to proper ventilation leads to prevention of a disease such as Coccidiosis.

SOURCE OF HEAT IN THE BROODER HOUSE

Majority of the respondents use charcoal pots as their main source of heat in the brooder house which represent 64.3% and the number of birds by this category is 67.37% followed by the managers who use incandescent bulbs representing 28.6% (**Fig 11**). This indicates that overheating could easily be controlled by those using the incandescent bulb by just putting some of the lights off or reducing its height if it is too close to the grounds whereas with the charcoal pot more heat is generated than needed and also in allowing for a little ventilation, ash from the charcoal could also be blown onto the day-old chicks leading to mortality.

NUMBER OF RESPONDENTS WHO SENDS THE DEAD BIRDS FOR POSTMORTEM

From the survey, it was also reported that 28.6% of the managers/owners had no post-mortem done on their dead birds in the first week while 71.4% did a post-mortem on the dead birds. In the week 1 those who did not do post-mortem recorded mortality of 0.61% whiles those who did record 0.5% mortality. This trend is seen throughout the other three weeks even though the percentage mortalities reduced as the birds aged/ as the week goes by **(Fig 12)**. From these, it can be deduced that mortality will be high among the respondents who do not perform post-mortem as they will not be able to know the diseases responsible for the death of the chicks or what caused mortality in the chick so as to prevent it next time. Trauma is one of the most common features found at postmortem examination. Bayliss (1986) and Gregory and Austin (1992) found signs of trauma in 35% of the DOA examined. In addition, Bayliss (1986) again pointed

out that 40% of the DOA was due to stress and suffocation after a post-mortem examination was done. So with these findings, knowledge can be imparted to the veterinarian so as to educate the managers so that caution can be taken to minimize this defect next time.

IV. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSION

From the research survey, it was found out that the inefficiency of the managers/owners, the type of breed of the day-old chicks, the source of the day-old chicks, source of feed of the day-old chicks, source of heat for the day-old chicks and diseases such as Coccidiosis and Gumboro were all associated or covary with early chick mortality.

5.2 RECOMMENDATIONS

After the research survey, it was realized that these major factors causing early chick mortality were as a result of poor management and poor sanitary conditions both from the hatcheries and also on the farms and lack of access to the veterinary services. I, therefore, recommend that;

- Correct sorting must be done at the hatcheries to remove deformed, weak birds, small chicks, and dehydrated chicks during packaging into the cartons.
- Farmers should seek advice and the services of Veterinary officers in the district.
- Also, container cartons should be disposed of after use to avoid infections in day-old chicks as some managers/owners claim they sell to other people as a form of business which is not only unhygienic but the source of infection.
- Out of the 28 (twenty-eight) respondents, 13 reported overheating in their brooder house and such practices should be checked to avoid death in chicks.
- Lastly, the litter material should be changed when wet or that portion removed immediately to avoid coccidian spores which cause coccidiosis.

CONFLICT OF INTEREST

I certify that there is no conflict of interest to declare regarding the material discussed in the manuscript.

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FIGURES 1-12

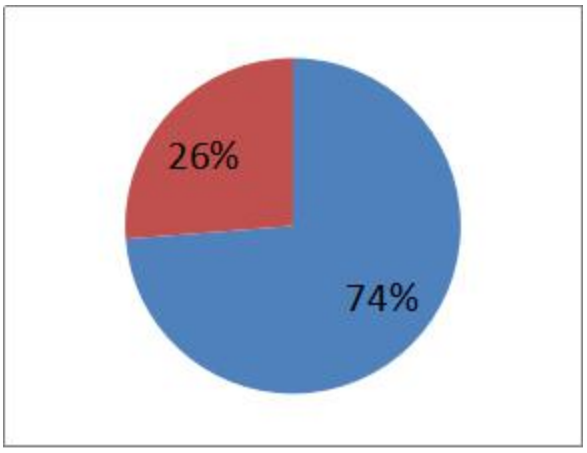

Fig.1b: Percentage of bird population by gender of the farmers

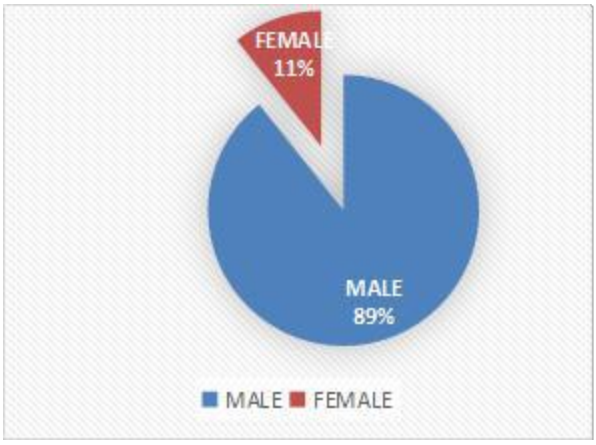

Fig.1a: Sex of Respondent

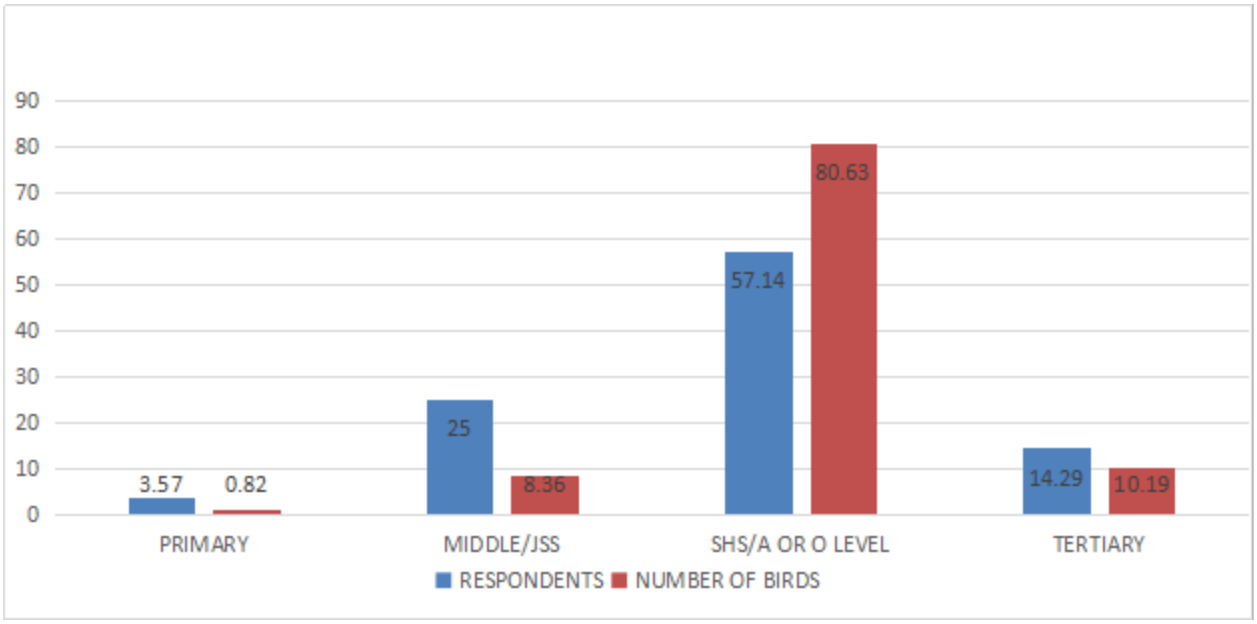

Fig.2: Level of Education of the Respondents and their corresponding number of birds

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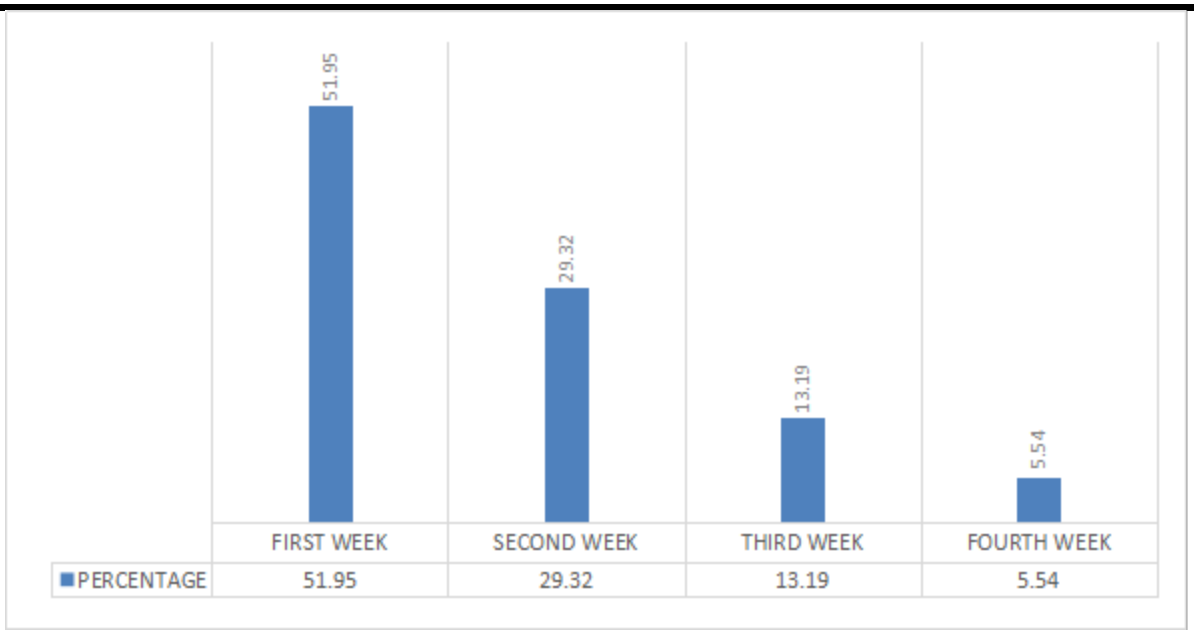

Fig.3: A chart showing mortality percentages for the first four weeks

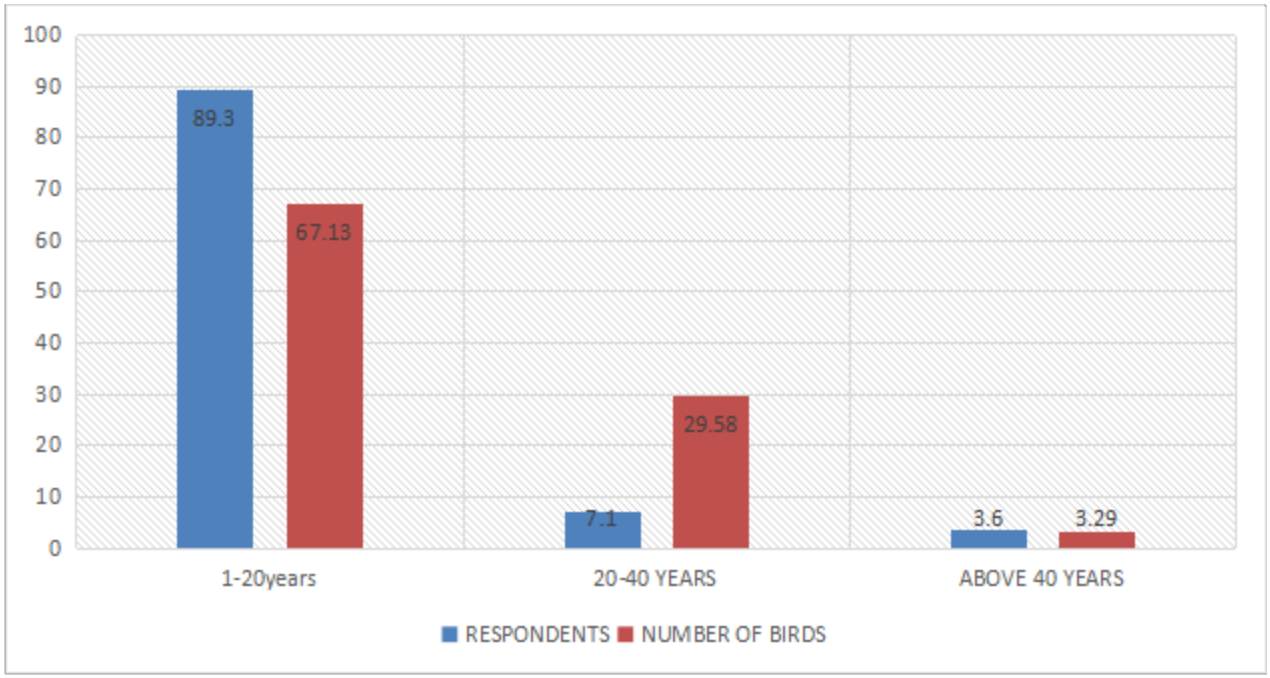

Fig.4: Experience group of the Respondents in the poultry industry

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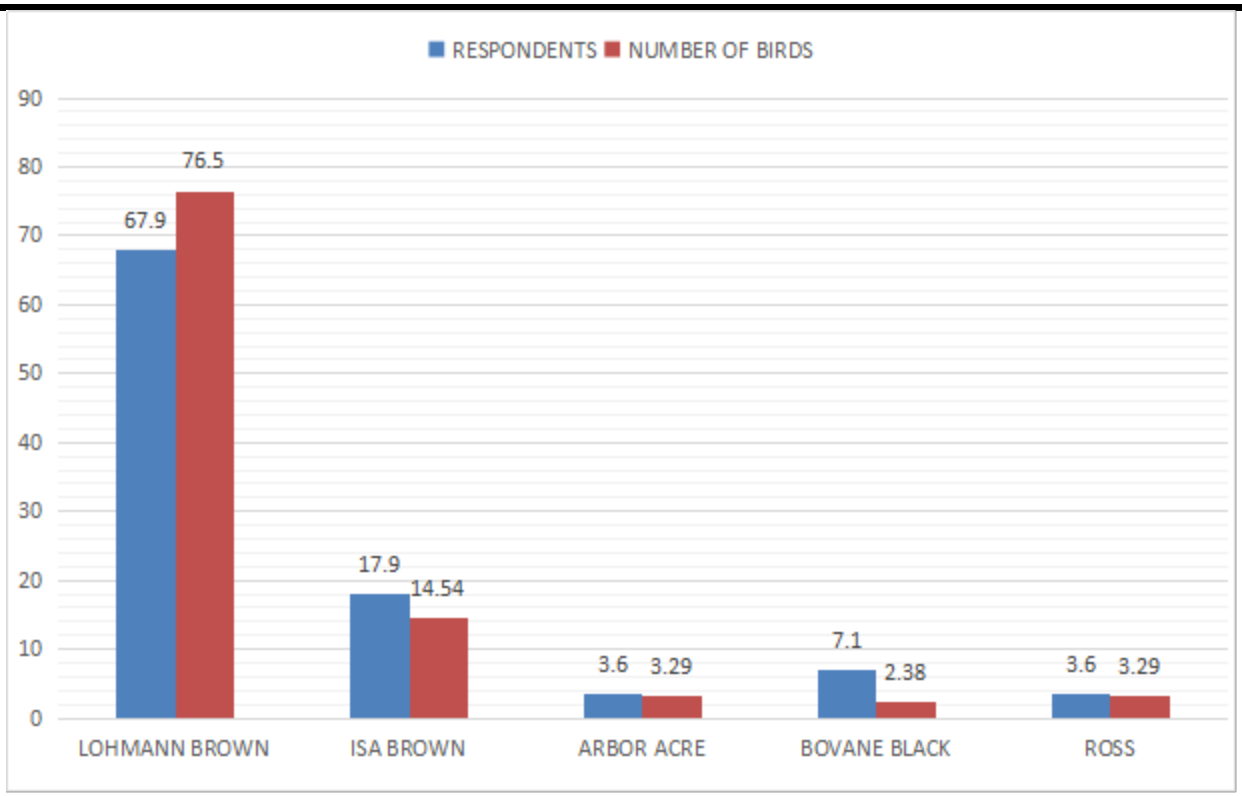

Fig.5: Breeds of day-old chicks reared by respondent

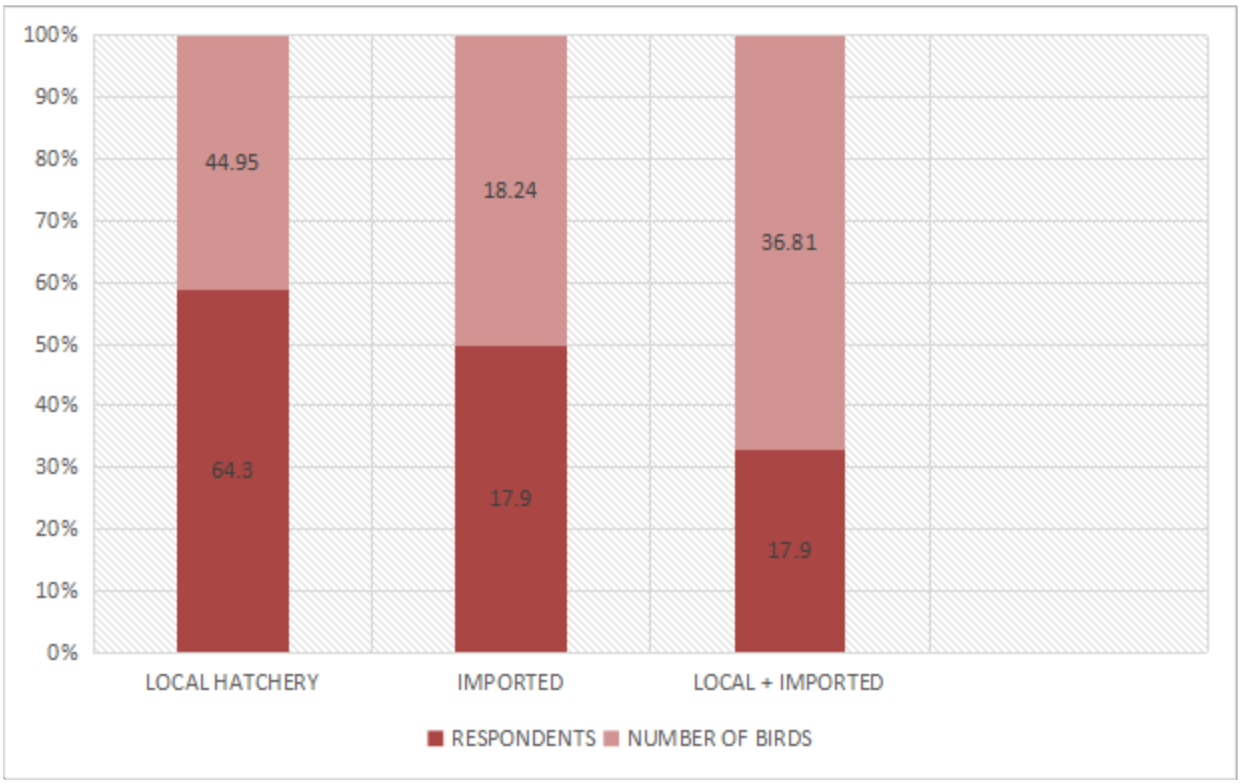

Fig.6: Source of day-old chick by the Respondents

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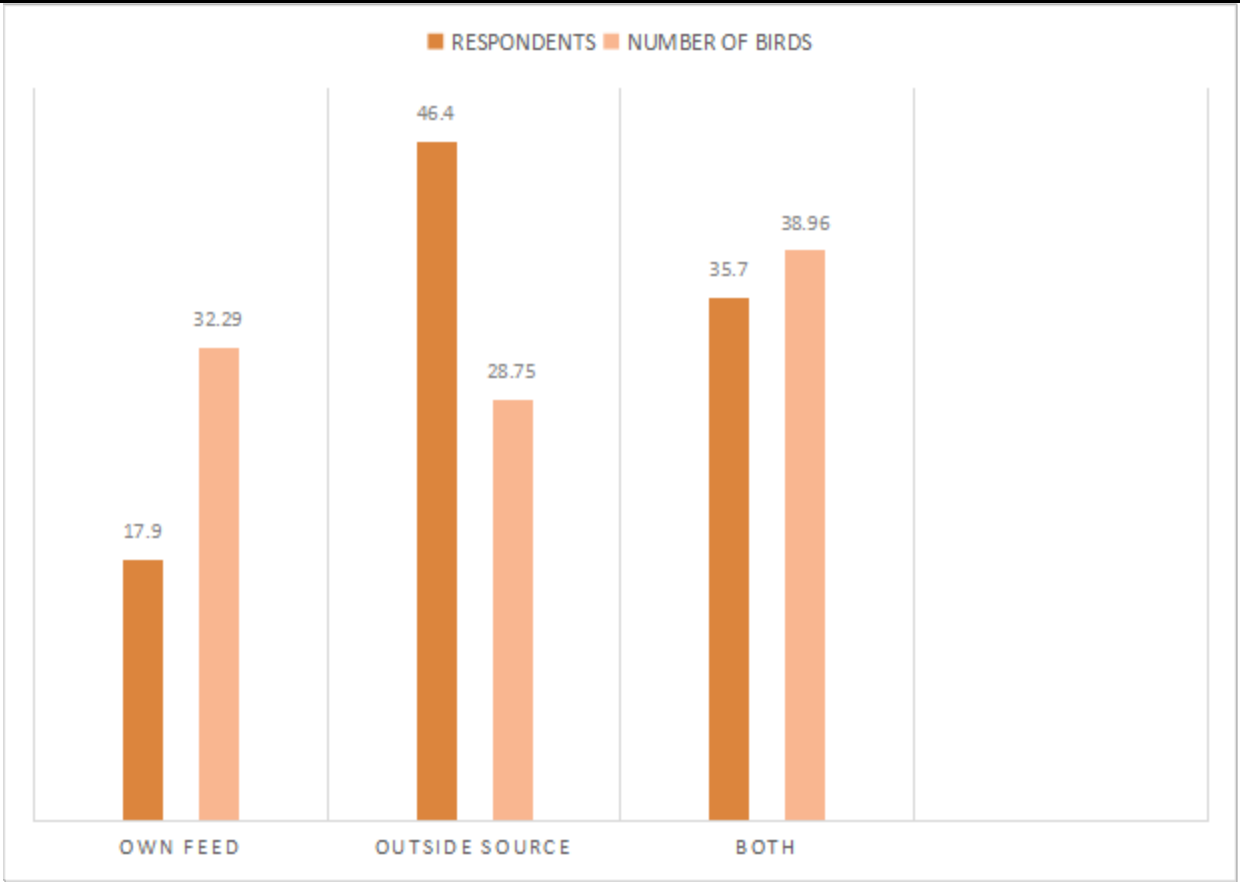

Fig.7: Source of feed of respondents

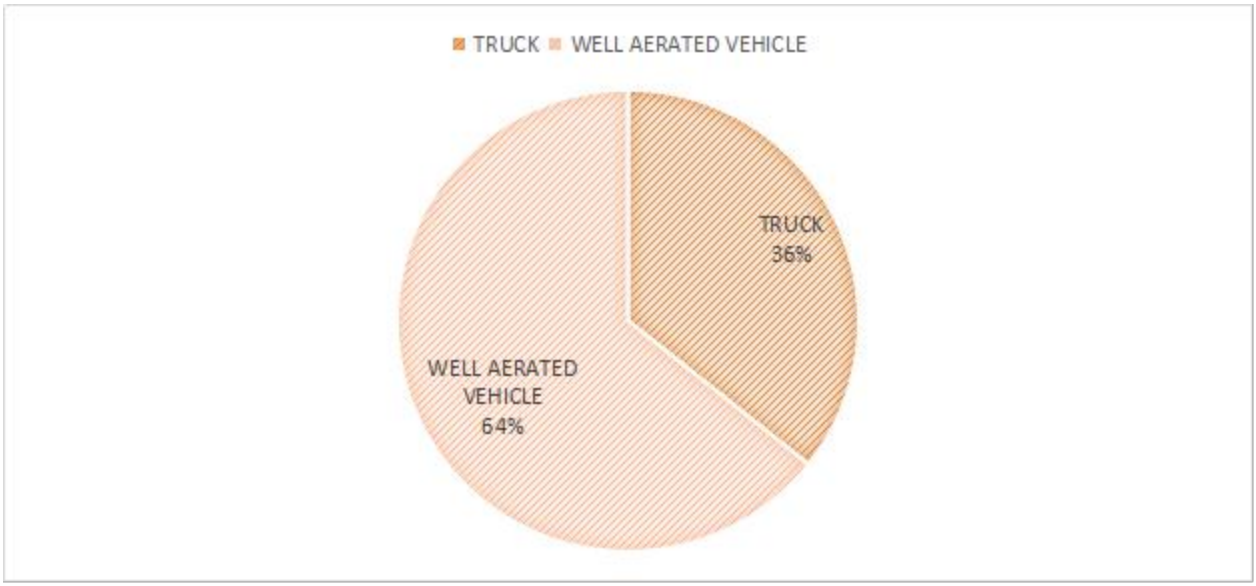

Fig.8: Means of transportation of day-old chicks by respondents

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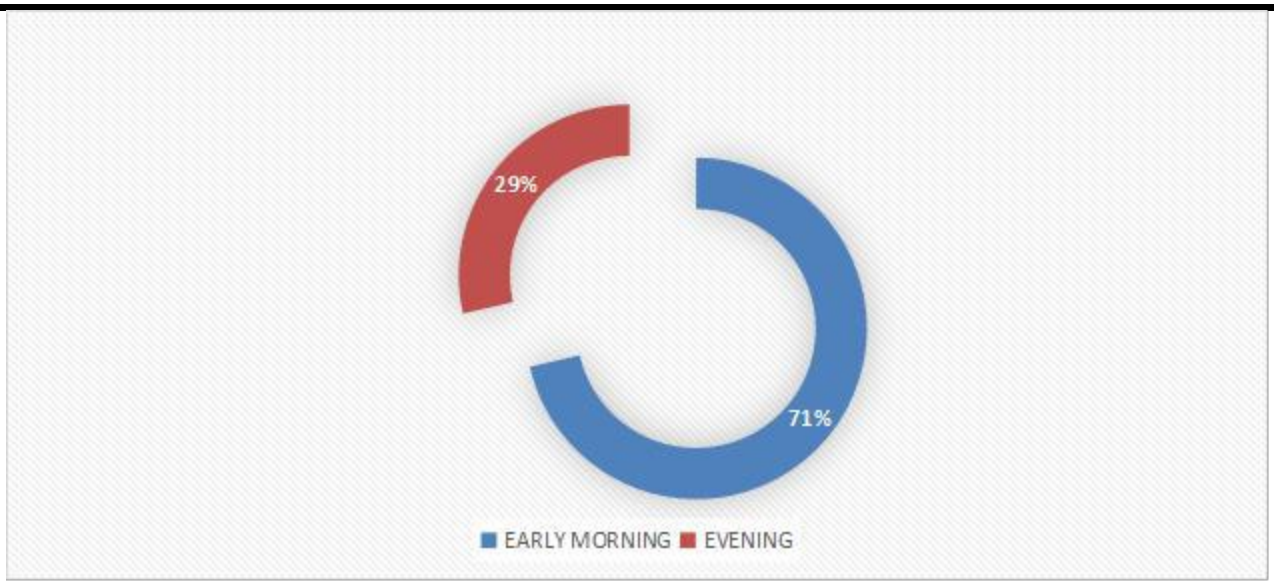

Fig.9: Time of transportation of day-old chick by respondents

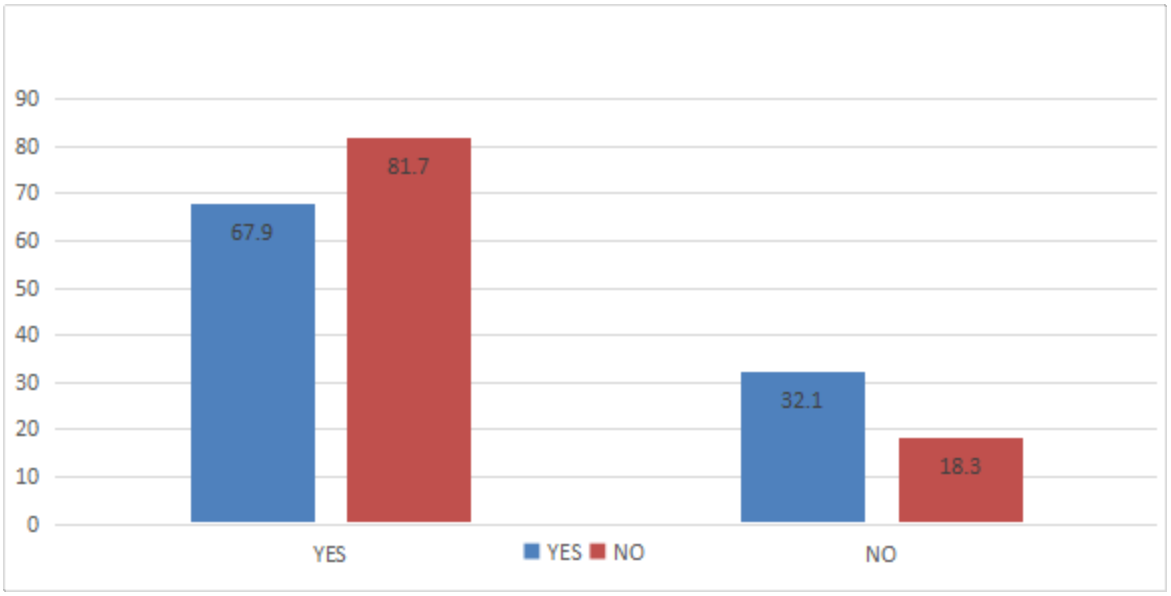

Fig.10: Temperature adjustment in the brooder house by the respondents

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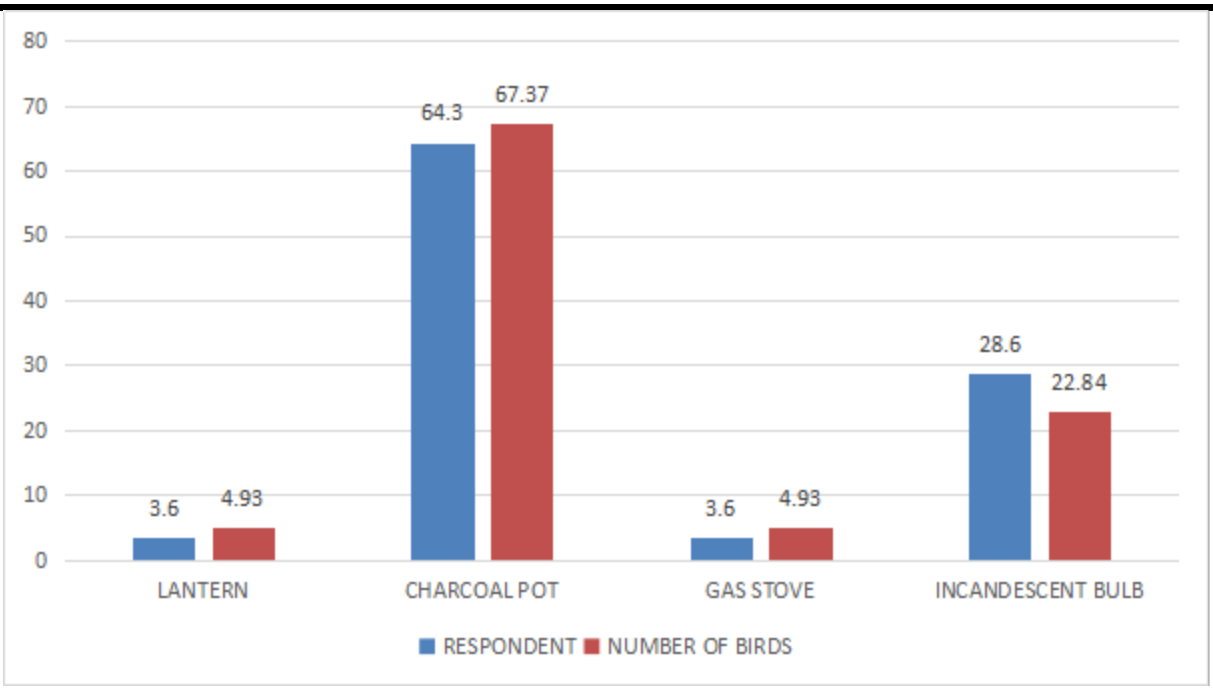

Fig.11: Source of heat in the brooder house by respondents

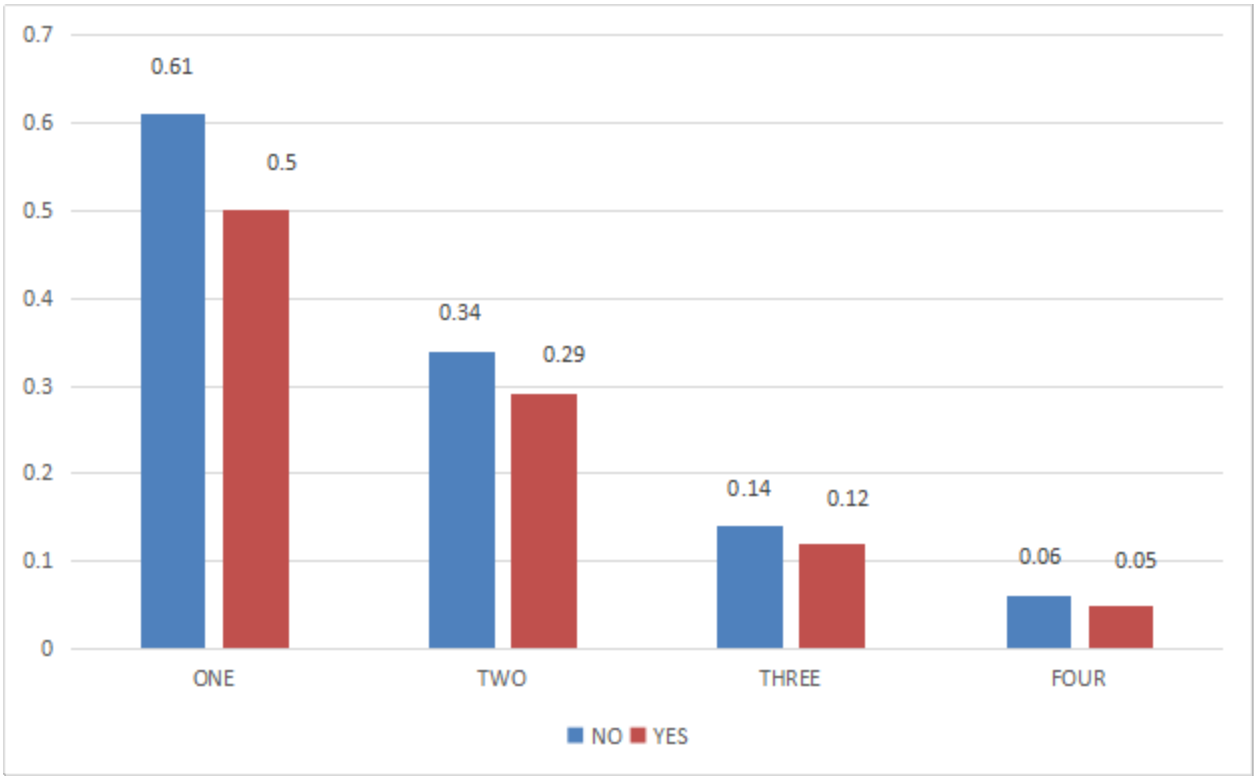

Fig.12: Number of Respondents to request for post-mortem examination

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TABLES 1-5

Table.1: Major diseases causing early chick mortality

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	TOTAL
COCCIDIOSIS	36.99 (118)*	46.66(84)	51.85(42)	47.06(16)	260
CRD	13.48 (43)	11.11(20)	8.64(7)	5.88(2)	72
YOLK INFECTION	6.27 (20)	8.33(15)	9.88(8)	23.53(8)	51
BACTERIAL INFECTION	6.27 (20)	5.56(10)	6.17(5)	2.94(1)	36
NEWCASTLE	3.13 (10)	2.78(5)	2.47(2)	0(0)	17
TOTAL	319	180	81	34	614

***- Figures in the brackets are the number of birds**

Table.2: Effect of experience and level of education of managers on mortality

EXPERIENCE					Total
	Primary	Mid/JSS	SHS/O level/A level	Tertiary	
1-20	10 (2.0)	89 (1.75)	162 (0.33)*	14(0.23)	275
20-40	0	0	20 (0.041)	18(0.29)	38
Above 40	0	0	6 (0.012)	0	6
Total number of mortality	10	89	188	32	319
Total number of birds	500	5090	49070	6200	60860

***- Figures in the brackets are the percentage mortalities**

Table.3: Breeds of birds and their percentage of mortality in the first four weeks

	BOVANE BLACK	ISA BROWN	ARBOR ACRES	LOHMANN BROWN	WHITE COBB
WK 1	34 (2.34)*	66 (0.75)	15 (0.75)	202 (0.43)	2 (0.10)
WK 2	13 (0.92)	50 (0.57)	10 (0.50)	105 (0.23)	2 (0.10)
WK 3	7 (0.50)	20(0.23)	3(0.15)	50(0.11)	1(0.10)
WK 4	3(0.21)	8(0.09)	1 (0.05)	21 (0.05)	1 (0.05)

***- Figures in the brackets are the percentage mortalities**

Table.4: Source of day-old chicks for the respondents

	LOCAL HATCHERY	IMPORTED	BOTH	TOTAL
WK 1	218 (0.80)*	34 (0.31)	67 (0.30)	319
WK 2	105 (0.39)	27 (0.24)	48 (0.22)	180
WK 3	45 (0.17)	14 (0.13)	22 (0.10)	81
WK 4	18 (0.067)	7 (0.063)	9 (0.040)	34
Total number of mortality	386	82	146	614
Total number of birds	27360	11100	22400	60860

***- Figures in the brackets are the percentage mortalities**

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Batch Investigation of Biogas Production from Palm Oil Sludge, Bambara Nut Chaff and African Wild Mango at Varying Meteorological Conditions

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***Abstract*—** *A custom response design study on the biogas production from blends of Palm oil sludge (POS), Bambara nut (VignaSubterranea) chaff (BNC) and African wild mango (AWM) was carried out. The anaerobic digestion was in the ratio of 3:1 and 2:1 of water to waste depending on the nature of the substrates as follows: System A was 100%POS; B: 100%BNC, C: 100%AWM;D: 50%POS+50%BNC and E: 60% POS+20% BNC+20% AWM. The wastes were charged into 32L capacity metal prototype digesters in a batch for 30 days retention period at an ambient temperature range of 26°C - 37°C. The cumulative gas yield from the five treatments systems (digesters) were different: the 50%POS+50%BNC had the highest cumulative gas yield (69.5L); followed by 100%BNC system (54.5L); 60%POS+20%BNC+20%AWM system (39.2L); 100%POS system (14.4L) and 100%AWM system (11.7L). 100%BNC system had the highest methane content (88.056%); followed by 60%POS+20%BNC+20%AWM system (88.007%); 100%POS system (83.025%); and 50%POS+50%BNC system (73.055%).The research has shown that 100% BNC had the least lag days (6 days), highest calorific value (25330.24KJ/Kg) and highest methane content (88.056%). African wild mango needs to be co-digested to produce flammable biogas. The TS, VS, BOD and TVC were seen to be consistently reducing throughout the digestion period. Meteorological conditions like solar radiation, air temperature and wind speed had significant effects on ambient temperature, slurry temperature, pH and daily gas yield.*

Keywords*— *Batch, Biogas production, Palm Oil Sludge, Bambara Nut Chaff, African Wild Mango, Meteorological Conditions.

Abbreviations: TS=Total Solid, VS=Volatile Solid, BOD=Biochemical Oxygen Demand, TVC= Total Viable Count

I. INTRODUCTION

In this era of industrialization and civilization energy turns out to be one of the bedrocks to achieving national economic status. Hence, the importance of energy cannot be over emphasized as it cuts across virtually every work we do from a little household to the largest cooperation company in the world. Some of the challenging facts facing the energy sector worldwide is the exhaustive nature of the commonly and generally used non-renewable energy sources such as petroleum, and the serious threats (uncontrolled emission of greenhouse gases) the use of such energy sources such as petroleum poses to the environment and mankind in general. Also alarming is the poor waste management strategies in the country; these wastes especially bio-degradable wastes constitute nuisance at the road sides, in our streets and sometimes in our homes. It has also been stated that uncontrolled greenhouse gases (CH_4, CO_2, CO etc) are emitted during the degradation of organic wastes. Subsequent upon these, there have been so many ameliorating technologies employed to ensuring a reduction of the effects such energy sources as it concerns man and his environment; however, biogas production through anaerobic digestion of organic wastes is one of such technologies. Anaerobic digestion is a multistage biochemical process that can stabilize many different types of organic material to produce methane and carbon dioxide by different groups of micro-organisms under anaerobic condition (Joaquin, 2008). Lo et al., (2010) in their work titled "Modelling biogas production from organic fraction

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of MSW co-digested with MSWI ashes in anaerobic bioreactors" stated that anaerobic digestion involves four steps which are: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The activities of micro-organisims present at different stages of anaerobic digestion however depends on various parameters such as temperature, pH, concentration of nutrients agitation, pre-treatment of feedstocks, carbon/nitrogen ratio, hydraulic retention time etc. Hence, in order to improve the overall efficiency of anaerobic digestion process, there have been many works on biogas production through anaerobic co-digestion of different wastes. Anaerobic co-digestion is defined as the treatment of a mixture of at least two different substrates with the aim of improving the efficiency of anaerobic digestion processes (Neczaj et al, 2012).

Oil palm is a common plant in the Southern part of Nigeria. Specifically the south-eastern part believes that it has some traditional values associated with it. Crude palm oil is one of the major products of palm oil processing plants with palm oil effluent as a by-product. This palm oil effluent is often accompanied by palm oil sludge which contains substantial quantities of solids left-over after the liquid must have drained. However, palm oil sludge are sources of pollution to the environment if discharged untreated, this is due to large oxygen depleting capabilities when present in aquatic systems (Hassan et al., 2013). According to Okwute et al., 2007 from their work titled "The environmental impact of palm oil mill effluent (POME) on some physico-chemical parameters and total aerobic bio-load of soil at a dump site in Anyingba, Kogi state Nigeria."studies on POME dump site revealed that the physico-chemical properties of the soil at this dump sites were obviously altered due to its acidic nature. Palm oil sludge are produced and discharged into the environment in millions of tonnes from both milling plants and individual households nationwide, thus, posing a big treat to the environment. Anaerobic digestion is the most suitable method for the treatment of effluents containing high concentration of organic carbon such as POME (Borja et al., 1996a). Projection depicts that 555, 457 and 409 million m^3 of methane gas could be produced under high, low and current status production rates scenario respectively up to 2030, if Nigeria harnesses the energy contained in palm oil mill effluent (Ohimain et al., 2014).

Vigna subterranean (Bambara nut) is a local bean plant commonly grown in the Northern part of Nigeria, but consumed in all part of the country. After it is processed the chaff are either used as blend for poultry feeds or they are thrown away, thus, depleting the environmental harmony. Previous work done on the biogas production potential of Bambara nut chaff showed that it had potentials for biogas production. According to Ofoefule et al. (2010) in their work titled "Biogas production from blends of Bambara nut (Vignasubterranea) chaff with some animal and plant wastes" observed that Bambara nut chaff has the potential for biogas production though the expected increased biogas yield and extended flammable time was not achieved by the co-substrates employed (cow dung, swine dung, cassava peel and field grass wastes). However, they proposed a chemical treatment to increase the pH to neutrality to enhance gas production and extend the retention time.

African wild mango (Irvingiagabonensis) is a plant which produces seeds rich in fat (around 70% on dry matter basis), traditionally used as a soup thickener in central and western African regions. However, it is obvious that much has not been done as it concerns biogas production from African wild mango.

Thus, the major objective of this work is to co-digest these three wastes to: (i) determine their physico-chemical properties and their effects on biogas production as well as waste treatment. (ii) to note the effect of African wild mango as a co-substrate with respect to increased gas production (iii) to determine the optimum mix ratio for optimized gas production. (iv) to have an idea of the level of waste treatment achieved at 30 days retention time.

II. MATERIALS AND METHODS

The study adopted custom response design. Bambara nut shells were collected from Ogige market in Nsukka and also from the university environment. African wild mango was gotten from Ibagwa plantation in Nsukka. Palm oil sludge was collected from a local palm oil processing plant in Ajuona community in Nsukka.

SAMPLE PREPARATION

The African-wild mango was chopped in smaller pieces and soaked in water at 100% level for 2 days. Thereafter, it was sieved to facilitate digestion due to the nature of the waste. The same was done for Bambara nut chaff for effective digestion and on-time gas production. After the soaking period, the feedstocks were then weighed out for charging.

EXPERIMENTAL SET-UP

Metallic model biodigesters (Plate 1) utilized for the study were each of 32.0 L working volume (fabricated locally at the National Centre for Energy Research and Development, University of Nigeria, Nsukka). Materials such as top loading balance (Camry Emperors Capacity 50 kg/110 Ibs), plastic water troughs, graduated transparent plastic buckets for measuring daily gas production, the pHep pocket-sized

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pH meter (Hanna Instruments), thermometers, pressure gauge, thermoplastic hose pipes, metallic beehive stand and biogas burner fabricated locally for checking gas flammability were used.

III. EXPERIMENTAL STUDY

The fermentation of the blends took place for 30 days at the prevailing ambient mesophillic temperature range of 26°C to 37°C. The ratio of the water to waste in each charging is as shown in table 1. This was based on the moisture content of the organic wastes at the point of charging the biodigesters. Palm Oil Sludge, Bambara Nut Chaff and African Wild Mango were co-digested to result to the following treatment blends: A (100%POS), B (100%BNC), C (100%AWM), D (50%POS+50%BNC) and E (60%POS+20%BNC+20%AWM). Table 1 shows details of the blending. Co-digestion is used to increase methane production from low-yielding or difficult to digest materials. Volume of gas produced, ambient and slurry temperatures, relative humidity and wind speed, insolation, pH and slurry pressure were monitored on daily basis throughout the period of digestion. Flammability check was also carried out on daily basis until the system produced flammable biogas and occasionally till the end of digestion period. The study was carried out at the exhibition ground of National Centre for Energy Research and Development, University of Nigeria, Nsukka.

Table.1: Substrates weight

Digesters	Quantity of undigested substrates	Amount of water (kg)	Total volume of slurry (L)	Volume Space for Gas (L)	Total volume of Digester (L)	Mix Ratio of slurry
A (100% POS)	8kg of POS	16	24	8	32	2:1
B (100% BNC)	4.8kg of BNC	19.2	24	8	32	4:1
C (100% AWM)	8kg of AWM	16	24	8	32	2:1
D(50% POS + 50% BNC)	3kg of POS + 3kg of BNC	16	24	8	32	3:1
E(60% POS + 20% BNC + 20% AWM)	4.8kg of POS + 1.6kg of BNC + 1.6kg of AWM	16	24	8	32	2:1

DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES

The methods used in this work to determine the physico-chemical properties of the undigested substrates are clearly defined as follows: The Meynell (1982) method was used to determine the: Total solids and Volatile solids while the A.O.A.C method (1990) was used to determine the: Moisture content, Ash content and Crude fibre content. The Pearson (1976) method was used in the determination of the Crude fat content with the use of Soxhlet extraction apparatus. The Micro-Kjedahl method as described in Pearson (1976) was used in the determination of Crude protein content while the method of surface viable count was used in the determination of the Total viable count (Number of living micro-organisms). The Energy content was determined with bomb calorimeter (model XRY-1A, make: Shanghai Changji, China), using A.O.A.C (1990) method).Walkey-Black (1934) method was used to determine the Carbon content while the ambient and slurry temperature was taken daily using a liquid in glass thermometer and the pH was ascertained using the Hanna instrument pH meter standardized using buffer solutions for pH 7.0.The pressure of the gas produced in the biogas digesters was measured daily using the sphygmomanometer. This water displacement method was used to determine the biogas volume while the Bacharach (PCA2) gas analyzer was used to determine the gas composition. A locally made gas burner was used to carry out the gas flammability tests. The population of the microbes in each of the treatment cases was determined at different times (at charging, flammable, peak of production and end of digestion), during the period of study to monitor the growth of the microbes at the various stages.

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Plate.1: The Anaerobic Biodigesters

Gas Analysis

The flammable gas compositions from the (100%POS), (100%BNC), (50%POS+50%BNC) and (60%POS+20%BNC+20%AWM) were analyzed using BACHARACH (PCA2) Gas Analyzer, made in United States.

Data Analysis

The data obtained for each of the systems were subjected to analysis using Microsoft Excel XP 2007. Meteorological data were obtained from Centre for Basic Space Science, University of Nigeria, Nsukka.

IV. RESULTS AND DISCUSSION

Table 2 shows the physicochemical properties of undigested wastes.

Table.2: Table of the physicochemical properties on the charging day (Day 0)

PARAMETERS	Sample A	Sample B	Sample C	Sample D	Sample E
Crude Fat (%)	0.7	0.75	0.6	0.95	0.6
Crude Protein (%)	1.4	1.14	1.31	1.45	1.44
Crude Nitrogen (%)	0.224	0.182	0.21	0.232	0.23
Carbon Content (%)	4.5	4.31	2.23	4.7	4.68
Total Solid (%)	8.46	9.3	7.37	10.4	9.5
Volatile Solid (%)	6.3	6.9	5.5	7.43	6.93
Ash Content (%)	3.9	4.7	3.2	6	5.3
Moisture Content (%)	89.29	88.68	88.24	85.19	89.47
Crude Fibre (%)	2.89	5.4	5.1	3.8	4.6
B.O.D (mg/l)	80	86	65.6	96	89.6
T.V.C (cfu/ml)	$40x10^5$	$45x10^5$	$34x10^5$	$63.33x10^5$	$50x10^5$
C/N	20.09	23.68	10.62	20.26	20.35

Table 3 shows the gas compositions for the various substrates.

Table.3: Table of gas composition of the flammable gas from experiment

Digester (Treatment)	**Retention Time(Day)**	**Cumulative Volume of Biogas(L)**	**Flammable Time(Day)**	**Component of Biogas (%)**			
				CO_2 (%)	**CO (%)**	**CH_4 (%)**	**Other components**
POS (100%)	30	14.4	23	20	0.0246	83.0246	3
BNC (100%)	30	54.5	5	15	0.0555	88.0555	3
AWM (100%)	30	11.7	-	-	-	-	3
POS (50%)+BNC (50%)	30	69.5	23	30	0.0055	73.055	3
POS(60%)+BNC(20%)+AWM(20%)	30	39.2	23	15	0.0065	88.0065	3

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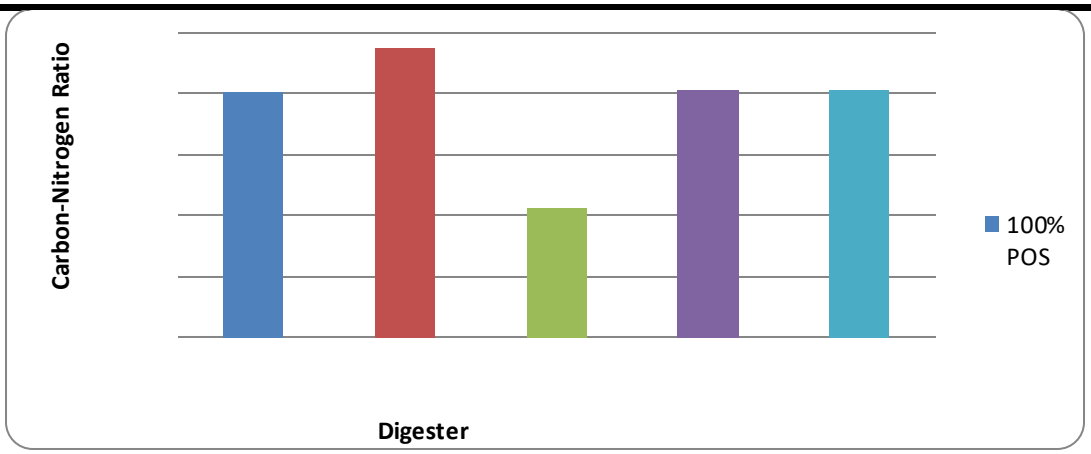

Fig.1: Carbon-Nitrogen Ratio of Digesters

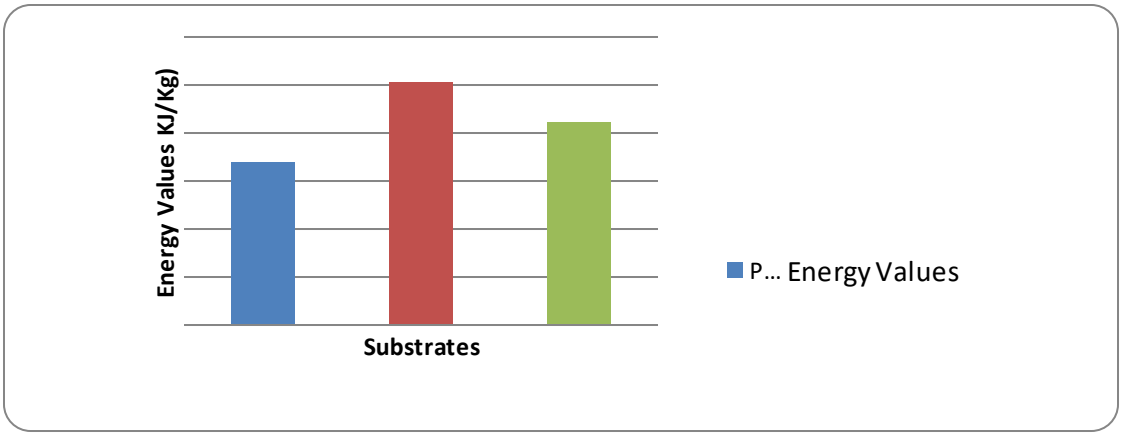

Fig.2: Energy values of Substrates

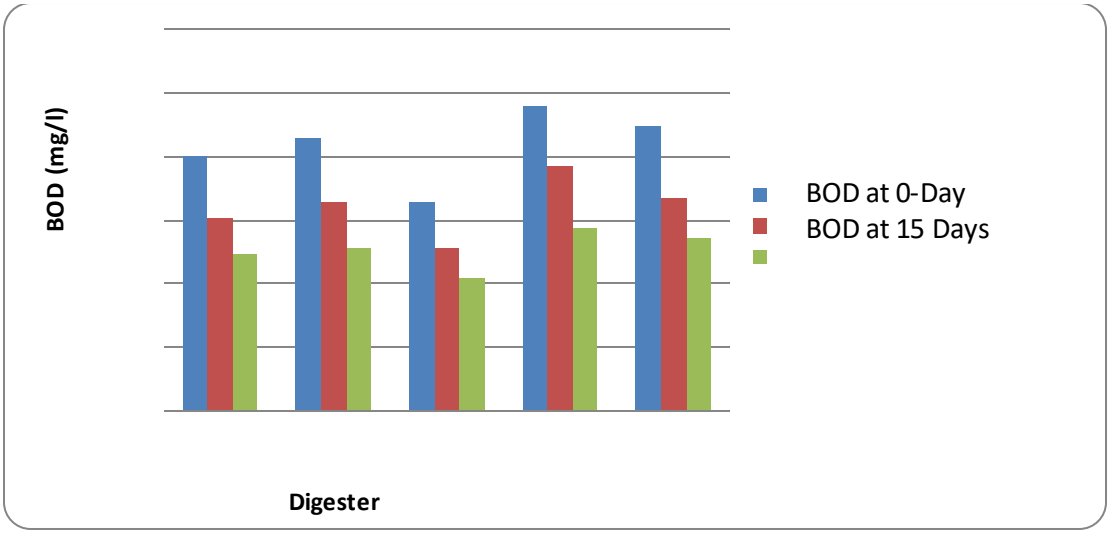

Fig.3: Weekly BOD Values

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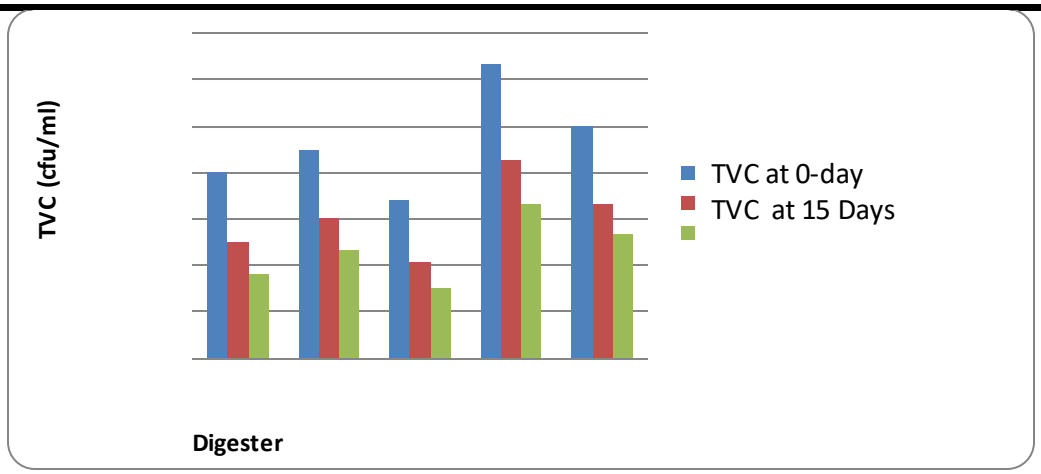

Fig.4: Weekly TVC Values

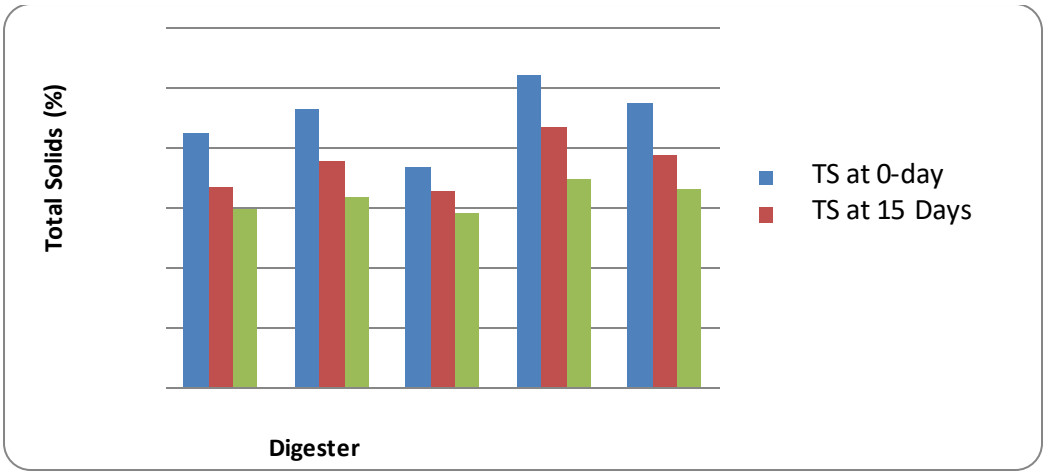

Fig.5: Weekly Total Solids

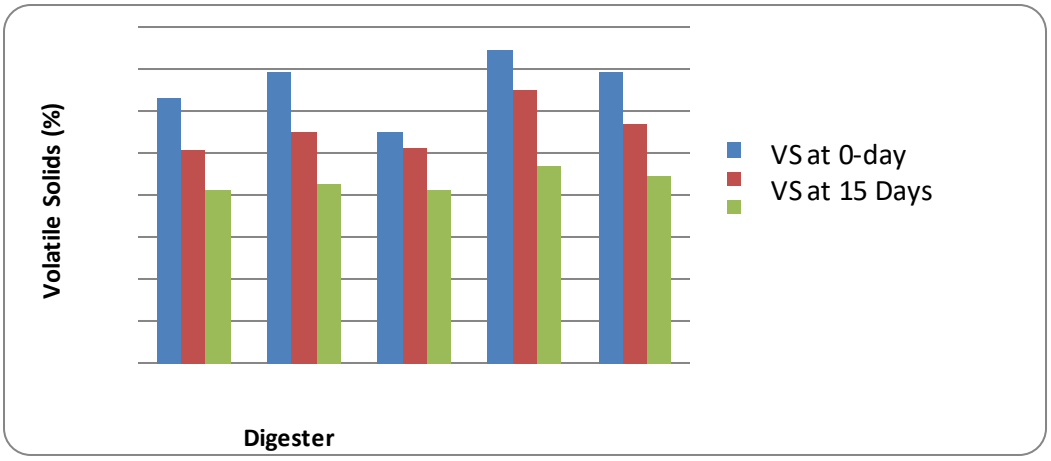

Fig.6: Weekly Volatile Solids

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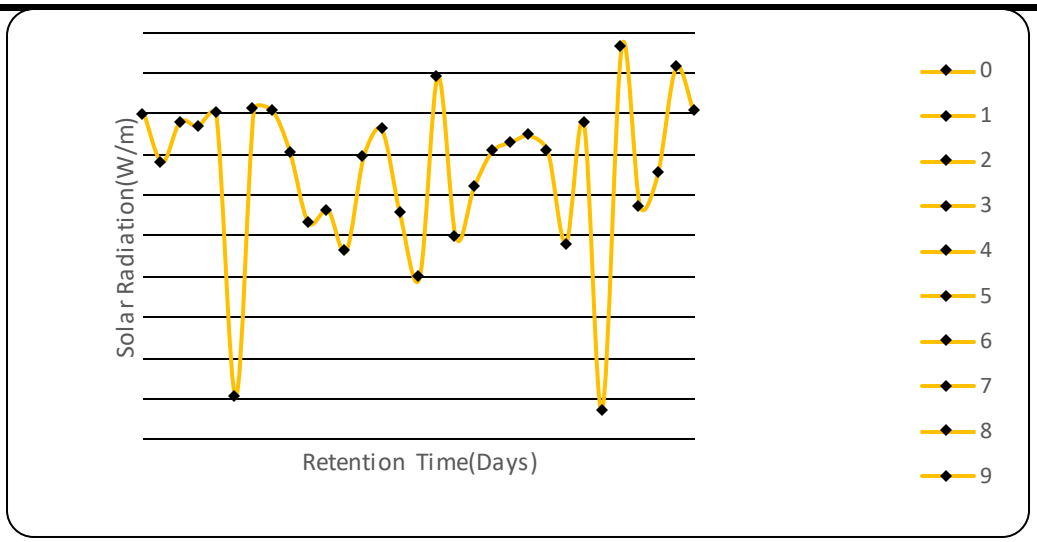

Fig.7: Solar Radiation Versus Retention Time

Fig.8: Air Temperature Versus Retention Time

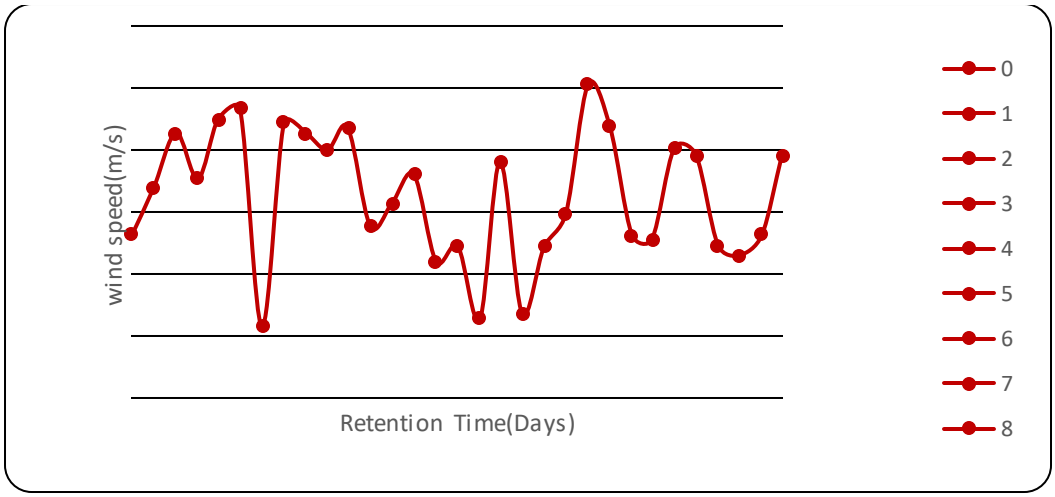

Fig.9: Wind Speed Versus Retention Time

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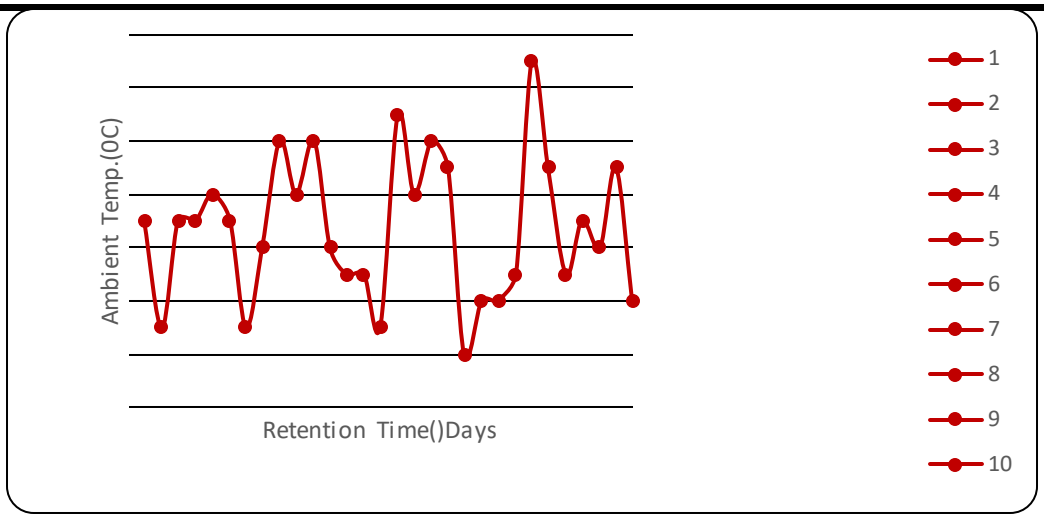

Fig.10: Ambient Temperature Versus Retention Time

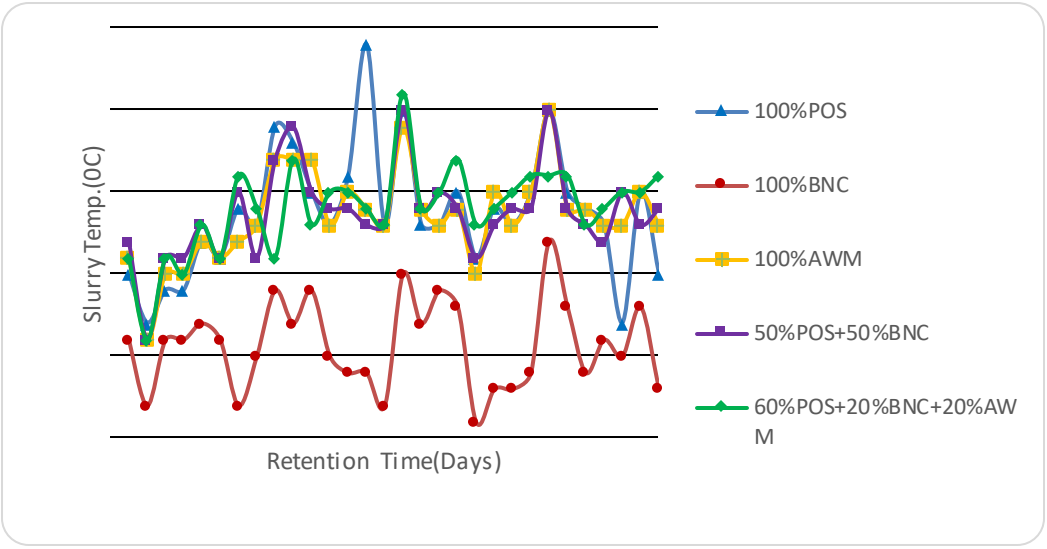

Fig.11: Slurry Temperature Versus Retention Time

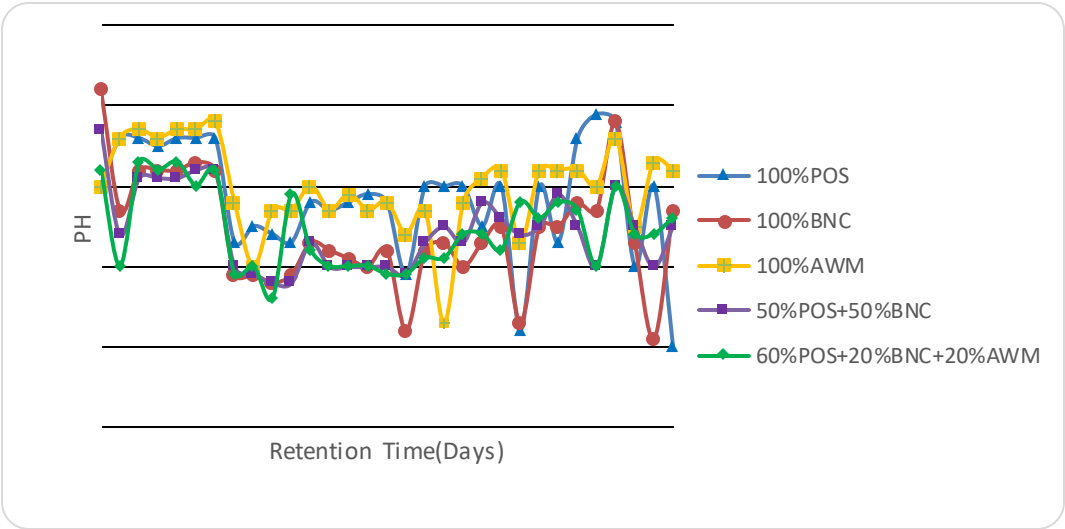

Fig.12: pH versus Retention Time

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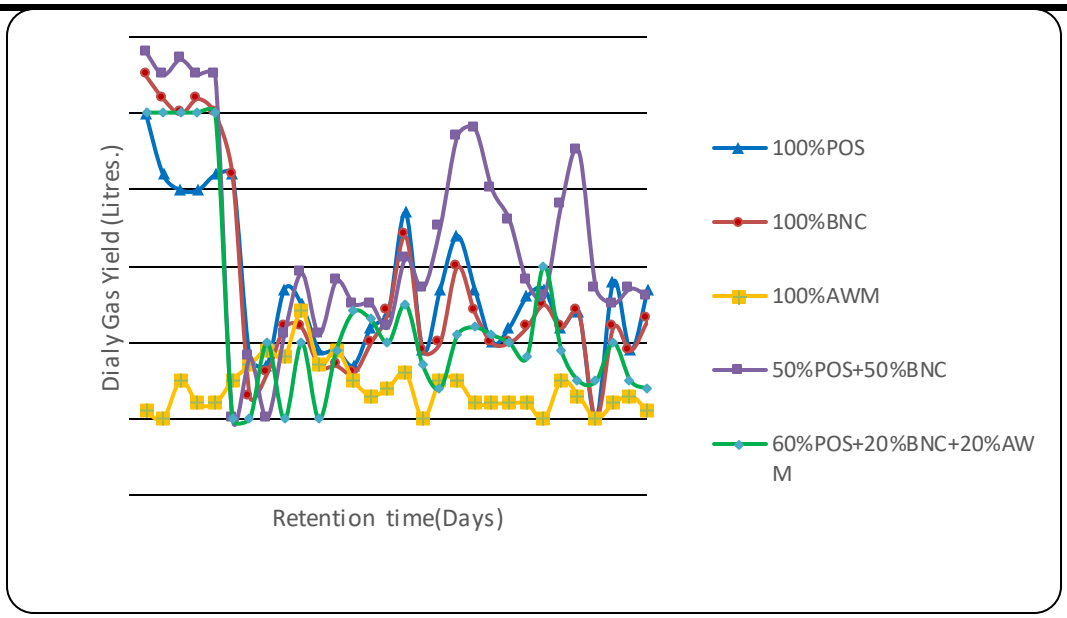

Fig.13: Daily Gas Yield Versus Retention Time

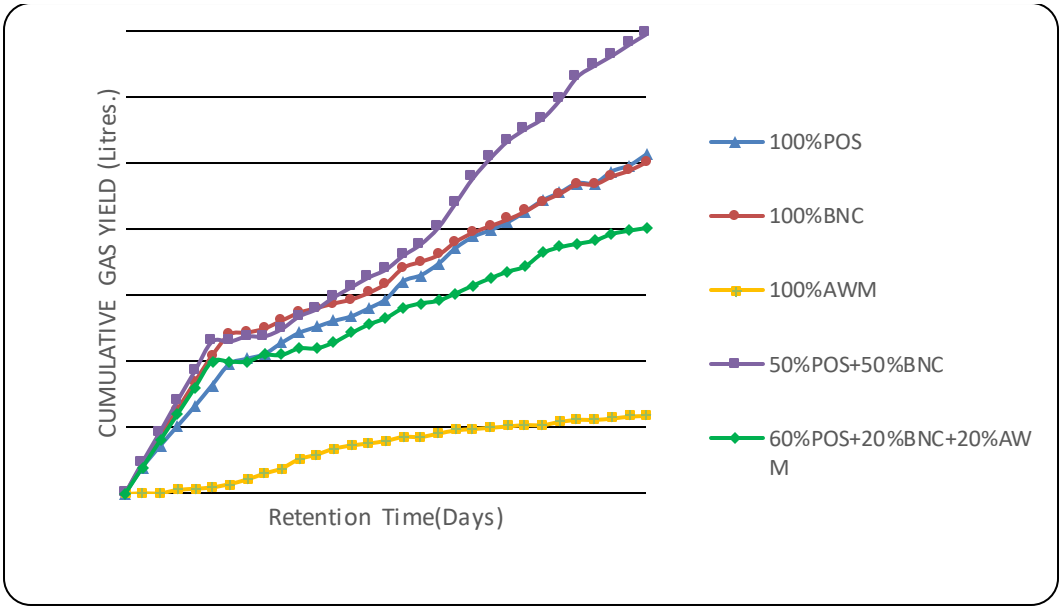

Fig.14: Cumulative Gas Yield Versus Retention Time

PROXIMATE ANALYSIS OF THE SYSTEMS

The proximate composition includes the ash, moisture, crude fibre and crude fat contents of the wastes. From table 2, The ash content of the 50%POS+50%BNC system was highest (6%) while that of 100%POS was least (3.9%).The 100%BNC system had the highest crude fibre content (5.4%) while the 100%POS had the least crude fibre content (2.89%). Each of the wastes for the systems had optimum moisture content because of the mix. Biological activities are increased when digester fluid are mixed to provide homogenous temperature and nutrient condition throughout the digester (Lay et al., 1997). The crude fat for each of the wastes was appreciable.

DIGESTERS' PERFORMANCE

The results of digester performances (from Table 3) indicated that 100% BNC system flamed on the 6th day while 100%POS, 50%POS+50%BNC and 60%POS+20%BNC+20%AWM systems flamed on the 23rd day. By having lesser number of lag days, the 100% BNC system is better in biogas production technology (Nagamani and Ramasamy, 1999). The cumulative gas

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yield from the five treatments were different: the 50%POS+50%BNC had the highest cumulative gas yield (69.5L); followed by 100%BNC system (54.5L); 60%POS+20%BNC+20%AWM system (39.2L); 100%POS system (14.4L) and 100%AWM system (11.7L) during the 30 days retention period. 100%BNC system had the highest methane content (88.056%); followed by 60%POS+20%BNC+20%AWM system (88.007%); 100%POS system (83.025%); and 50%POS+50%BNC system (73.055%).

EFFECT OF C/N RATIO ON THE SYSTEMS

From the results of Table 2 and figure 1, the C/N ratio of (100%POS), (100%BNC), (50%POS+50%BNC) and (60%POS+20%BNC+20%AWM) CD, were seen to be within the range of the optimum C/N. Consequently, each of these digesters flamed. Digesters 100% AWM had low C/N ratio that possibly led to ammonia accumulation and consequently could not flame. C/N ratio is an important indicator for controlling biological systems. During anaerobic digestion, microorganisms utilize carbon 25 to 30 times faster than nitrogen (Yadvika et al., 2004). To meet these requirements, microbes need 20 to 30:1 ratio of C to N.

CALORIFIC VALUES OF SUBSTRATES

The energy contents (figure 2) show that palm oil sludge and bambara nut chaff are good feedstock for biogas production if properly utilized while African wild mango has to be co-digested to produce biogas. Bambara nut chaff had the highest calorific value (25330.24 KJ/Kg); followed by African wild mango (21053.77JJ/Kg) and then Palm oil sludge (16935.98KJ/Kg).

EFFECT OF WEEKLY BOD, TOTAL VIABLE COUNT, TOTAL SOLIDS, VOLATILE SOLIDS; AND ANAEROBIC DIGESTION AS A WASTE MANAGEMENT TECHNOLOGY

Biochemical Oxygen Demand (BOD) is the amount of dissolved oxygen needed (i.e. demanded) by aerobic biological organisms to breakdown organic materials present in a given water sample at certain temperature over a specific time period. This is a quantitative expression of the ability of microbes to deplete the oxygen in waste water. It is also the amount of oxygen required for the biological decomposition of organic matter in wastewater by bacteria under aerobic conditions. This depletion is caused by the microbes consuming organic matter in the water via aerobic respiration. Total Viable Count (TVC) gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mould in a sample. The count actually represents the number of colony forming units (cfu) per gram (or per ml) of the sample.Total solid shows the total solid matter constituent of the entire organic waste both degradable and non-degradable. The volatile solid is the true organic matter available for bacterial action during digestion. Figures 3, 4, 5 and 6 show reductions in weekly BOD, TVC, TS, VS respectively. This is expected as the wastes stabilized. Anaerobic digestion is the most important method for the treatment of food waste because of its techno-economic viability and environmental sustainability. The use of anaerobic digestion technology generates biogas and preserves the nutrients which are recycled back to the agricultural land in the form of slurry or solid fertilizer. The relevance of biogas technology lies in the fact that it makes the best possible utilization of food wastes as a renewable source of clean energy since there is always reduction in BOD, TVC, TS and VS.

THE EFFECTS OF SOLAR RADIATION, WIND SPEED AND AIR TEMPERATURE ON AMBIENT TEMPERATURE, SLURRY TEMPERATURE, PH AND DAILY BIOGAS YIELD

There was variation in solar radiation resulting to highest solar radiation (582.604W/m) on the 26th day and least (135.655W/m) on the 25th day. Air temperature had highest (30.309^{o}C) on the 24^{h} day and least (23.196^{o}C) on the 25^{h} day. Wind speed had highest (1.813m/s) on the 21st day and least (1.035m/s) on the 6th day (figures 7, 8 and 9). The variation in these climatic conditions gave rise to variations in ambient temperature, slurry temperature, pH, and daily volume of gas produced (figures 10, 11, 12, 13 and 14).

V. CONCLUSION

This study has shown that wastes such as palm oil sludge, bambara nut chaff and African wild mangowhich have been termed nuisance to the environment can be utilized to produce biogas which can be used as an alternative to the widely known and used fossil fuel. The digestate after biogas has been produced can also be used as fertilizer to improve plant growth and enhance soil capability in producing.

From the results, it can be seen that the palm oil sludge and bambara nut chaff substrates are excellent in producing flammable biogas; capable of being utilized for any purpose such as cooking. African wild mango if co-digested can produce flammable biogas. The research has shown that 100% BNC had the least lag days, highest calorific value and highest methane content.

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This study has shown a new source for wealth creation and at the same time a means of decontaminating the environment by waste recycling and transformation. This wastes that are consumed in large quantities in homes can be used to produce biogas, this will help them lose the name attached to them as being nuisance to the environment.

VI. RECOMMENDATIONS

The following has been recommended as a result of findings from this work:

- The gas produced should be further purified to enhance its scope of utilization such as in welding and automobiles.
- A method of gas collection which is safe and highly reliable should be enhanced.
- Highly advanced technological equipment should be constructed for the storing the gas separately from the digesters.
- Equipment that can purify and utilize the biogas that has been produced can be fabricated; this will encourage people to use biogas.
- Researches should be carried out to discover means of improving the methane quality produced and also the quality of the bio-fertilizer left after digestion.

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Mesophillic Anaerobic Co-Digestion of Horse Dung, Plantain Peel and Egg Sheel at Different Climatic Conditions

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***Abstract*—** *This study centered on biogas production from locally available animal and kitchen wastes: horse dung (HD), plantain peel (PP) and egg shell (ES) using five 32-Litres metallic prototype digesters. The anaerobic digestion was in the ratio of 3:1 of water to waste for all the samples as follows: Sample A was 100%HD, Sample B; 100% PP, Sample C; 100% ES, Sample D; 50%HD+50%PP and Sample E; 60% HD+30% ES+10% PP. The retention time was 30 days. Parameters like pH, daily biogas production, ambient and slurry temperatures, solar radiation, wind speed, air temperature alongside the physico-chemical properties of wastes were monitored. The cumulative gas production yield was 81.8L, 22.3L, 51.9L, 109L, and 75.2L for samples A, B, C, D and E respectively. The flammable time was 10^{th}, 13^{th}, 16^{th}, 6^{th}, 10^{th} day for samples A, B, C, D and E respectively. The result revealed that sample D: 50%HD+50%PP gave the highest yield of biogas (109L) and flamed earlier than the other samples (6 days) while sample B: 100%PP had the lowest yield of biogas (22.3L). The results also showed that the sample that had the highest composition of methane in the biogas produced was Sample C: 100%ES with 90.3995% while the lowest composition of methane was found in Sample B: 100%PP to be 79.9963%. The TS and VS were seen to be consistently reducing while TVC and BOD reduced (immediately the microbes got acclimatized to the environment) showing the level of waste treatment achieved during the digestion period of 30 days.*

Keywords*— *Anareobic, Co-Digestion, Horse Dung, Plantain Peel, Egg Shell, Climatic Conditions.

Abbreviations: TS=Total Solid, VS=Volatile Solid, BOD=Biochemical Oxygen Demand, TVC= Total Viable Count

I. INTRODUCTION

Today, solid waste management and rising energy cost are two major problems facing the world in the recent days. Secondly the issue of global warming and climate change are strongly receiving public attention and have become a major environmental concern both at national and international level. The increasing concentration of atmospheric greenhouse gases as a result of culpable human activities represents the major cause for this problem (Lassey, 2008).

Possible methods to solve these problems are conversion of the solid wastes into methane gas via anaerobic digester. Anaerobic digestion can be used to treat all types of biodegradable waste, including food waste. It is used to stabilize mixed waste after the removal of recycleable materials. The realistic solution to reduce methane emission from manure storage will be using anaerobic digester in a controlled biogas plant so that methane can be collected as biogas (Külling et al., 2002). In this way atmospheric methane emission from the manure storage could dramatically be decreased. Biogas generated can be utilized for various energy services, such as heat, combined heat, and power. In addition, the biogas can be used as vehicle fuel, after removal of carbon dioxide and hydrogen sulphide in an up grading system (Lantz et al, 2007).

Co-digestion for anaerobic digestion means more than one feedstock is used at a time to produce biogas. Co-digestion is used to improve the methane yield from low yield feedstocks. Care must be taken to select compatible feedstocks that enhance methane yields and avoid materials that may inhibit biogas and methane production. Agricultural feed stocks have successfully been co-digested with restaurant bio wastes, food processing and crop residues (EPA 2012). The quantity, availability, and cost of co-digestion feed stocks are important factors to consider. Other factors to consider include: regulations and

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permitting; digester capacity; mixing of the feed stocks; and nutrient.

Literature contains substantial biogas production from different wastes in the locality. Nwankwo, 2014 did a research on the digestion of plantain peel (PP) and the codigestion of plantain peel with swine droppings (PP-SD) to observe the biogas production ability in a 50litres metal prototype biodigester. The waste (PP and PP-SD) were subjected to anaerobic digestion for a period of 44days. The cumulative biogas yield for the plantain peels alone (PP) was80.10dm^3 while that of plantain peels mixed with swine droppings (PP-SD) was 163.30m^3. The PP-A commenced flammable gas production on the 2nd day while, PP-SD commenced flammable gas production on the 30th day. The PP-SD had the highest cumulative gas yield though with a slow onset of gas flammability. The overall result indicates that the low gas yield of PP could be significantly enhanced by blending it with swine droppings.

Ezekoye, 2013 carried out a research on Plantain/almond leaves and pig dung used as substrates in anaerobic bio digester for producing biogas by batch operation method within the mesophilic temperature range of 20.0 to 31.0°C. The study was carried out to compare biogas production potential from plantain/almond leaves and pig dung wastes. The cumulative biogas produced from the plantain/almond leaves was 220.5L while the cumulative biogas from the pig dung was 882.5L. The methane component of gas from pig dung was 70.2% while that for plantain/almondleaves with algae was 72.7%. The biogas from the almond/plantain leaves became combustible onsixteenth day while the biogas from the pig dung was combustible on fourteenth day. Results showedthat pig dung produced more biogas than the almond/plantain leaves within the same period.

Kusch et al, 2008 conducted an experiments on methanogenesis from horse dung were conducted in laboratory-scale batch reactors in order to determine the substrate performance in a solid-phase digestion process, more specifically in terms of potential energy recovery and suitable process technology. Dung from a horse stable with straw bedding was used. The temperature was kept in the mesophilic range. In the percolation process (with process water sprinkled over the stacked biomass) a proportion of 10-20% of solid inoculum (pre-digested horse dung) was found to be suitable. Comparative experiments with both percolation and flooding revealed a higher biogas production per volume for the flooded process, as no addition of solid inoculum was necessary. Methane yield from fresh material was similar in both processes: around 170 L(N) CH(4) per kg VS added was obtained in six-week cycles with untreated material under optimized conditions. Methane production was increased after chopping the substrate. Pre-aeration resulted in decreased methane production.

Hadin and Eriksson, 2016 stated that horse keeping is of great economic, social and environmental benefit for society, but causes environmental impacts throughout the whole chain from feed production to manure treatment. According to national statistics, the number of horses in Sweden is continually increasing and is currently approximately 360,000. This in turn leads to increasing amounts of horse manure that have to be managed and treated. Current practices could cause local and global environmental impacts due to poor performance or lack of proper management. Horse manure with its content of nutrients and organic material can however contribute to fertilisation of arable land and recovery of renewable energy following anaerobic digestion. At present anaerobic digestion of horse manure is not a common treatment. In this paper the potential for producing biogas and biofertiliser from horse manure is analysed based on a thorough literature review in combination with mathematical modelling and simulations. Anaerobic digestion was chosen as it has a high degree of resource conservation, both in terms of energy (biogas) and nutrients (digestate). Important factors regarding manure characteristics and operating factors in the biogas plant are identified. Two crucial factors are the type and amount of bedding material used, which has strong implications for feedstock characteristics, and the type of digestion method applied (dry or wet process). Straw and waste paper are identified as the best materials in an energy point of view. While the specific methane yield decreases with a high amount of bedding, the bedding material still makes a positive contribution to the energy balance. Thermophilic digestion increases the methane generation rate and yield, compared with mesophilic digestion, but the total effect is negligible.

Ofili et al., 2010 researched on the quantity of biogas yield fromanaerobic digestion of rabbit waste and swine dung. Thesame volume of digester was used in this experiment toaccurately compare the volume of the biogas yield. Themaximum volume of biogas produced from the 45 litresdigester used was 8.2 litres and 6.8 litres respectively forswine dung and rabbit waste. It was observed that the biogas production from swine dung was greater than that of rabbit waste. These studies focused on anaerobic digestion of one substrate and or co-digestion of only two subtrates.

Co- digestion was not extended to more than two wastes. This necessitated embarking on this study. Hence, the aim of this work is to anaerobically co-digest horse dung,

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plantain peel and eggshell.These wastes were chosen in this experiment because they are available within the experimental locations.

II. MATERIALS AND METHODS

The study adopted customresponse design. Horse dung was gotten from Obollo-Afor market Udenu L. G. A, egg shell was gotten from Dawuba fast food restaurant behind Ekpo Refectory, University of Nigeria, Nsukka and plantain peels were gotten from Odenigbo Junction Nsukka and some fromOgige market, Nsukka. Metallic model biodigesters (Plate 1) utilized for the study were each of 32.0 L working volume (fabricated locally at the National Centre for Energy Research and Development, University of Nigeria, Nsukka). Materials such as top loading balance (Camry Emperors Capacity 50 kg/110 Ibs), plastic water troughs, graduated transparent plastic buckets for measuring daily gas production, the pHep pocket-sized pH meter (Hanna Instruments), thermometers, pressure gauge, thermoplastic hose pipes, metallic beehive stand and biogas burner fabricated locally for checking gas flammability were used.

Experimental Study

The fermentation of the blends took place for 30 days at the prevailing ambient mesophillic temperature range of 24°C to 34°C. The ratio of the water to waste in each charging was 3:1. This was based on the moisture content of the organic wastes at the point of charging the biodigesters. Horse dung, plantain peel and egg shell were co-digested to result to the following treatment blends: A (100%HD), B (100%PP), C (100%ES), D (50%HD+50%PP) and E (60%HD+30%ES+10%PP). Table 1 shows details of the blending. Co-digestion is used to increase methane production from low-yielding or difficult to digest materials. The moisture content of the respective wastes determined the waste to water ratios used. Volume of gas produced, ambient and slurry temperatures, relative humidity and wind speed, insolation, pH and slurry pressure were monitored on daily basis throughout the period of digestion. Flammability check was also carried out on daily basis until the system produced flammable biogas and occasionally till the end of digestion period. The study was carried out at the exhibition ground of National Centre for Energy Research and Development, University of Nigeria, Nsukka.

Table.1: Substrates weight

DIGESTER	Horse Dung (kg)	Egg Shell (kg)	Plantain Peel (kg)	Water (kg)
A (100%HD)	6	-	-	18
B (100%PP)	-	-	6	18
C (100%ES)	-	6	-	18
D (50%HD+50%PP)	3	-	3	18
E (60%HD+30%ES+10%PP)	3.6	1.8	0.6	18

The ratio of water to waste is 3:1

DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES

The methods used in this work to determine the physico-chemical properties of the undigested substrates are clearly defined as follows: The Meynell (1982) method was used to determine the: Total solids and Volatile solids while the A.O.A.C method (1990) was used to determine the: Moisture content, Ash content and Crude fibre content. The Pearson (1976) method was used in the determination of the Crude fat content with the use of Soxhlet extraction apparatus. The Micro-Kjedahl method as described in Pearson (1976) was used in the determination of Crude protein content while the method of surface viable count was used in the determination of the Total viable count (Number of living micro-organisms). The Energy content was determined with bomb calorimeter (model XRY-1A, make: Shanghai Changji, China), using A.O.A.C (1990) method).Walkey-Black (1934) method was used to determine the Carbon content while the ambient and slurry temperature was taken daily using a liquid in glass thermometer and the pH was ascertained using the Hanna instrument pH meter standardized using buffer solutions for pH 7.0.The pressure of the gas produced in the biogas digesters was measured daily using the sphygmomanometer. This water displacement method was used to determine the biogas volume while the Bacharach (PCA2) gas analyzer was used to determine the gas composition. A locally made gas burner was used to carry out the gas flammability tests. The population of the microbes in each of the treatment cases was determined at

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different times (at charging, flammable, peak of production and end of digestion), during the period of study to monitor the growth of the microbes at the various stages.

Plate.1: The Anaerobic Biodigesters

Gas Analysis

The flammable gas compositions from the 100% HD, 100%PP, 100%ES, 50%HD+50%PP and 60%HD+30%ES+10%PP were analyzed using BACHARACH (PCA2) Gas Analyzer, made in United States.

Data Analysis

The data obtained for each of the systems were subjected to analysis using Microsoft Excel XP 2007. Meteorological data were obtained from Centre for Basic Space Science, University of Nigeria, Nsukka.

III. RESULTS AND DISCUSSION

Table 2 shows the physicochemical properties of undigested wastes.

Table.2: Table of the physicochemical properties on the charging day (Day 0)

PARAMETERS	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D	SAMPLE E
Moisture Content %	88.33	84.48	87.93	88.46	85.19
Ash Content (%)	2.60	2.10	3.40	3.7	1.80
Crude Fibre(%)	3.80	3.40	4.10	4.60	2.51
Crude Fat (%)	0.50	0.80	0.60	0.55	0.90
Crude Protein (%)	1.75	1.23	1.40	0.96	2.01
Crude Nitrogen (%)	0.175	0.196	0.224	0.154	0.18
Carbon Content (%)	4.2	4.15	4.55	4.63	4.3
Magnesium (ppm)	0.5710	0.5742	0.7383	0.9286	0.7285
Calcium (ppm)	3.7753	1.8090	5.1124	4.4045	3.8539
Volatile Solid (%)	8.70	11.07	8.73	7.13	11.03
Total Solid (%)	10.33	12.87	9.93	8.77	13.40
B.O.D (mg/l)	59.2	73.6	62.4	51.2	68.8
Phosphorus (%)	0.60	0.90	1.40	1.10	1.00
Total Viable Count (TVC) (cfu/ml)	41.67×10^5	56.67×10^5	46.67×10^5	37.50×10^5	50.83×10^5
C/N	24.0	21.174	20.313	30.065	23.889

Table 3 shows the gas compositions for the various substrates.

Table.3: Table of gas composition of the flammable gas from experiment

Sample	Flammable Time/Lag Time (days)	Retention Time (days)	Cum vol of Biogas (L)	Composition of Biogas (%)				
				CO_2 (%)	CO (ppm)	CO converted to (%	CH_4 (%)	Others
A	10	30	81.8	17	10	1×10^{-3}	79.999	3
B	13	30	22.3	17	37.3	3.7×10^{-4}	79.9963	3
C	16	30	51.9	6.6	5	5×10^{-4}	90.3995	3
D	6	30	109.0	14.4	8	8×10^{-4}	82.5992	3
E	10	30	75.2	9.6	8	8×10^{-4}	87.3992	3

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Table 4 shows the energy content/ calorific values of the substrates

Table.4: Energy Contents/Calorific Values of substrates

Egg shell	13938.63KJ/Kg
Plantain peel	20634.86KJ/Kg
Horse dung	21351.17KJ/Kg

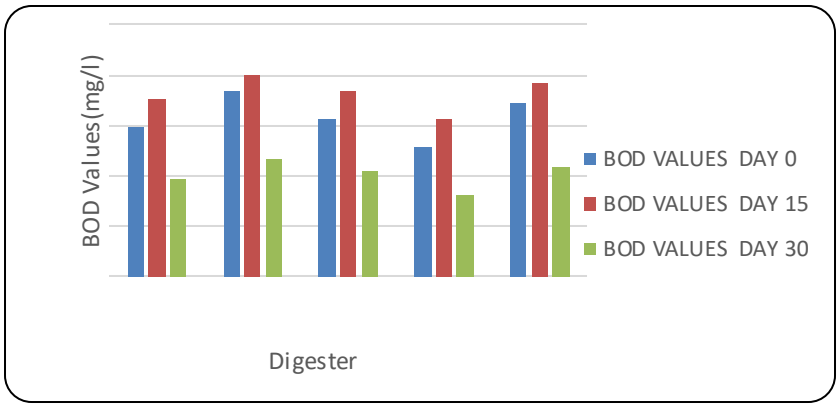

Fig.1: Weekly BOD Values

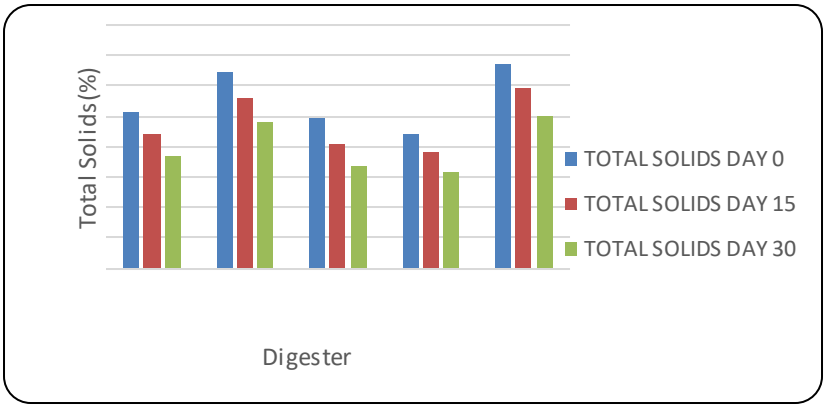

Fig.2: Weekly Total Solids

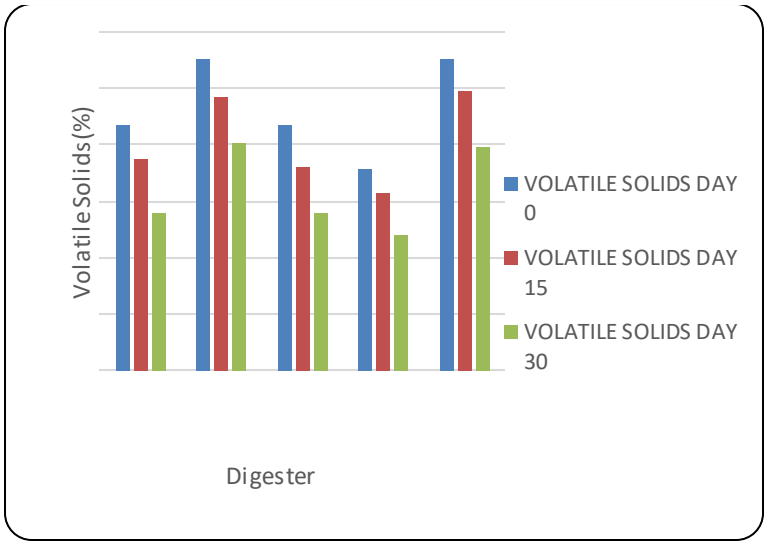

Fig.3: Weekly Volatile Solids

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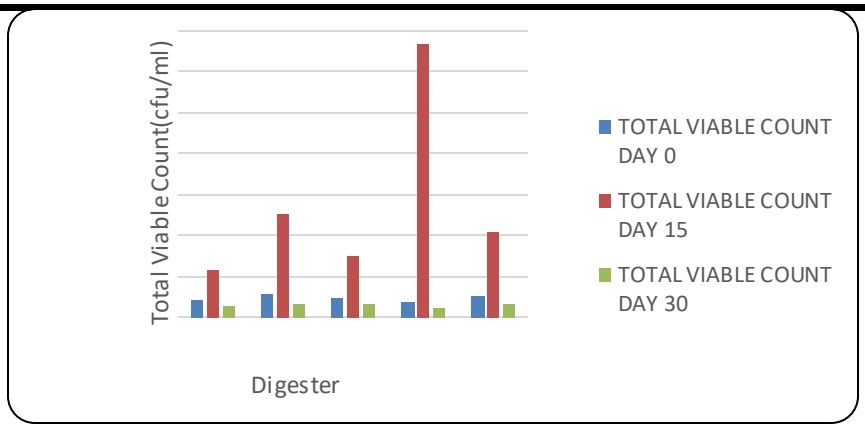

Fig.4: Weekly Total Viable Count

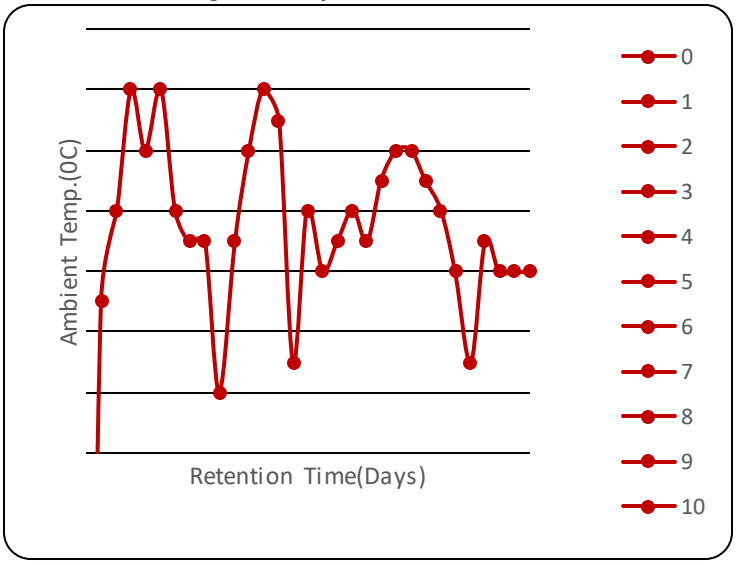

Fig.5: Ambient Temperature versus Retention Time

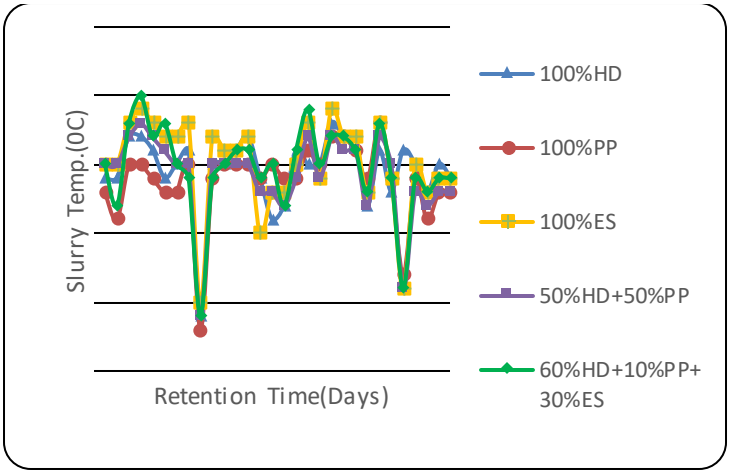

Fig.6: Slurry Temperature versus Retention Time

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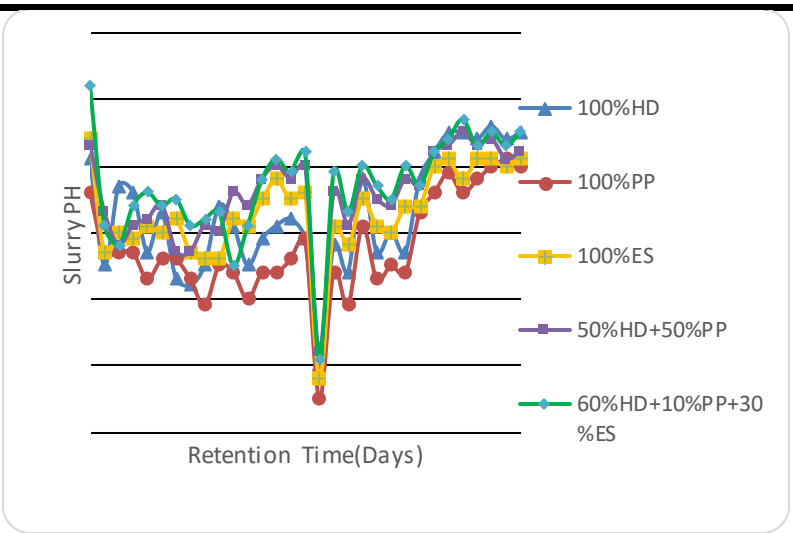

Fig.7: Slurry pH verus Retention Time

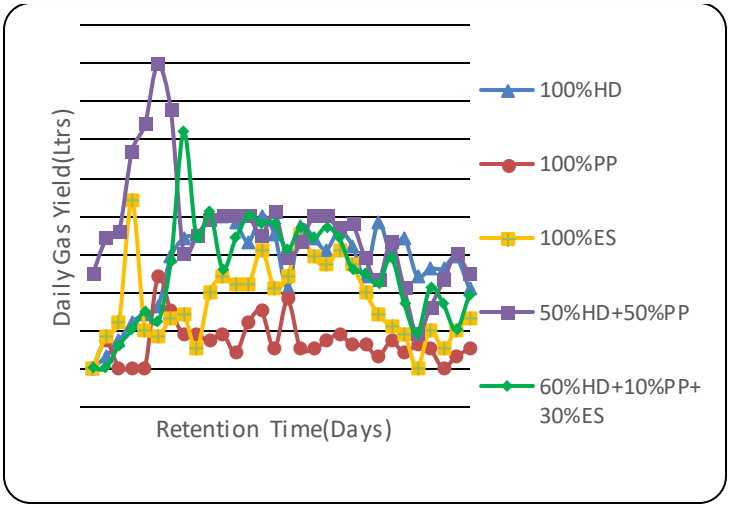

Fig.8: Daily Gas Yield versus Retention Time

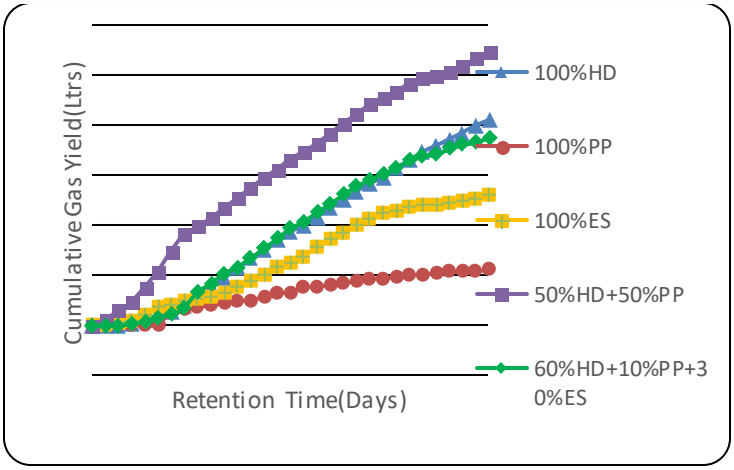

Fig.9: Cumulative Gas Yield versus Retention Time

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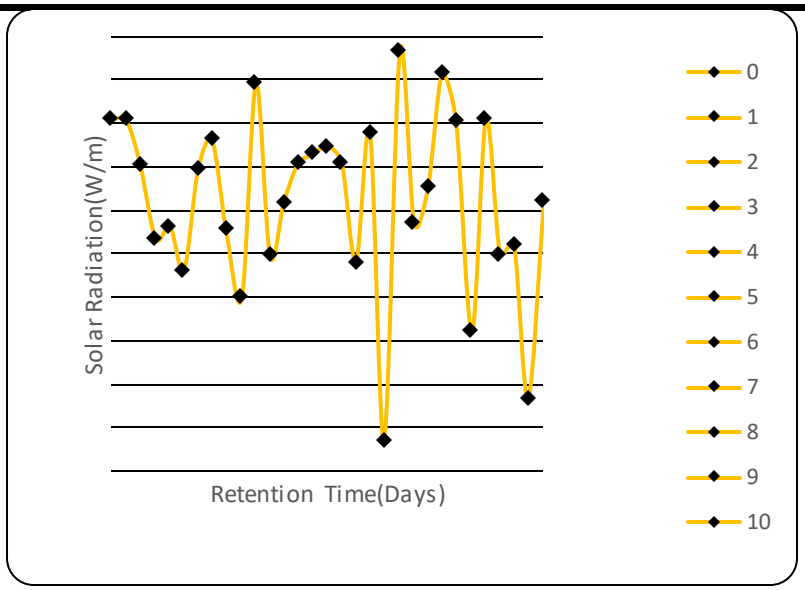

Fig.10: Solar Radiation versus Retention Time

Fig.11: Air Temperature versus Retention Time

Fig.12: Wind Speed versus Retention Time

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DIGESTERS' PERFORMANCE

The results of digester performances (from Table 3) indicated that 100% HD system flamed on the 10th day; 100%PP system flamed on the 13th day; 100%ES system flamed on the 16th day, 50%HD+50% PP system flamed on the 6 day while 60%HD+30%ES+10%PP system famed on the 10th day. By having lesser number of lag days, the 50%HD+50% PP system is better in biogas production technology (Nagamani and Ramasamy, 1999). The cumulative gas yield from the five treatments were different: the 50%HD+50%PP had the highest cumulative gas yield (109L); followed by 100%HD system (81.8L); 60%HD+30%ES+10%PP system (75.2L); 100%ES system (51.9L) and 100%PP system (22.3L) during the 30 days retention period. 100%ES system had the highest methane content (90.3995%); followed by 60%HD+30%ES+10%PP system (87.3992%); 50%HD+50% PP system (82.5992%); 100%HD system (79.999%) and 100%PP system (79.9963%).

EFFECT OF C/N RATIO ON THE SYSTEMS

From the results of table 1, the C/N ratio of 100%HD, 100% PP, 100%ES, 50%HD+50%PP and 60%HD+30%ES+10%PP were seen to be within the range of optimum C/N ratio. Consequently all the digester systems flamed. C/N ratio is an important indicator for controlling biological systems. During anaerobic digestion, microorganisms utilize carbon 25 to 30 times faster than nitrogen (Yadvika et al., 2004). To meet these requirements, microbes need 20 to 30:1 ratio of C to N.

CALORIFIC VALUES OF SUBSTRATES

The energy contents (table 4) show that the substrates are good feedstock for biogas production if properly utilized.Horse dung had the highest calorific value, followed by plantain peel and then eggshell.

EFFECT OF WEEKLY TOTAL SOLIDS AND VOLATILE SOLIDS

Total solid shows the total solid matter constituent of the entire organic waste both degradable and non-degradable. The volatile solid is the true organic matter available for bacterial action during digestion. There was generally a reduction in the weekly trend for total solids and volatile solids for each of the systems (figures 2 and 3).

EFFECT OF WEEKLY BOD AND TOTAL VIABLE COUNT

Biochemical Oxygen Demand (BOD) is the amount of dissolved oxygen needed (i.e. demanded) by aerobic biological organisms to breakdown organic materials present in a given water sample at certain temperature over a specific time period. This is a quantitative expression of the ability of microbes to deplete the oxygen in waste water. It is also the amount of oxygen required for the biological decomposition of organic matter in wastewater by bacteria under aerobic conditions. This depletion is caused by the microbes consuming organic matter in the water via aerobic respiration. Total Viable Count (TVC) gives a quantitative idea about the presence of microorganisms such as bacteria, yeast and mould in a sample. The count actually represents the number of colony forming units (cfu) per gram (or per ml) of the sample. The BOD and TVC on 0-day were lower than those on the 15th day since the microbes needed to acclimatize with the environment because there was no seeding. However, the values of BOD and TVC for the 30th day were lower than those of the 15th day; indicating stabilization. These trends were generally observed for each of the digester systems (figures 1 and 4).

THE EFFECTS OF SOLAR RADIATION, WIND SPEED AND AIR TEMPERATURE

There was variation in solar radiation resulting to highest solar radiation (582.604W/m) on the 20th day and least (135.655W/m) on the 19th day. Air temperature had highest (30.309°C) on the 18th day and least (23.196°C) on the 19th day. Wind speed had highest (1.813m/s) on the 15th day and least (0.84m/s) on the 25th day (figures 10, 11 and 12). The variation in these climatic conditions gave rise to variations in ambient temperature, slurry temperature, pH, and daily volume of gas produced (figures 5, 6, 7 and 8).

IV. CONCLUSION

This study has shown that wastes such as horse dung, plantain peel and egg shellwhich have been termed nuisance to the environment can be utilized to produce biogas which can be used as an alternative to the widely known and used fossil fuel. The digestate after biogas has been produced can also be used as fertilizer to improve plant growth and enhance soil capability in producing.

From the results, it can be seen that the three substrates and their combinations are excellent in producing flammable biogas; capable of being utilized for any purpose such as cooking. The research has shown that even though egg shell had the lowest calorific value; it had the highest methane content. 50% HD+50%PP had the least lag time (6 days).

This study has shown a new source for wealth creation and at the same time a means of decontaminating the environment by waste recycling and transformation. This wastes that are consumed in large quantities in homes can

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be used to produce biogas, this will help them lose the name attached to them as being nuisance to the environment.

V. RECOMMENDATIONS

The following has been recommended as a result of findings from this work:

- The gas produced should be further purified to enhance its scope of utilization such as in welding and automobiles.
- A method of gas collection which is safe and highly reliable should be enhanced.
- Highly advanced technological equipment should be constructed for the storing the gas separately from the digesters.
- Equipment that can purify and utilize the biogas that has been produced can be fabricated; this will encourage people to use biogas.
- Researches should be carried out to discover means of improving the methane quality produced and also the quality of the bio-fertilizer left after digestion.

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Size Distribution and Growth *Mackerel scad* (*Decapterusmacarellus*) in the Ambon Waters

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Abstract*—Mackerel scad (Decapterusmacarellus) is one of the small pelagic fish that has important economic value and caught throughout the year around the waters of Ambon. Utilization of these fish is not only profitable economically but also can have a negative impact if not pay attention to aspects of sustainability. The purpose of this research is to know the distribution of long frequency and growth pattern. This research was conducted for six months (September 2016-February 2017) around Ambon waters. Samples of fish analyzed amounted to 1548 individuals. The average length of the minimum fish caught is 9.5 cm and the average size of the maximum length of fish caught is 31.8 cm. Fish growth patterns obtained are isometric.*

Keywords: Decapterusmacarellus, size distribution, growth pattern, Ambon Waters.

I. INTRODUCTION

Ambon Waters is a one of the waters which has a lot of Fishery Resources in Maluku. The site of ambon waters which is front on the Banda Sea makes this waters also has a lot of variety and kind of pelagic fish and demersal fish. Mackerel Scad (*Decapterusmacarellus*) is a one of pelagic fish which was caught over the year, around Ambon Waters. This kind of fish was caught by using purseseine with a Fish Aggregating Device (FADs). Comparing with any kind of mackerel fish like *Decapturesrusselli* and *Decapturesmacrosoma*, the characteristic of Mackerel Scad is Off-shore. This kind of fish is living as a group with other small pelagic fishes (Chan *et al*, 1997). Tiews*et al*., (2001) A spreading of Mackerel Fish in Indonesia inculdes, Java Sea, Strain of Makassar, Ambon, Ternate (East Indonesia). Besides, became a source of an animal protein to be consumed by local people, this fish also can be a fish bait to the pole and line fishery and has become an export commodity to a making of katsuobushiin Japan. (Widodo, *et al* 1999).

An utilization and an effort of catch which has done straight away toward the source of *Dercapterusspp* should be based on the amount of the available supply in order to make a fish supply can be continued.An utilization of *Decapterusmacarellus* resource in Ambon Waters was indicated that it has experienced a biological overfishing and economic overfishing (Sangadji*et al*,2014; Pattikawa, J.A*et al*, 2018). An information of *Decapterusmacarellus* utilization from biologic aspect still lack of doing for kicking the continuing management. The purpose of this reasearch is to know size distribution and the pattern of growth of *Decapterusmacarellus* in Ambon Waters. An information of *Decapterusmacarellus* should be investigated and done straight away, and also include the fish biological information. The data about length distribution and the growth pattern of mackerel was useful to describe a condition of fish supply, recently and was expected to be able in giving biological information and data base for the importance of the fish management.

II. MATERIALS AND METHODS

This research was conducted for Six Months (Sept. 2016 to February 2017) represent two seasons of catch, such as; Two Change Season and West Season around Ambon Waters (**Fig 1**).Sample of Mackerel Scad was achieved from the result of pursesein haul which operated around Ambon Waters every month as long as six months. The sample was taken random at the fishing landing and fish landing site around Ambon. Sample collection was done every two weeks during a reserach. Sample analyzing of Mackerel Scad was done at the Biological Laboratory of the Faculty of Fishery and Marine Science, Pattimura University. The sample of fish length was measured by using gauge board with carefulness of 0,1 cm. Type of measuring which has done was the total length of body such as the length from a top of the head or a top of the mouth to the end of the tail. The weight was measured by using digital scales with a carefulness of 0,1 gram.

Length distribution was made based on the class with the interval 0,5 cm on every month of research (Sept. 2016-February 2017). The relation between the length and the weight were analyzed by using Effendie (1979) as follow : $W = a L^b$.

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Which : W = fishWeight (gr) . L = fish length (cm). a and b = Constanta.

Formula above will be equal with using natural logarithm on the two variable as follow :

$$\text{Ln } W = \text{Ln } a + b \text{ Ln } L.$$

In determining the relation between length - weight as long as the research used regression and correlation analyzing. In finding about b mark whether it is equal or not with 3, will be used t test according to Effendi (1979) as :

B = tangent of regression angle.

Sb =Standard deviation of value b.

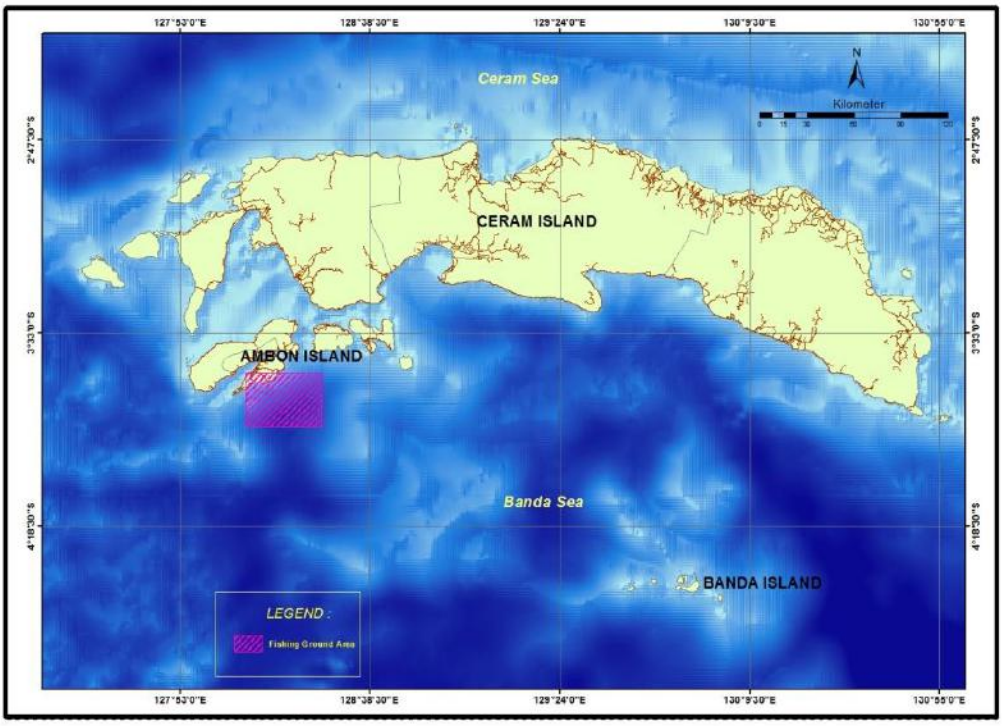

Fig. 1: Map of Mackerel scad (Decapterusmacarellus) fishing operations in the Ambon Waters.

III. RESULTS AND DISCUSSION

3.1. Size Distribution

A total sample of 1.548 *Decapterusmacarellus* individuals collected over six months was obtained in a total length range of 9.5-31.8 cm (**Table 1**).The length size every month had a variation, followed by the domination of size presence frequency. For Sept. 27,2 cm, Oct. 19,9 cm, Nov. 17,5 cm, Dec. 19,7 cm, Jan. 22,5 cm, Feb. 21, 35 cm. The size of catch dominant fish was imature except on september in the size of 27,2 cm. Some of research result by Widodo (1999) in Gorontalo Waters, Tilamuta, Tomini Bay, and Maluku Sea shows the size of fish length was 9-30 cm, Hariati (2004) in Banda Aceh Waters, the fork length of 16-32cm., Iksan andIrham (2009) in North Maluku Waters 21,1-31,1 cm, Silooy&Soumokil (2007) in the South of Ambon Waters, the length of the fish 14,0-27,0 cm, Widyastuti andZamroni, (2017) in Tomini Bay 17,25-30,25 cm FL with the ripe condition on August and Pattikawa (2018) in the North Ambon Waters, the length 11,0-24,5 cm. Presence Frequency indicates that *Decapterusmacarellus* was caught over the year in Ambon Waters, it was also revealed by a purseseine fisherman. Widodo (1999), said that the top of this fish season happened twice in a year around April-May and November in Maluku Sea. Yulius (2013) at LombeBay , The season of catch on August - November. Commonly, In Ambon Waters and surroundings, the season of the catch happened on Sept-November (Second Change), this thing was assumed because there was a upwelling proccess in Banda Sea (Gordon and Susanto 2001;Sediadi 2004;Haruna *et al*, 2018).

Table.1: Size distribution of Decapterusmacarellus during the study

Period (month)	Number (indiv)	Length class (cm)	Mid Length (cm)	Minimum (cm)	Maximum (cm)	Mode (cm)
Sept-2016	354	12-29,5	20,8	12,3	29,3	27,2
Oct-2016	205	15-26,5	21	15,5	26,5	18,9

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Nov-2016	360	9,5-32,0	20,65	9,5	31,8	17,5
Dec-2016	202	12,0-24,0	18	12,1	23,9	19,7
Jan-2017	196	18,5-26,5	22,5	18,6	26,4	19,7
Feb-2017	231	12,0-31,0	21,35	12,1	30,6	20,6
Total	1.548					

3.2. Growth

Based on the result of length-weight relationship of *Decapterusmacarellus*shows that there is a tight relation between length and weight. This relation can be seen from a milling of correlation coefficient mark (r) as much as 0,936-0,984 close to +1 (**Table 2**.) Silooy and Soumokil (2007) also got correlation coefficient mark (r) as much as 0,9168. Ongkers*et al* (2016) got a mark (r) around Latuhalat Waters as much as (r) = 0,942. Pauly (1984) revealed Coefficient of Growth (b) to the relation of length - weight can be used to see the pattern of fish growth. If B = 3 then can be said that the pattern of growth was isometric which means the rate of additional length as same as additional weight. In reverse, if B (not)= then the growth of fish is allometric as Negative Allometric (b<3) which is the growth of length was faster than the growth of weight and Positive Allometric (b>3) which means the growth of weight faster than the growth of length. Based on the result then the growth coefficient mark (b) of Marckerel during the research has a milling about 2,927-3,165. Isometric Growth as the ascension of length had a same level with the ascension of weight happened on September, December, and February, Positive Allometric Growth as ascension of weight was faster than ascension of length on December, meanwhile Negative Allometric Growth as ascension of length was faster than an ascension of weight on October & January. The difference of the b mark of the fish was assumed that there was an effect of food availability, Spawning Time, and the time and pressure of different catch and effecting the living and the growth of fish.

Table.2: Length-weight relationship of Decapterusmacarellus during the study

Periode	n	W= a L^b	Correlation coefficient (r)	Growth Pattern
Sept-2016	354	$W = 0{,}0073L^{3{,}0748}$	0,984	Isometric
Oct-2016	205	$W = 0{,}0116L^{2{,}9296}$	0,951	Negative allometric
Nov-2016	360	$W = 0{,}0058L^{3{,}1685}$	0,985	Positive allometrics
Des-2016	202	$W = 0{,}0073L^{3{,}090}$	0,936	Isometric
Jan-2017	196	$W = 0{,}0106L^{2{,}9716}$	0,940	Negative allometric
Feb-2017	231	$W = 0{,}0086L^{3{,}0403}$	0,976	Isometric

IV. CONCLUSION

The avarage of size distribution of Mackerel in Ambon Waters on the size 9,5-31,8 cm. The Variant Growth on the milling of 2,927-3,165, isometric pattern on September, December, and February, negative allometric on October & January, meanwhile positive allometric on November.

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Exploring MAS: A Reliable Molecular tool for Development of Multiple Disease Resistance in Tomato (*Solanum lycopersicum* L.) through Gene Pyramiding

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***Abstract* —** *Tomato is principally subjected to severe yield losses due to adverse effects of a number of factors particularly biotic factors (pathogens). The lacks of tomato genotypes resistance to diseases have increased both the financial cost and environmental impact of tomato production while reducing the yield and quality. Therefore developing tomato genotypes with multiple resistances to diseases is a desirable goal. However, the most effective strategy of controlling reduction in yield and quality of tomato caused by various biotic factors during crop productivity encountered by poor resource farmers is by utilization of host resistance. Recently, technological advancement has led to the development of DNA based molecular markers closely linked to genes in tomato chromosomes that bestowed resistance to various kinds of biotic factors. Gene transfer of preferred traits into a single tomato genotype is now possible through the deployment of marker assisted selection (MAS) process, which now hastens the advancement of resistance tomato cultivars in its lowest population with gene pyramiding or stacking. In this review, we present the eco-friendly molecular tools with emphases on MAS for the improvement of biotic resistance in tomatoes which can be more explore in identifying land races that have the potentials of subduing the attack of pathogens if improved genetically.*

Keywords— Disease resistance, gene pyramiding, Tomato, Marker Assisted Selection (MAS).

I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is cultivated as a major fruit and vegetable crop both in the tropics and subtropics of the world and is of great economic importance to many smallholder farmers as well as consumers. When eaten either fresh or in processed form, it supplies vitamin A and C in the human diet and equally serves as an antioxidant which protects cells from oxidants that have been linked to cancer (Giovannucci, 1999; Agarwal and Rao, 2000; Rao and Rao, 2007). The less effort required in tomato cultivation compared to other crops and the short maturity duration of most tomato genotypes; could have led to the appreciable number of smallholders increasing their initial cultivable land areas thereby changing from subsistence farming to commercial cultivation.

Generally, low yield of tomatoes are due to a number of factors, that is, biotic, abiotic, or their natural genetic composition. These factors include, lack of appreciable number of improved well-performing varieties or genotypes, poor fruit setting due to heavy rains and excessively high temperatures; which limit pollination, more specifically fecundation plus pollen viability; pests and diseases (Villareal, 1979; Lyons et al., 1985; Ladipo, 1988). However, diseases caused by pathogens such as fungi, bacteria, viruses and nematodes have been playing significant roles in reduction of potential yield of many tomato varieties either directly or indirectly for many years as evident from reports in literature. Management approaches to reduce or mitigate the extent of damage caused by these pathogens in the past have mainly through manipulation of the environment through cultivation practices (such as intercropping, mixed cropping systems, crop rotation and so on), which prevent an increase in the disease causal agents of infection. The use of Agrochemicals has also been encouraged in the past but this approach is not eco-friendly and also poses many health

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problems in addition to their adverse effect it might possibly caused on fruit quality. Chemical control is also known to give good results for some pathogens, but poor results against others, such as bacteria, and practically no effects on viruses.

It is noteworthy that technological advancement in innovative genetic preferences and breeding for pathogen-plant resistance in tomato is a cost effective and feasible option to that can contribute to yield in an eco- friendly environment. Since the domestication of many plants including tomato, differences in varietal response to various stresses have been observed (Ragimekula et al., 2013). The emergence of new strains of pathogens necessitates development of highly improved approaches to enhance the capability of tomato varieties to survive numerous pathogen attacks at once. In cultivated tomato generally, there is absence of resistance in their gene pools and so, effort has been to identify host resistance against diseases in wild tomato species in the last decades (Zamir et al., 1994; Ji et al., 2007, 2009; Hanson et al., 2006; Garcia et al., 2007; Anbinder et al., 2009).

Through conventional breeding programmes along with desired genes of interest, other genes also continue to the next filial generations, which make screening a difficult task in conventional breeding techniques. In spite of their drawbacks, conventional breeding techniques are also important for the conservation of wild type germplasms, hybridization between opposite parental lines and so on.

Today, for continued yield improvement, new technologies especially the use of DNA molecular markers, currently serve as a new technological tool to detect the presence of allelic variation in the genes underlying the economic traits in tomato varieties. DNA markers have enormous potential to improve the efficiency and precision of conventional breeding through marker-assisted selection (MAS) by reducing the reliance on laborious and fallible screening procedures (Ragimekula et al., 2013). The technique enables the transfer of desired genes of interest in tomato with appropriate combination. This short review therefore, briefly highlights the molecular markers presently available for breeding programs in tomato with classical examples of successful results where genes/loci from resistant tomatoes have been pyramided into other susceptible tomato elite. We also give suggestions for further research work in this area crop productivity.

II. MARKER ASSISTED SELECTION: A MOLECULAR TOOL IN TOMATO BREEDING

MAS are a technological based process by which a marker is indirectly used for the selection criteria of genetic factor(s) or determinants of a trait including disease tolerance. It is used as a substitute for or to assist phenotypic screening. It involves selecting plants carrying traits of interest in the genomic regions expressing the traits through the use of molecular markers. Basically, selection of plants using MAS is based on the genotype of the associated markers rather than the traits. But the success of using MAS depends on many other factors, including the underlying genetic control of the trait(s) of interest (Foolad and Panthee, 2012). With the advent of MAS in tomato breeding programs, its application enables individual plants to be selected based on their genotypic characteristics during the selection process. Since, heterozygotes cannot be easily distinguished from homozygotes through conventional breeding, MAS can be used instead to achieve this purpose by selecting parents and as well increase the chance of backcrossing for reliable linkages between markers and traits of interest such as disease resistance, and fruit quality. Furthermore, MAS can be used to investigate heterosis for hybrid crop production (Reif et al., 2003).

There are many more advantages of using MAS in tomato breeding, for example, in testing seed purity of large lots of tomato, quick germplasm screening for disease resistance or fruit quality (Foolad and Panthee, 2012). This is because it is simpler than phenotypic screening, reduces time, effort to employ and resources to be used. Additionally, MAS can be done at the seedling stage by eliminating undesirable plant genotypes even when dealing with traits that seem to be complex.

III. MOLECULAR MARKERS USED IN TOMATO BREEDING

The progress in plant breeding is now easy and quick for qualitative traits controlled by major genes with easily identifiable effects on phenotype unlike quantitative characters which is still more complicated. .The use of molecular tools has significantly improved tomato breeding programs, allowing application of advanced molecular breeding techniques in tomato that have led to the improvement of cultivable tomato varieties that are resistant to diseases. Through MAS, this objective has been achieved over the years. Fortunately, tomato was one of the first crops for which molecular markers were suggested as indirect selection criteria for breeding purposes (Rick and

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Fobes, 1974; Tanksley and Rick, 1980; Tanksley, 1983). However, with the development of new molecular markers and maps in tomato, MAS has become a routine practice in many tomato breeding programs, in particular in the private sector, for several purposes including (1) to assess hybrid purity from overseas production by screening seed lots with a panel of molecular markers, (2) for quick and effective germplasm screening for disease resistance or fruit quality and (3) for marker assisted backcrossing (MAB) after reliable linkages between markers and simple traits of interest are discovered (Foolad and Panthee 2012). Most of the molecular markers used in tomato breeding programs use the polymerase chain reaction (PCR). These markers are all DNA based techniques designed to detect and exploit naturally occurring DNA polymorphisms which provides a unique type of markers to serve as an index of genetic worth of the entire genome. The main consideration for the use of DNA markers in MAS is that, markers should be tightly linked to target loci or genes preferably less than 5 centimorgan genetic distances.

Several DNA based molecular markers have been used in breeding for tomato biotic stress resistance as evident from various literatures (**Table 1**). These molecular markers include, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), cleaved amplified polymorphic sequence (CAPS), single nucleotide polymorphism (SNP) and Sequence Characterized Amplified Region (SCAR). Depending on research and interest needs, each suitable molecular marker of preference in any tomato breeding program must fulfill the following conditions that characterize its suitability: (1) must be polymorphic, (2) display co-dominant inheritance, (3) randomly and frequently distributed throughout the genome, (4) easy and cheap to detect and (5) should be reproducible. However, PCR-based markers are more attractive for MAS, due to the small amount of DNA template required and more efficient handling of large population sizes (Ragimekula et al., 2013). With the advent of these molecular markers, their applications in tomato breeding have been useful in: (1) early generation selection of transgressive segregants to increase the speed and efficiency of developing new varieties, (2) introgression of genes from wild type, (3) gene pyramiding, which is of emphases of this review (4) DNA fingerprinting of tomato varieties to characterize genetic diversity for effective management and utilization of germplasm resources, (5) construction of high saturated (density) maps of tomato crop that provides assessment of segment of genome for economic use, and so on. Hence, the steps to follow for gene pyramiding scheme using MAS are described in (**Fig.1a, b)** respectively.

IV. GENE PYRAMIDING USING MAS: AN EXCELLENT APPROACH IN TOMATO BREEDING PROGRAMS

Gene pyramiding or preferably gene stacking in tomato involves the transfer or pyramiding of more than one gene resistance or tolerance to a particular disease (s) into a single tomato genotype (**Fig. 1b**). Since there are likely chances of each resistance gene to be overcome by a new virulent race (VR), there is need to place all available major genes of VR into a single genotype which will reduces the probability of a new race conferring virulent genes at all the corresponding loci. And so, to achieve this objective, gene pyramiding is an essential technique to be adopted. This breeding methodology combines both the conventional (artificial hybridization) and molecular techniques in improving new tomato genotypes carrying the genes of interest. Pyramiding of resistance genes into a single line for each disease is a strategy to prevent the breakdown of resistance against specific disease (s). Such genes are found mostly in the wild types of tomato varieties. Basically, with the introduction of MAS, plant breeders now make use of this technique to confer resistance by several different genes or loci with the help of markers that are closely linked against such diseases like *Tomato leaf curl virus* disease (TLCV), *Tomato spotted wilt virus* (TSWV), Bacterial spot, Bacterial speck diseases, late blight, *Fusarium* wilt and so on (**Table 1**). To obtain the desired population with required gene combinations without unwanted genes, it is essential to make some backcrossing with the recurrent parent (**Fig. 1b**). With the help of molecular markers linked to the gene, selection of tomato progenies with desired traits in a population are easily identified for several effective backcrossing schemes whereas progenies not carrying the conferred gene are discarded. Gene pyramiding helps in development of a pedigree designed to accumulate all desired genes in a single genotype and as well fix the target genes into a homozygous state to derive the ideal genotype from one single genotype (**Fig.1b**).

V. CONCLUSIONS

There are several biotic factors that affect the productivity of tomato. But the present development of molecular markers has led to an increase in the use of disease resistant quantitative trait loci (QTL) in tomato breeding. Gene pyramiding is an important breeding strategy for tomato and

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improvement of germplasm of agricultural crops, but requires that breeders should consider the minimum population size that must be evaluated to have a chance of obtaining the desired genotype. Considerably, molecular marker genotyping can facilitate the process by reducing the number of generations that breeders must evaluate to ensure that they have the desired gene(s) combination introgressed to the desired genotype of interest. Hence, in order to reduce the risks of pathogen adaptation, resistance breeding schemes through the use of MAS by gene pyramiding should be more thoroughly explored with deployment strategies of resistant genotypes and combination strategies of diverse disease control methods. Since the use of MAS has made it possible to identify and select tomato plants with different numbers of resistance genes with related resistance performance, hence, exploring diverse tomato genotypes genome should be intensified. However, to pyramid multiple resistance genes into a single cultivar of tomato, tomato breeders should be able to monitor the effects of the genes stacked in the same cultivar, which is not readily possible through phenotypic measurements. Therefore through the use of marker assisted pyramiding in tomato breeding programs, breeding for qualitative disease resistance has been proven to be useful and should be effectively utilized on tomato germplasms that have high yielding ability but with significant rate of susceptibility to pathogens.

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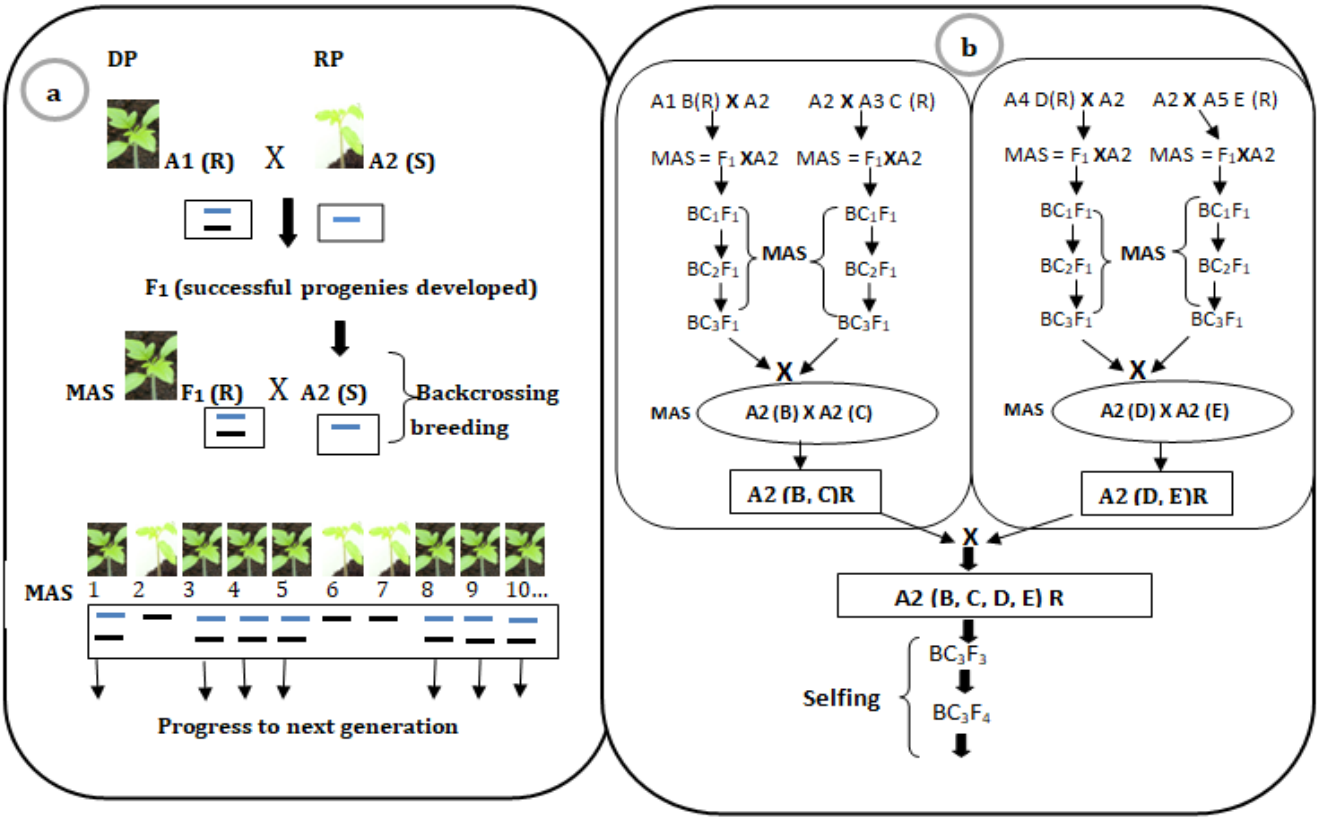

Fig.1 (a) General overview of marker assisted backcross in a tomato breeding program; DP (donor parent); RP (recipient parent); X (crosses); R (Resistant genotype);S (Susceptible genotype); A1,B,C,D,E (represents different tomato genotypes resistant to different diseases; A2 (high yielding genotype but susceptible to A1,B,C,D,E) (b) Basic flow diagram showing steps involved in gene pyramiding of multiple biotic resistance (R) genes into a single genotype using marker assisted backcross breeding.

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Table 1: Examples of some selected successful tomato genotypes improved by pyramiding using MAS; biotic stress resistance; genes/QTLs; linked markers and their donor parents.

Traits pyramided	Gene/QTL(s)	Genotype (s) improved by pyramiding	Donor Parent(s)	Type of available linked markers	Reference
Tomato yellow leaf curl virus X *Tomato spotted wilt virus*	*Ty-1,Ty-3,Sw-5*	Cuban tomato (LD3)	LA2779, LA1969,	CAPS, RAPD(*Eco*R1), SCAR,	Consuegra et al., 2015
Tomato yellow leaf curl virus X Tomato mottle virus	*Ty-1,Ty-3*	LA2779	LA1932, LA1938	CAPS, RAPD(*Eco*R1), SCAR,	Ji et al., 2007
Late blight X *Tomato yellow leaf curl virus* X *Bacterial wilt* X *Fusarium wilt* X *Gray leaf spot* X *Tobacco mosaic virus*	*Bwr-12, Ty-2, Ty-3, Tm2^2, Ph-2, Ph-3, Sm*	CLN3241	CLN2777G, G2-6-20-15B, LBR-11	CAPS, SCAR	Hanson et al., 2016
Fusarium wilt X *Late blight*	*I1 , Ph-3*	Accession 1008, 017878,107868, 0101 and 1002	Not defined	SSR , SCAR	Akbar et al., 2016
Bacterial spot X *Bacterial speck*	*Pto , Rx3*	Crosses between parents	Ohio 9834, Ohio 8819, Ohio 981205	CAP (*Rsa1*, *BsrB1*)	Yang and Francis, 2005
Tomato yellow leaf curl viruses (monopartite and bipartite)	*Ty-2,Ty-3*	Crosses between: FLA478-6-1-11 and CLN2498C,CLN1621E	*Ty*- stock of: LA3473,CLN2585D,CA4,GC171, TY-172	CAPS, SCAR, SSR	Prasanna et al., 2014
Tomato spotted wilt virus X Late blight	*Sw-5, Ph-3*	Crosses between NC946 and NC592	NC946, NC592	CAPS , SCAR	Robbins et al., 2010
Tomato leaf curl virus	*Ty-1,Ty-2*	Pbc, H-86 crosses with the parents	EC538408, EC520061, H-24	CAPS, SSR	Kumar et al., 2014

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Traits pyramided	Gene/QTL(s)	Genotype (s) improved by pyramiding	Donor Parent(s)	Type of available linked markers	Reference
Tobacco mosaic virus X *Fusarium wilt* X *Root-knot nematode disease X Leaf mildew*	*TM-2[a], I-2, Mi-1, Cf-9*	L11, L19, L46 and L51	Longkeeper, Jia Powder	CAPS	Zhu et al., 2010
Phytophthora infestans	*Ph-2**	AD17, 137, P15250, SeI8	Heline, Momor, Motelle, Ontario, Pyrella, Stevens		Barone A (Not yet published .See reference section for link to this information)
*Fusarium oxysporium f.*sp. *radicis-lycopersici* X *Tobacco mosaic virus X Verticillium dahliae*	*Frl, TM-2[a], Ve**	AD17, 137, P15250, SeI8	Heline, Momor, Motelle, Ontario, Pyrella, Stevens	CAPS	
*Fusarium oxysporium f.*sp. *lycopersici* X *Meloidogyne* spp. X *Verticillium dahliae*	*I2*, Mi, Ve**	AD17, 137, P15250, SeI8	Heline, Momor, Motelle, Ontario, Pyrella, Stevens	CAPS	
Pseudomonas syringae	*Pto*	AD17, 137, P15250, SeI8	Heline, Momor, Motelle, Ontario, Pyrella, Stevens	CAPS	
Pyrenochaeta lycopersici	*py-1*	AD17, 137, P15250, SeI8	Heline, Momor, Motelle, Ontario, Pyrella, Stevens	SCAR	
Tomato spotted wilt virus	*Sw-5*	AD17, 137, P15250, SeI8	Heline, Momor, Motelle, Ontario, Pyrella, Stevens	CAPS	

* = Search for the suitable markers is still in progress as reported by **Barone A**

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Aorta Artery and Branches in Tucanuçu (*Ramphastostoco* – Muller, 1776)

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***Abstract*—** *Tucanuçu (Ramphastostoco – Muller, 1776) is the largest Tucano birds and possess remarkable characteristics, such long orange beak and black spot on end, its black plumage, an area of bare orange skin around the eyes and blue eyelids, being a symbol of avian diversity found in South American ecosystems, whose anatomy and systemic description are little studied, fundamental process to understand biological system of this animal. Thus, this work aimed to perform an anatomical description of aorta artery and respective branches in Tucanuçu through a comparative study, using 2 male and 2 female specimens. In this sense, the present work shows unprecedentedly discovered about this animal that vascular pattern shows similarities with other birds and Aorta being the main vessel that emerges from left ventricle base of the heart. The first branches of Aorta are three coronary arteries: Right, Left and Dorsal. Brachiocephalic Aa.andCervicobrachialarises from cranial face of aortic arch and directed: Brachiocephalic a. which is left limbs, to head, neck and left thoracic member and Cervicobrachial to neck and right thoracic limb.*

***Keywords*— Circulatory System, Comparative Anatomy, Wild Animals and Birds.**

I. INTRODUTION

Tucanuçu (*Ramphastostoco* – Muller, 1776) is the largest Tucano birds **[1]** and possess remarkable characteristics, such long orange beak and black spot on end, its black plumage, an area of bare orange skin around the eyes and blue eyelids **[2]**. It is a bird Piciforme order and Ramphastidae family, found in all central part of Brazilian territory and some parts of Amazon, exhibiting abundance in Cerrado and Atlantic Forest **[1,3,4]**. Due unmistakable and unmistakable characteristics, Toucanos birds are considered the symbol of avian diversity in South American continent. However, this species is threatened as victim of anthropic actions, including animal trafficking **[5,6]**.

This bird stands out for it large size, reaching up to 66 cm in length and its beak, 23 cm, which, although large and disproportionate to the body, is light, representing only 5% of its body weight, which can weight 540 grams **[7]**. They are large birds that occupy forest canopy and whose diet is substantially based on fleshy fruits **[3,8,9]**, insects and arthropods, but also can loot nests of other birds and feed their eggs or puppies **[10]**.

The presence of fruits in Tucanuçu diet, it has an important ecological function in dispersion of seeds, fundamental for regeneration of forests **[11]**. The planet presents complex pattern climates, which, in turn, play an important role in creating complex patterns of vegetation and types of community found, including Cerrado, which can be considered complex biome **[12]**. The Cerrado is the second largest Brazilian biome, consisting of a mosaic of plant formations ranging from open fields to dense forest formations **[13,14]**. It occupies about 20% of national territory, limiting itself almost all Brazilian biomes (except southern fields and coastal ecosystems), there are still regions of enclaves Cerrado in Amazon, Caatinga and Atlantic Rainforest caused changes in biodiversity **[15]**.

Cardoso da Silva and Bates (2002) **[16]** suggest that bird distribution patterns follow same dynamics of flora diversification of Cerrado environments. The group of birds presents an expressive concentration of species in Cerrado, since practically half of species registered in Brazil can be found in this biome. Major revisions of

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Cerrado avifauna indicate existence of 837 species in this area **[17]**.

The aorta is the main arterial trunk of the body, beginning at the base of left ventricle and divided into *Ascending* and *Descending Part*, and presents many ramifications. In domestic birds, the first branches of *Ascending Part* are *Right Coronary* and *Left CoronaryAa*.that born cranially at aortic valve fixation. Each coronary artery branches in *Superficial* and *Deep Branches*, unlike mammals. The *Deep Branch* is larger than *Superficial* and travels through interventricular septum. The right coronary artery is larger than left. The following branches of Aorta are: *Right* and *Left Brachiocephalic Arteries*, very prominent, which originate *Right* and *Left Common Carotid Arteries* and *Right* and *Left Subclavian Arteries***[18]**.

Aorta Arch is the transition between Ascending and Descending Parts and extends dorsally and to the right and reaches the apex level of right lung doubles caudomedially continuing then as *Descending Part of Aorta* or simply *Descending Aorta*, which extends to tail region, when is called *Median Caudal Artery*. Each *Brachiocephalic Artery* is divided into *Common Carotid Artery*, *Axillary Artery* and *Pectoral Trunk*. The *Descending Aorta*, along its path through thorax and abdomen, emits several pairs of *Dorsal Arteries* which are named according to the segment of column in *Dorsal, Caudal, Sinsacral*and *Intercostal*. At the fifth costal level, descending aorta emits two large branches, *Celiac A.*and *Mesenteric cranial a.*, the two main abdominal visceral arteries, other visceral branches of descending aorta are *Renal Arteries* and *Mesenteric Caudal*. In the pelvic portion of birds, the substantial branches of aorta are *External* and *Sciatic Iliac Arteries* as the major branches of this portion of aorta and the main arteries for pelvic limb of birds **[18]**.

Together, anatomical and systemic descriptions of any specie are fundamental for biological system knowledge and the importance in ecosystem. However, anatomy of aorta and its branches in Tucanuçu is little studied. Considering the importance of circulatory system for this animal anatomy, this study aimed investigate and describe the anatomy of Abdominal Aorta and its branches in Tucanuçu as a literary subsidy for different areas of knowledge and preservation programs.

II. MATERIAL AND METHODS

The present paper is a descriptive anatomical study with two male and two female specimens of Tucanuçu (*Ramphastos toco*), obtained from natural or accidental death on the roadsides of Brazilian Southeast of Goiás, under authorization of SISBIO nº 37072-2. Considering the descriptive approach of this work, statistical analysis is not necessary. All procedures were conducted in accordance with ethical principles and approved by the Institutional Ethics in Research Committee at the Federal University of Uberlândia (CEUA/UFU nº 067/12).

The study was made in the research laboratory of human and comparative anatomy of Federal University of Goiás – RC, as previously described by our group **[19]**, were the arterial system was dissected and inject with Latex Artecola, colored with red pigment Wandalar, through the carotid artery. Subsequently was made a fixation with aqueous 10 % formaldehyde solution to conservation.

The preparation of anatomical pieces was performed under consecrated techniques in Macroscopic Anatomy and abdominal wall was laterally open to visceral exposure, then all abdominal part and digestive system secluded, exposing dorsal wall of abdomen. With an anatomical forceps, adipose tissue and other tissues were removed to expose abdominal aorta artery and its branches. A stereomicroscope MOTIC SMZ-168, with magnification of 10X was used to dissection procedure.

The Nikon® D7000 18-105 digital camera was used to photographical documentation and description nomenclature adopted is the standard of NominaAnatomicaVeterinaria (2012) **[20]**, elaborated by the International Committee on Veterinary Gross Anatomical Nomenclature.

III. RESULTS

Aorta is the main artery that leaves heart of Tucanuçu, it born at base of left ventricle and emits three coronary arteries: *Right, Left* and *Dorsal*. The first part of Aorta in Tucanuçu, *Ascending Aorta* is very short, it follows cranially, slightly inclined to the right and soon curves strongly to the right and dorsally, like *Aortic Arch*. The *Aortic Arch* emits *Brachiocephalic Aa.*to left and *Cervicobrachial* to the right. A *Brachiocephalic a.* is a prominent blood vessel that born from cranial faces of aortic arch and then branches into *Left Carotid a.* and *Left Subclavian a.*. A *Cervicobrachial a.* arises from aortic arch and provides branches to neck and right limb and from *Aortic Arch* in dorsal surface, born an artery for lungs, trachea, and esophagus (Figure 1).

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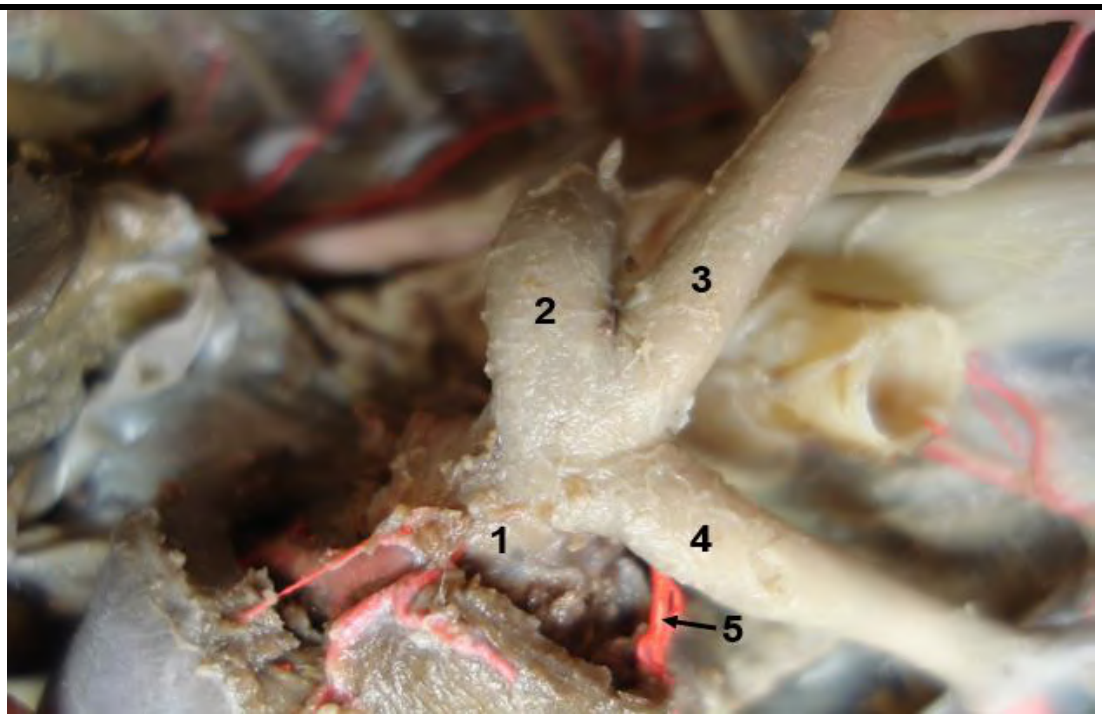

Fig.1: Ventral view of the thoracic cavity of the Tucanuçu: 1- Ascending Aorta, 2- Descending Aorta, 3- Cervicobrachial Artery, 4- Brachiocephalic Artery, 5-Pulmoesophageal Artery.

The descending aorta begins at cranial pole of right lung, curves medially to medial plane, when follows the ventral aspect of spine, crossing entire thorax, without providing collateral. The first major branch of descending aorta is a visceral artery, *Celiac A.*, and then, on ventral side, *Mesenteric Cranial A.* born, a large artery which together *Celiac A.*, supplies nearly all abdominal viscera. Throughout *Abdominal Part* of Aorta, in the vicinity of *Celiac* and *Mesenteric Cranial A.*, several small arteries, as *Intercostals Aa.* intended for chest wall and abdominal wall. The next branches of Aorta are small *Renal Cranial Aa.*, intended for cranial lobes of kidney (Figure 2).

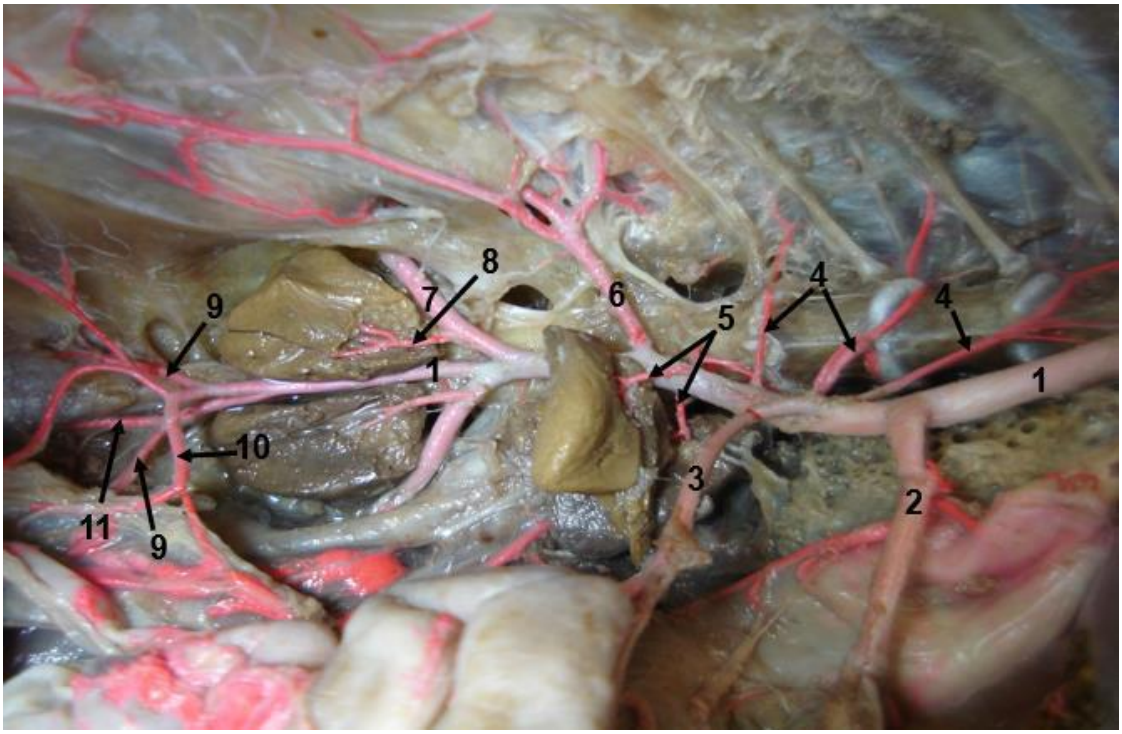

Fig.2: Ventral view of the thoracoabdominal cavity of the Tucanuçu:1- Descending aorta, 2- Celiac artery, 3- Cranial Mesenteric Artery, 4- Intercostal Arteries, 5- Cranial Renal Arteries, 6- External Iliac Artery, 7- Sciatic Artery, 8- Caudal Renal Arteries, 9- Internal Iliac Artery, 10- Caudal Mesenteric Artery, 11- Medium Caudal Artery.

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Then, on each side, a large *ExternalIliac A.*, which dorsally crosses renal lobe in lateral direction, but does not provide any branches before reaching abdominopelvic wall. Thereafter, a large *Sciatica A.* appears on each side, as penultimate branch of aorta, which is destined for pelvic limb. Both *Sciatic Arteries* cross laterally caudal lobe of the kidney and path emits a branch for renal lobe, then follows freely until Ileum-Sciatic foramen, where leaves pelvis. Aorta follows caudally, now much smaller in caliber, on ventral surface of synsacrum and divides into *Internal Iliacs Aa.*and *Medial Caudal*. *Internal Iliac* provides branches to intrapelvic structures and medial flow goes to *uropygium*. A large *Caudal Mesenteric Artery* arises from *Internal Iliac* and suppresses intra-pelvic viscera and caudal part of intestine (Figure 3).

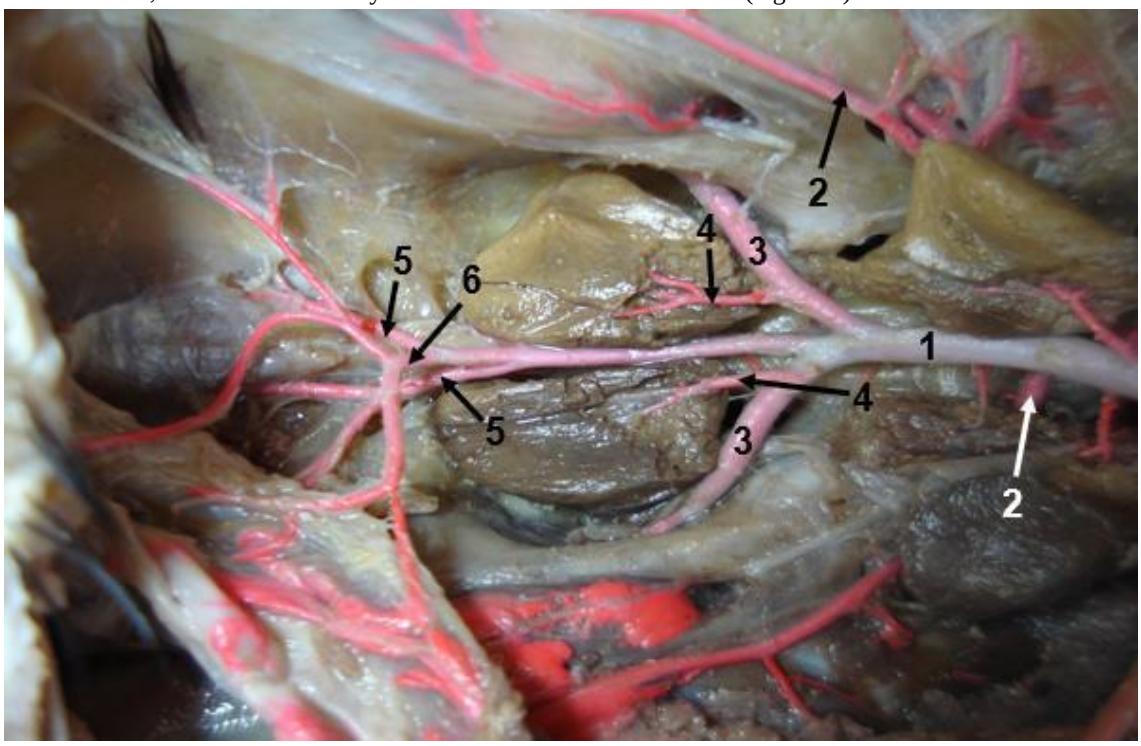

Fig.3: Ventral view of the thoracoabdominal cavity of Tucanucu: 1 - Descending Aorta, 2- External Iliac Artery, 3 - Ischiatic Artery, 4 - Caudal Renal Artery, 5 - Internal Iliac Artery, 6 - Caudal Mesenteric Artery.

IV. DISCUSSION

Spite the ecological concern situation, Tucanuçu (*Ramphastostoco* – Muller, 1776) is one of the main birds found in "Centro de Triagem de AnimaisSilvestres – CETAS – Catalão" and other Wildlife Rehabilitation Centers, and a large part of animals destined to these organizations originate in traffic and many of them are dies **[21]**.

Considering the results obtained in this work, was observed that Ascending Aorta of Tucanuçu is very short and follows cranially, slightly inclined to the right and soon curves strongly to the right and dorsally, constituting Aorta Arch, in agreement with descriptions in domestic birds **[18,22,26]** and domestic pigeon (*Columba livia*) **[27]**. Neto (2013) **[28]**, describes, in pigeons, the branches of *Aorta A.*, and portrays *Celiac A.*, as the first major branch of descending aorta, in agreement with Getty in Sisson & Grossman (2008) **[18]** and Schwarze&Schröder (1970) **[2]**, in domestic Gallus and Mutuns of gender Crax and Mitu to Gonçalves*et al.* **[29]**.

The *Brachiocephalic Aa.* and *Cervicobrachial* are two prominent blood vessels found in Tucanuçu that arise from cranial face of aortic arch and go to head, neck and thoracic limbs, in accordance with descriptions of Getty in Sisson & Grossman (2008) **[18]** and Schwarze&Schröder (1970) **[23]** in Domestic Rooster. In Tucanuçu, the *Brachiocephalic a.* is a left branch, of *Aortic Arch*, which divides into *Common Carotida*, *Subclavian A.* and *Cervical Ascending A.*, while *Cervicobrachial a.* is a right branch, which emits a large *Cervical A.* and remains laterally as *SubclaviaA.*. From Aortic Arch, in Tucanuçú, an artery for lungs, trachea, and esophagus born from dorsal surface in accordance with descriptions of Getty in Sisson & Grossman (2008) **[18]** and Schwarze&Schröder (1970) in domestic Gallus **[22]**.

According to Getty in Sisson & Grossman (2008) **[18]** and Schwarze&Schröder (1970) **[22]**, in domestic Gallus, the *Descending Aorta*, along its path through thorax and abdomen, emits numerous pairs of Dorsal Arteries are named according to the segment of

column in *Dorsal Intercostals*, *Sinsacral* and *Caudal*, but in Tucanuçu also arises several arteries, the *Intercostals Aa*.intended for thoracic wall and abdominal wall, without constituting itself in numerous branches.

Celiac A. and *Mesenteric Cranial A.*, the two main abdominal visceral arteries, other visceral branches of descending aorta are renal arteries and caudal mesenteric **[18]**. The findings in Tucanuçú are consistent with descriptions of Getty in Sisson& Grossman (2008) **[18]**, Schwarze&Schröder (1970) **[22]**, Baumel (1981)**[23]**, Baumel*et al.* (1993) **[24]** and Dyce *et al.* (2004) **[25]** in Domestic Gallus and Geeverghese*et al.*(2012) **[26]** in Domestic pigeon (*Columba livia*).

In Tucanuçu, the next major branches of descending aorta are External Iliac a., Ischiatic and Internal Iliac, in agreement with citations in domestic Gallus by Getty in Sisson & Grossman (2008) **[18]**, Schwarze&Schröder (1970) **[22]**, Nickel *et al.*, (1977) **[27]**, Baumel (1981) **[23]**, Baumel*et al.* (1993) **[24]** and Dyce *et al.* (2004) **[25]** and in Domestic pigeon (*Columba livia*) to Geeverghese*et al.*(2012) **[26]**.

V. CONCLUSION

The present study shows unprecedentedly relates about aorta artery and respective branches in Tucanuçu (*Ramphastostoco*), showing that the first major branch of descending Aorta is an odd visceral artery, *Celiac a.* and then, on ventral face, *Mesenteric Cranial a.*, the largest visceral branch of descending aorta, which together with *Celiac A.* supplies almost all of abdominal viscera. In Tucanuçu, *Renal Cranial Aa.*arises from aorta, but averages and flows are from *Ischiatic a.*. Also, several small branches are found destined to structures abdominal cavity, such as: gonads, adrenals and other tissues. Thus, this work shows great similarity with others birds and particularities about this specie, contributing to anatomical description and understanding of an important blood vessel in Tucanuçu biological system.

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Variation in Soil Properties along a Catena in kwambai Taraba State, Nigeria

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Abstract*—The study was carried out to determine the variation in soil properties within units and along a catena, significant of variation to soil mapping and soil management at kwambai, Takum, within latitude 7o5 N and longitude 9o5 D E to 10o 10oE .Taraba State. Soil sample were collected from upland, slope and lowland areas of the catena at the depth of 0-15cm and 15-30cm at 3 composite,18 samples were collected. Soil properties analyzed include pH, Total Acidity EC(dS/m), Ca(Meg/Kg), Mg(Meg/Kg, K(Meg/Kg), Na(Meg/Kg), Base saturation%, TEB(Meg/Kg) ECEC(Meg/Kg), P(ppm), N%,O.C%,C:N ratio. Sand has the highest fraction in the soil. The soil is predominantly sandy clay loam. Clay loam in lowland surface, loam and sandy clay are less according to textural class. Grayish brown dominate the soil, dull brown in upland subsurface, others are brown, orange,and brownish grey.In upland area EC, Ca, Mg ,TEB, N and OC decrease with depth while others increase with depth. In slope area Total acidity, Ca, Mg, TEB, ECEC and P Increase with depth while others decrease with depth. In Lowland area Ca, Mg, Na, TEB, Base saturation, ECEC, and N Increase while others decrease with depth There is Significance differences in soil properties down the catena. There is no change in EC. C:N ratio has the highest coefficient of variation of 40.8% while base saturation has the lowest with 1.2% in surface down the catena, the highest in subsurface is total acidity with 55.5% and the lowest is C:N ratio with 1.0%. The following recommendations were made. More samples to be collected during Mapping, the area interval should be reduced. Management can be easily be done in the soil. Application of lime to correct soil acidity should be moderate; fertilizer application should be not be uniform because of variation in the Landscape area.*

Keywords*— *Catena, Variation, depth, Mapping, management, properties.

I. INTRODUCTION

One of the major problem of soils often ignored by soil scientists is soil variation is no stranger to a penologist since the very essence of his profession dates back to the early recognition that soil are systematically related to landscape. The variation of soil properties affects soil Performance in that a uniform application of treatment in
especially variable soil results in over-application in some parts of the field and under application in others. Cambardella,C.A & Karlen,D.L.(1999).Details characterization of soils and their variation along different topography positions has not been investigated in depth Butros I. Hatter et.al (2010).Soil as a natural body is inherently heterogeneous because of the many factors that contribute to soil formation and the complex interactions of those factors, L.M.Maniyunda ,et al;(2015). Variability of soil properties may be attributed to several sources. Apart from inherent soil differences, variation in soil properties are due to soil forming factors(climate, parents materials, organisms, relief and time)and differences in weathering rates M.Mzuku,R.Khosla et.al (2005).

Mapping of soil properties is an important role in the knowledge about soil properties and how it can be use sustainable, O.A.Denton et.al (2017).If the top soil pH is above 5.5surface and subsurface is above 4.8 only maintenance level of liming is require, Bolland M.et.al (2004).Soil organic carbon accounts for less than 5% on soil layers and diminishes with depth, soil
organic carbon can be greater than 10% while the poorer or heavily exploited soils level are lively to be less than 1% ,R A. Webster et.al (2014.)Carbon to Nitrogen (C:N) ratio is often used as a determinant for the health of a soil, Xu et.al (2016).The bulk of soil fungi and bacteria are found to be concentrated in the surface 10cm of the soil as there is high availability of SOM and oxygen(Brady and Weil,2002) P is medium along a catena in soil in Yola Adamawa state, Emmanuel A et.al (2018)

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II. MATERIALS AND METHODS

2.1 Study Area

The study area is located within latitude 7o5 N and longitude 9o5 D E to 10o 10oE Takum local government area is located in southern Taraba state. It share boundary with Donga to the North, Ussa to the west, Benue and Cameroon to the south and Wukari to the East.Total land mass is 45km2 with the population of 135,349 according to 2006 Census.

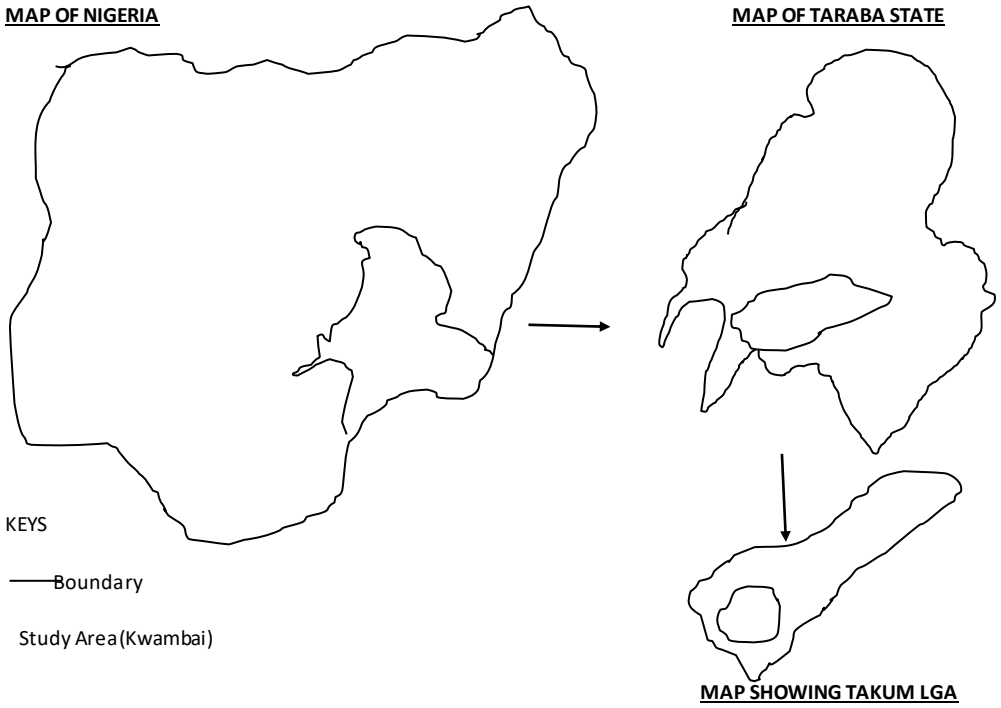

Fig.1: Showing the location of the study area

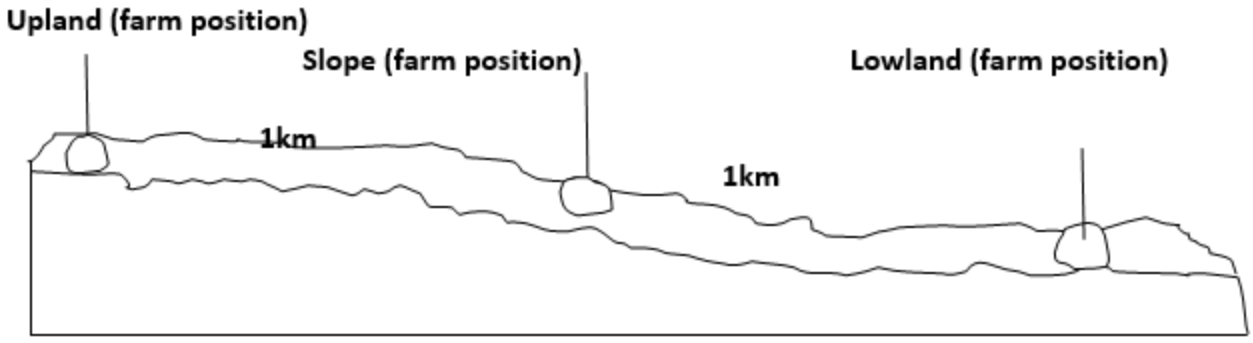

Fig.2. Landscape, farm position of the study area

Climate; Raining season start march and end October, dry season reach it peak in January and February when the dusty North east trade wind blow across the area. The annual temperature range from27^{o}C to 31^{o}C.the climate of the study area provide conducive atmosphere for cultivation of most stable food. The area has a rainfall of 2000 to 2500mm. Vegetation of the study area is Guinea savannah which is made up of dense grasses and trees. Topography of the study area, has a gentle slope .The angle of the slope is about 8^{o} using abney level. The relief of the area favour rainfall and temperature.

2:2 Sampling Procedure

Field work reconnaissance survey was carry out to identify a representative landscape in the catena, in Kwambai area of Takum local government of Taraba state landscape position such as upland, slope and lowland were located.

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For the propose of this study three location or intervention sites chosen within the landscape positions with interval of 1km each. This was base on the level of farming activities. Samples were collected at two depths (0-15 cm) and (15-30 cm) 3composite was located in each landscape position. The total of 18 samples were collected in the landscape position kwambai.

2.3 Laboratory Analysis

The soil samples were air-dried for 24-28 hours, crushed and passed through a 2mm sieve. Sample from sieve were collected with labeled put inside include site location, survey number and laboratory numbers. Soil sample were analyzed for pH in both water and 0.01m potassium chloride solution(1:1) using glass electrode pH meter. Total nitrogen was determined by the macro-kjeldahl digestion method,
available phosphorous(Av P) was
determined using Olsen's extraction method UV/visible Spectrometer. Available K is part of the exchangeable bases ,the exchangeable bases(Ca^{2+} Mg^{2+} K^{+} and Na^{+}) were measured by atomic absorption spectrophotometer after extraction with ammonium acetate at pH 7.OC samples were titrated using 0.5N $FeSO_4$ deep bottle green colour as end point. Hydrometer method was used to determine the particle size distribution. The cation exchange capacity (CEC) was determined by
extraction with ammonium acetate .percentage base saturation was calculated by dividing the sum of the charge equivalents of the base cations by CEC of the soil and multiplying by 100. Total acidity was determine through titration .EC was taking using EC meter. TEB is the sum of exchangeable cation in the soil.C:N This is when Organics carbon divided by nitrogen.

III. RESULTS AND DISCUSSION

Table 1 In particle size distribution Sand is high range from (44.25-61) ,clay(19.70-36.75) and silt(6.55-34.45).it follows the same train in Slope sand(44.6-65.05), Clay(30.35-36.95) and silt(12.4-20.6) and
lowland sand(38.2-53.85),clay(31.6-39.00) and silt(14.55-27.00).There is medium variation of particle size distribution down the catena.

Textural class Shows that sandy clay loam is more in the catena, leaving clay loam for lowland surface and small part of upland subsurface and slope .Sandy clay occupied little space in upland subsurface and low land subsurface .little loam in
Upland surface .

Surface has more grayish brown and then light brown red, subsurface has more dull orange and then dull brown. Slope surface has brown, dull brown, and grayish brown, subsurface has more orange and then bright brown. Lowland surface has grayish brown, brownish grey and grayish grey, subsurface has more grayish brown and then brownish grey. The catena has more grayish brown

Table.1: soil texture class and color

Landscape position	Particles Size distribution%			Texture class	Soil color
	Sand	Silt	Clay		
Upland surface					
0-15cm	44.25	22.55	33.70	Sandy clay loam	7.5 YR 5/2 grayish brown
0-15cm	45.85	34.45	19.70	Loam	7.5 YR 7/2 Light brown red
0-15cm	61.85	6.55	31.6	Sandy clay loam	7.5 YR 5/2 grayish brown
Subsurface					
15-30cm	49.4	15.8	34.8	Sandy clay loam	7.5 YR 6/3 dull brown
15-30cm	45.85	17.4	36.75	Clay loam	7.5 YR 6/4 dull brown
15-30cm	49.05	14.55	36.4	Sandy clay	7.5 YR 6/4 dull brown
Slope surface					
0-15cm	46.20	20.6	33.2	Sandy clay loam	7.5YR 6/3 brown
0-15cm	65.05	16.0	33.35	Sandy clay loam	7.5YR 6/3 DULL brown
0-15cm	44.6	20.6	34.8	Clay loam	7.5 YR 4/2 grayish brown
subSurface					
15-30cm	52.25	17.4	30.35	Sandy clay loam	7.5YR 6/6 orange
15-30cm	50.65	12.4	36.95	Sandy clay loam	7.5YR 6/6 orange
15-30cm	49.05	14.53	36.95	Sandy clay loam	7.5YR 5/6 bright brown
Lowland surface					
0-15cm	41.05	19.95	36.4	Clay loam	7.5 YR 4/2 grayish brown
0-15cm	42.05	19.35	39	Clay loam	7.5 YR 5/1 brownish grey

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0.15cm	42.65	27	38	Clay loam	7.5 YR 5/2 grayish grey
Subsurface					
15-30cm	38.2	18.1	36.4	Sandy clay	7.5 YR 5/1 brownish grey
15-30cm	45.5	17.75	34.8	Sandy clay loam	7.5 YR 5/1 grayish brown
15-30cm	53.85	14.55	31.6	Sandy clay loam	7.5 YR 5/1 grayish brown

Table 2 shows that pHs in upland area are slightly acidic increasing with depth, in slope and lowland area, pH are moderate and decrease with depth. Maintenance level of liming is require because the pH is above 5.5 in upland and above 4.8 in subsurface .this is with the conformation with Bolland M. et'al (2004)

Total acidity is moderate in upland, slope and lowland. Upland and slope increase with depth while lowland decrease with depth.

EC in upland, slope and lowland are medium. Upland and slope is unsuitable with moderately leaching and slightly decrease with depth, lowland is low and remain the same with depth.

Exchangeable base, Ca is moderate in upland, slope and lowland. it may be due to plant uptake or leaching. Upland decrease with depth, while slope and lowland increase with depth. Mg is high in upland area and decrease with depth. Mg is low in slope surface and high in subsurface. It is high in lowland and increase with depth .K is moderate in upland and it increase slightly with depth .k is moderate in slope and high in lowland (it may be as a result runoff) slope and lowland decrease with depth. Na increase with depth in upland and lowland area Na is high in lowland and it decrease with depth in slope. Exchangeable base fall mostly within low and medium range. Confirmation with (Brady and Weil,2002)this may be due to low decomposition of organic matter and slow release of chemical element into the soil. Base saturation slightly increases with depth in upland and low land. it decrease with depth in slope.

TEB and ECEC increase with depth in slope and lowland.TEB decrease while ECEC increases with depth .They are not medium. This is different with the finding of (Emmanuel A et.al 2018).P fall within medium, confirmation with (Emmanuel A et.al 2018) Who observed that P in the soil of some selected farmland located at Modibbo Adama University Adamawa State shows that P is medium., P increase with depth in upland and decrease with depth in slope and lowland .it confirm the increase in upland but contradict decrease in slope and lowland (Osujieke et.al 2016) .OC is medium (FDLAR 2012),OC decrease with depth in upland ,slope and lowland. this is confirmation with (Brady and Weil,2002) that The bulk of soil fungi and bacteria are found to be concentrated in the surface 10cm of the soil as there is high availability of SOM and oxygen. Organic carbon is high in upland and lowland. it is low in the slope, it may be due to plant uptake, rain and runoff down the catena. It is also with agreement with those observed by (Emmerson WW.1991).Nitrogen is low down the Catena l(FDLAR 2012).N decrease with depth in upland ,slope and increase with depth in lowland area. C:N ratio increase with depth in upland ,decrease with depth in slope and lowland. it may be due to leaching or plant uptake.

Table.2: Physio Chemical properties

Landscape Position	**pH 1:LS H_20**	**Total acidity**	**EC (dS/m)**	**Exchangeable (emolekg^{-1}**				**Base Saturation(%)**	**TEB (Meg/kg)**	**ECEC (emoleKg^{-1})**	**P(MegKg)**	**N(%)**	**OC(%)**	**C:N**	**Texture Class**
				Ca	mg	k	Na								
Upland															
Surface	6.25	0.30	231	5.60	3.00	0.37	0.13	96.8	9.10	9.10	18.00	0.45	1.07	2.38	SCL
0-15cm	6.36	0.30	261	3.40	6.40	0.29	0.16	94.4	10.2	10.8	15.20	0.58	1.30	2.24	L
0-15cm	6.18	0.40	280	3.20	4.60	0.32	0.20	98.3	8.12	8.52	17.90	0.48	1.11	2.31	SCL
0-15cm															
Subsurface															
15-30cm	6.40	0.60	261	3.80	4.40	0.42	0.15	93.6	8.78	9.38	16.50	0.33	0.83	2.58	SCL
15-30cm	5.93	0.30	251	4.40	4.40	0.36	0.20	96.9	9.36	9.66	17.50	0.45	1.11	2.47	CL
15-30cm	6.85	0.20	241	4.80	2.00	0.36	0.20	97.4	7.36	7.56	19.20	0.36	0.82	2.28	SC
Slope Surface															
0-15cm	6.95	0.30	281	2.40	2.40	0.43	0.16	94.7	5.59	5.69	13.15	0.25	0.75	2.20	SCL
0-15cm	5.98	0.20	230	2.80	3.80	0.47	0.24	97.1	6.84	7.04	14.2	0.28	0.66	2.36	SCL
0-15cm	5.62	0.30	245	3.80	2.60	0.39	0.20	95.9	6.99	7.29	14.5	0.53	1.13	2.13	CL
Subsurface															
15-30cm	5.10	0.30	242	2.80	3,60	0.38	0.15	95.8	6.89	7.21	13.5	0.22	0.59	2.68	SCL
15-30cm	5.95	0.40	233	5.80	5.20	0.51	0.19	96.7	11.7	12.1	20.0	0.25	0.57	2.28	SCL

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15-30cm	6.93	0.70	205	2.40	5.40	0.29	0.20	92.2	8.29	8.99	12.2	0.17	0.39	2.29	SCL
Lowland															
Surface															
0-15cm	6.31	0.30	227	2.60	7.00	0.46	0.16	97.1	10.2	10.5	17.8	0.42	1.14	2.36	CL
0-15cm	5.91	0.50	250	2.40	2.00	0.38	0.24	90.9	5.02	5.52	16.5	0.36	0.80	2.22	CL
0-15cm	5.38	0.20	235	4.00	4.40	0.59	0.20	97.8	9.19	9.39	20.3	0.19	1.09	4.41	CL
Subsurface															
15-30cm	5.83	0.40	210	2.80	4.80	0.36	0.20	95.3	8.16	8.56	14.3	0.45	1.10	2.44	SC
15-30cm	5.85	0.30	227	3.20	5.80	0.47	0.24	97.1	9.71	10.1	17.5	0.28	0.50	2.03	SCL
15-30cm	5.81	0.20	275	4.80	4.00	0.37	0.24	97.7	9.41	6.61	19.0	0.53	1.11	2.09	SCL

KEY:L=LOAM, CL=CLAY LOAM,SC=SANDY CLAY,SCL=SANDY CLAY LOAM.

3.1 Trend in soil properties down a catena (mean and CV%)

From table 3,pH surface(6.26>6.18>5.86) ,and subsurface(6.36>5.98>5.83) Aweto A.O and Enaruvbe G.O (2010)The decline in soil pH is partly due to the decline of Exchangeable cations,especially especially magnesium, and base saturation. Total acidity surface(0.33>0.26<0.33) and subsurface (0.36<0.46>0.30).Soil properties are not always uniform in all the segment of the catena kravchenko and Bullock(2000). EC,surface(2.57>2.52>2.32) and subsurface (2.56>2.27<2.37), Ca surface(4.06>3.00=3.00) it may be due to exploitation through farming or leaching in slope and lowland. subsurface(3.56<3.66>3.60), Mg surface(4.66>2.90<4.46) subsurface (3.16<4.73<4.86) K surface(0.32<0.43<0.47) it may be due to water runoff from the upland. Subsurface (0.38=0.38<0.40) ,Na surface(0.16<0.20=0.20) and subsurface (0.18=0.18<0.22), base Saturation surface(96.5<95.9<96.7) subsurface (95.9>94.9<96.7,TEB surface(9.14>6.40<8.13) and subsurface(8.5<8.96<9.09).ECEC surface(9.57>6.60<8.47) and subsurface (8.86<9.43<9.44),P surface(17.03>13.9>8.2) and subsurface (17.66>15.2<16.9), N surface (0.50>0.35>0.32) subsurface (0.38>0.21<0.42).The total Nitrogen shows Variation in descending in surface, but not descending or ascending order in Subsurface According to Majawliwa et.al (2015) ,OC surface(1.16>0.84<1.01) subsurface (0.92>0.15<0.92) C:N (2.31>2.23<2.99) and subsurface (3.37>2.14<2.18) .

Coefficient of variation in Surface C:N and N (2.9-42.8%),Ca and Mg,(24-36.4%),OC and ECEC (10.3-30.8%).K, Na and TEB,(9.3-21.2%) are medium, Base saturation(1.2-3.9%),Ec and pH (1.4-11%) P and Total Acidity (9.2-19.2%) are low. In subsurface Total acidity and Mg(11.5-55.5%) have high variation. K, P, N, OC, ECEC, Ca and TEB (7.8-32.6%) have Medium variation. Ec and Na(3.9-15.5%), C:N,Base Saturation and pH (1.0-9.1%) have low variation along a catena.

Table.3: Variation of soil properties across the catena(Mean and CV%)

Variables	Properties	Upland		Slope		Lowland	
		Mean	CV %	Mean	CV %	Mean	CV %
Surface(0-15cm)	Ph	6.26	1.4	6.18	11	5.86	7.8
	Total Acidity	0.33	15.2	0.26	19.2	0.33	19.2
	EC(dS/m)	2.57	9.3	2.52	10.3	2.37	4.6
	Ca(Meg/Kg)	4.06	29.0	3.00	32.7	3.00	24
	Mg(Meg/Kg)	4.66	25.6	2.90	29.0	4.46	36.4
	K(Meg/Kg)	0.32	12.5	0.43	9.3	0.47	21.2
	Na(Meg/Kg)	0.16	16.6	0.20	2.0	0.20	20.0
	Base saturation%	96.5	2.0	95.9	1.2	95.2	3.9
	TEB(Meg/Kg)	9.14	11.3	6.40	13.7	8.13	33.7
	ECEC(Meg/Kg)	9.57	11.9	6.60	12.8	8.47	30.8
	P(ppm)	17.03	9.2	13.9	5.0	18.2	10.6
	N%	0.50	12.0	0.35	42.8	0.32	36.3
	O.C%	1.16	10.3	0.84	28.5	1.01	17.8
	C:N ratio	2.31	2.9	2.23	4.9	2.99	40.8
Subsurface(15-30)	Ph	6.36	7.1	5.98	3.8	5.83	3.4
	Total Acidity	0.36	55.5	0.46	43.4	0.30	38.3
	EC(dS/m)	2.56	3.9	2.27	8.4	2.37	13.9
	Ca(Meg/Kg)	3.56	29.1	3.66	11.5	3.60	10.1

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Mg(Meg/Kg)	3.66	50.5	4.73	11.1	4.86	38.3
K(Meg/Kg)	0.38	7.8	0.38	28.9	0.40	15.0
Na(Meg/Kg)	0,18	15.5	0.18	11.1	0.22	9.0
Base saturation%	95.9	2.1	94.9	2.5	96.7	1.3
TEB(Meg/Kg)	8.5	12.0	8.96	27.4	9.09	9.0
ECEC(Meg/Kg)	8.86	12.8	9.43	26.1	9.44	8.2
P(ppm)	17.66	8.2	15.2	27.1	16.9	14.2
N%	0.38	15.7	0.21	19.0	0.42	28.5
O.C%	0.92	17.3	0.51	21.5	0.92	32.6
C:N ratio	3.37	6.1	2.14	9.I	2.18	1.0

3.2 Significance Of Soil Variation To Soil Mapping

There is high and moderate variation of soil properties across the landscape position(catena).The soil change with some meters in confirmation of (Aweto A.O and Enaruvbe G.O 2010) from their finding soil properties are not always uniform in all slope segment of the catena. soil thickness was observed it may be due to heavy implement on the farm or grazing by ruminant animals. In case of sampling more samples should be collected. Sample interval should be reduced preferably small scale.

3.3 Significant of Soil Variation to Soil Management.

From table 1 The dominant soil is sandy clay loam then; clay loam, sandy clay and loam. Management can be easily done in the above soil. Application of lime to correct acidity should be maderate. Fertilizer application should not be uniform due to the variation of properties of some landscape area Cambardella,C.A & Karlen,D.L.(1999). Zero or no tillage should be practice to reduce compaction.

IV. CONCLUSIONS

Generally the variation in soil properties is moderate within the landscape position of the catena excluding Base saturation, EC, pH, P, Total acidity are low in surface while Total acidity and Mg are high, EC,Na,C:N,
Base Saturation, pH are low in subsurface. The results indicate that the density of observations for those soil properties exhibiting high and medium variation should be increase during sampling, soil mapping and for soil management purpose.

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Using Cheese whey for the Production of Carotenoids, Ergosterol and Novel Functional Foods of Industrial interest though a series of Optimized Bio- and Chemical- Processes

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Abstract*—The increasing industrial demand for the production of innovative functional food (lactose free products) as well as bio-compounds with nutritional value (such as carotenoids and other metabolites like ergosterol), that could find several applications in industrial food sector (Research and Development department) has increased interest regarding their potential production (employing applied biochemistry and biotechnology principles in their optimized production processes). Reduction of total production costs has encouraged the usage of low-cost or negative valued agroindustrial by-products or waste streams to industrial food sector. Cheese whey (after being deproteinized) was treated, either with acid (HCL) or commercial β-galactosidase (from Aspergillus oryzae), aiming at hydrolyzing initial lactose contained in a unique mixture of cheese whey, delivered by different cheese whey making processes, from Aegean islands (Greece). Regarding HCL (37% vv^{-1}) catalyzed hydrolysis of unique Aegean islands (Greece) delivered cheese whey, the highest glucose concentration of 3873.66 mgL^{-1} achieved after 120 min of reaction, at 100 ^oC at a pH range ranking from 1 to 1.1. As for enzymatic catalyzed hydrolysis process of cheese whey, using β-galactosidase (from Aspergillus oryzae), maximum production of 18.78 gL^{-1} glucose, achieved at 55 ^oC and pH= 5, after 12 hours of enzymatic hydrolysis (when the initial cheese whey concentration and initial enzymatic activity was 1200 gL^{-1} and 9 UmL^{-1}, respectively). Evaluating potential usage of cheese whey hydrolysates in industrial food sector, it was concluded that a 6h enzymatic process was adequate for the production of glucose-rich streams, that could find several applications toward the production of novel functional foods (free of lactose or low lactose content), improving their sensorial and technological properties (while addressed to those suffering from lactose intolerance). Regarding bioreactor fed-batch bioconversions by Rhodotorula glutinis, using enzymatically prepared cheese whey hydrolysates as generic feedstock (with initial glucose concentration of 18 $g\ L^{-1}$), the highest production of total carotenoids, ergosterol and total dry weight achieved was 127.3 ± 0.41 $\mu g\ g^{-1}$ (or 2023.03 ± 0.41 μgL^{-1}), 170.78 ± 0.38 $\mu g\ g^{-1}$ (or 2703.917 ± 4.37 μgL^{-1}), respectively. The flow aeration rate was maintained at 2vvm.The pH value was regulated by using 5M NaOH and 10% (vv^{-1}) H_2SO_4, at optimum range (6.2-6.5). The dissolved oxygen concentration at the bioreactor, was regulated at 30% of saturation. Cheese whey, a by-product of cheesemaking process, could form an ideal feedstock through the designing and development of optimized series of bioprocesses leading to the development of a novel biorefinery that could produce several value-added products with high nutritional value and several commercial marketed outputs.*

Keywords — cheese whey, applied biosciences, biotechnology, carotenoids, ergosterol, optimized processes, research and development.

I. INTRODUCTION

Carotenoids represent one of the most important classes of components in food, effecting their acceptability, used widely in industrial food sector as coloring agents or additives (their color vary from the yellow to red range), affecting the major sensory characteristics (color and acceptability) of foods. Carotenoids are lipid-soluble

pigments (the majority of them are C_{40} terpenoids), acting as membrane-protective antioxidants scavenging O_2 and peroxyl radicals. Thus, their antioxidant activity could be attributed to their structure. Interest in carotenoid production has been increased lately, owning to their related beneficial effects in human health as well as the growth of certain areas of industrial food sector such as agriculture, aquaculture and poultry industry (Dimou *et al*., 2019a; 2019b). Carotenoids are used as coloring additives in food systems, while enriching them with provitamin-A, often increasing their antioxidant activity (Dimou *et al*., 2019a). These bioactive compounds have been used as additives for the production of a wide range of food (such as cooked sausages, soft drinks, baked goods), pharmaceutical and cosmetic formulations. Besides, humans do not synthesize carotenoids de novo but take them in the diet, using them as precursors for the production of retinoids such as vitamin A (Dimou *et al*., 2019b). In fact, carotenoids are bioactive phytochemicals that have been credited of reducing risks of development of degenerative diseases such as cancer, cardiovascular diseases, macular degeneration and cataract (Dimou *et al*., 2017a). These bioactive compounds could be used for the production of several novel functional foods and nutrient supplements. It is expected that the growing demand for these healthy value-added products will boost carotenoid bioprocessing as a fundamental player to meet the requirements of consumers and industry. Carotenoids are primarily produced by filamentous fungi and yeasts as well as by some species of bacteria and algae (Dimou *et al*.,2019c; 2019d). Among microbial sources used for the production of biomas -enriched metabolites such as carotenoids and ergosterol of commercial interest, *Rhodotorula glutinis* is one of the most well-known species (Martinez *et al*., 2009; Dimou *et al*., 2019c; Koutelidakis *et al*.**,** 2019). Ergosterol is a biological precursor of vitamin D_2, produced through the implementation of suitable bioprocessing system, using yeasts as microbial strains. Ergosterol exists in yeast cell wall membranes and mitochondria. It has been reported, that hyphomycetes and ascomycetes, contain ergosterol in variant concentrations ranging from 2.3 to 11.9 μg of ergosterol/mg of dry weight. Also, the ergosterol content for *Cladosporium* sp, *Candida* sp, and *Alternaria* sp, varies from 0.4 to 14.3 μg/mg according to Pasanen *et al*. (1999). Bioprocessing derived ergosterol could be used as a dietary supplement and/ or food additive, for the production of ergosterol rich functional products (Corrêa *et al*., 2018). Research in that area is very limited (Dimou *et al*., 2019c; Koutelidakis *et al*., 2019). Trying to improve the yield of metabolite products and subsequently decrease the cost of bioprocessing, optimization of culture conditions including the usage of cheap agroindustrial byproducts or waste streams as nutrient supplements, form a current emerging tendency toward the production of bioderived value added products of high nutritional value and industrial interest (Dimou *et al*., 2015; 2016a; 2016b; 2016c; 2017a; 2017b; 2019a; 2019b; 2019c). Several agroindustrial by-product or waste streams from industrial food sector such as sugarcane, sugar beet molasses, cheese whey, hydrolyzed beans, corn meal, corn steep liquor, soybean oil, wine lees, grape pomace have been used for the production of value-added products (Dimou *et al*., 2015; 2016; 2017a; 2017b; 2019a; 2019b; 2019c; Koutelidakis *et al*., 2019; Kopsahelis *et al*., 2018).

Indeed, such media are very complex and further pretreatment steps may be needed so as to increase the bioavailability of nutrients to microorganisms (Dimou *et al*., 2015; 2016; 2017a; 2017b; 2019a; 2019b; 2019c. Cheese whey, a byproduct stream derived from cheese making process, constitutes a promising raw material for bioprocessing, since nearly 55% of milk nutrients (proteins, lactose and minerals salts) remain in that fraction, during cheese processing. Worldwide production of cheese whey is approximately 145×10^9 kg $year^{-1}$ (Dimou *et al*., 2019a). To the best of our knowledge there is no literature cited publication studying potential concurrent production of carotenoids, ergosterol and other nutrient supplements rich in glucose, using cheese whey, as low cost agroindustrial substrate.

The objective of this study was the evaluation of potential production of bio-carotenoids, ergosterol and other value-added functional feedstocks, with nutritional value, using cheese whey as raw material, through the development of bio-science based processes. Another goal of this research was the optimization of processes (enzymatic or acid catalyzed) leading to concurrent production of novel nutrient supplements rich in glucose, which could then be further exploited toward the production of either innovative functional foods or as a generic feedstock leading to the production of bio-carotenoids and ergosterol, using *Rhodotorula glutinis* strain.

II. MATERIALS AND METHODS

2.1 Substrates

Cheese whey effluents derived from different cheese production processes leading to the production of the very well-known Greek types of cheese: "Kalathaki", "Anthotiro", "Feta'' and "Melixloro", were obtained from local dairy plants from Aegean islands (mainly from Lemnos). For all the experiments performed, cheese whey used was a mixture, containing: 40 % vv^{-1} cheese whey derived from "Kalathaki" production; 10 % vv^{-1} cheese whey formulated during "Anthotiro" production; 40 % v v^{-1} cheese whey derived from "Feta" production and 10 % v v^{-1} cheese whey derived from "Melixloro" production.

The cheese whey mixture content was: 95% water; 65 g L^{-1} dry weight; 25 gl^{-1} ash; 38 gL^{-1} lactose; 0.3 gL^{-1} glucose; 58 mgL^{-1} phosphorus; 1.8 gL^{-1} soluble proteins and 0.1% total nitrogen. Cheese whey was evaluated as raw material for the bioproduction of value added products of nutritional value that could be used either as glucose rich streams free of lactose towards the production of novel functional foods or as a generic feedstock for the production of biomass-enriched metabolites (carotenoids and ergosterol) of commercial food and nutrition industrial interest.

2.2 De-proteinisation of cheese whey

De-proteinisation of cheese whey performed in an autoclave at 121ºC for 20min. After coagulation and precipitation of denaturated proteins the sample was centrifuged at 1000×g and 20ºC for 15 min. The supernatant was transferred to Erlenmeyer flasks and immediately used for hydrolysis and media formulation. The sediment, consisted of precipitated proteins, were stored at -40 ^{0}C.

2.3 Production of hydrolysates: Acid hydrolysis of cheese whey

The acid hydrolysis of cheese whey performed in 2000 mL Erlenmeyer flasks, placed in a shaking water bath (TC-202P Circulating, Ametek, Brookfield). Three sets of experiments were carried out to evaluate the effect of a) pH (1-5), b) reaction time (10-120 min), c) temperature (50-120 ºC) during acid hydrolysis of cheese whey, catalyzed by 1N HCl (37 %, Sigma Aldrich). Mixing of the suspension took place using magnetic stirrers. Samples were collected at random intervals. Remaining solids were separated by centrifugation (10min, 3000g). The supernatant was used for the analyses of glucose concentration. Hydrolysis yield was expressed as the percentage of lactose concentration of cheese whey to final glucose concentration of cheese hydrolysate. After the end of acid hydrolysis, cheese whey hydrolysates were pretreated, as described previously Dimou *et al*. (2012a; 2012b) so as to formulate a suitable nutritional supplement for *R. glutinis* bioconversions. All the aforementioned experiments were performed in triplicate.

2.4 Production of hydrolysates: Enzymatic hydrolysis of cheese whey

The enzymatic hydrolysis of cheese whey performed in 2000 mL Erlenmeyer flasks, by *Aspergillus oryzae* β-galactosidase of 3 UmL^{-1}, 6 UmL^{-1}, and 9 UmL^{-1} enzymatic activity, in orbital rotation shaker (MaxQ 4000 Benchtop, ThermoFisher Scientific), at 160 rpm. The effect of a) temperature (10°C and 55°C), b) reaction time (0h-12h), c) enzymatic activity (3 UmL^{-1}, 6 UmL^{-1}, and 9 UmL^{-1}), d) initial cheese whey concentration (500 gL^{-1}, 800 gL^{-1}, 1200 gL^{-1}) and e) pH (1-7) regarding glucose production was studied. The enzymatic concentrations of 3 UmL^{-1}, 6 UmL^{-1}, and 9 UmL^{-1} corresponded to 0.1 gL^{-1}, 0.2 gL^{-1}, and 0.3 gL^{-1} of *A.oryzae* β-galactosidase, respectively. At random intervals samples were taken and simultaneously submitted to heating at 100°C for 10 min, so as to inactivate the enzyme. After the end of acid hydrolysis, cheese whey hydrolysates were pretreated, as described previously Dimou *et al*. (2012b; 2015; 2016a; 2019a; 2019c) and Koutelidakis *et al*. (2019) so as to formulate a suitable nutritional supplement for *R. glutinis* bioconversions. The treated samples were stored at -40ºC for further determination of glucose. All the experiments were performed in triplicate.

2.5 Micro-organism and cultivation conditions

A strain of *Rhodotorula glutinis* CCY 20-2-26, donated from culture collection, Bratislava, Slovakia was evaluated for potential bioproduction of carotenoids and ergosterol. Initially the lyophilized culture was hydrated in YM medium (yeast/malt extract: 3 gL^{-1} yeast extract, 3 gL^{-1} malt extract, 5 gL^{-1} peptone and 18 gL^{-1} glucose) at 28ºC for 72h. Then the culture was transferred to slant tubes containing YMA medium (yeast/malt extract agar: this medium contains the same composition as YM medium including also 20 gL^{-1} agar) and incubated at 26 ºC for 72h. After growth the slants were kept at 4 ^{0}C and sub-cultured every 2 months. Stock cultures were also preserved at -80 ºC, containing 200 gL^{-1} glycerol. Inocula obtained from cultures grown on YM slants at 28 ºC for 24 hr. A loop of the yeast cells was transferred to 250 mL Erlenmeyer flasks containing the production medium and incubated at 26 ºC for 40 h, in a rotary shaking incubator (Lab-Line Incubator-Shaker) at 160 rpm.

2.6 Production of value-added products: carotenoids and ergosterol

Prior to each cultivation cheese whey and cheese whey hydrolysates were treated as described earlier (Dimou *et al*.; 2015; 2016a; 2018). Yeast fermentations carried out in 250 mL Erlenmeyer flasks (50 mL broth) placed in a rotary shaker and agitated at 160 rpm, using 1mL of preculture standard medium as inoculum. Crude enzymatic prepared hydrolysates, of variant glucose concentration (3.5-18 gL^{-1}), used as nutrient supplements, aiming at evaluating potential concurrent bio-carotenoids and ergosterol production. Fed-batch bioreactor bio-conversions performed as described earlier, according to Dimou *et al*., (2015; 2016a; 2018). Inoculations took place using a 10 % (vv^{-1}), preculture as inoculum. The flow aeration rate was maintained at 2 vvm. pH-value was regulated by using 5M NaOH and 10% (vv^{-1}) H_2SO_4, at

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optimum pH range (6.2 - 6.5). The dissolved oxygen concentration at the bioreactor, was regulated at 30% of saturation, by controlling the agitation speed at the range of 200-500 rpm. Concentrated cheese whey hydrolysates, containing a variant concentration of glucose ranking from 15 to 20 gL^{-1}, used for feeding. The salts solution as well as yeast extract concentrations, used as bioprocessing feedstock standardized mixture contained: 0.15g L^{-1} $MgSO_4 \cdot 7H_2O$, 5 gL^{-1} K_2HPO_4, 5% NaCl and 5 gL^{-1} yeast extract. All yeast fermentations were carried out in triplicate. Samples of 5mL were taken at random intervals.

2.7 Analytical methods

Glucose concentration measured according to the dinitrosalicylic acid (DNS) method Miller, 1959. Ergosterol and carotenoids were extracted and assayed as previously described by Dimou *et al*. (2019c) and Koutelidakis *et al*. (2019). β-galactosidase activities of the commercial enzymes were assayed according to Cruz *et al*.,1999 and Koutelidakis *et al*. (2019)**.** The total cell dry weight was determined according to Dimou *et al*. (2015; 2016).

III. RESULTS AND DISCUSSION

3.1 Acid catalyzed hydrolysis of cheese whey

The highest glucose concentration of 3873.66 ± 2.239 mgL^{-1} was obtained after 120 min of acid catalyzed (deproteinized) cheese whey hydrolysis, at 100 ^oC, at pH values around 1. As it can be seen in Table-1, as pH value of cheese whey medium increase from 1 to 5, final produced glucose concentration is decreased. On the contrary, as reaction time increases from 10 to 120 min, final produced glucose concentration is increased, reaching its maximum accomplished concentration of 3873.66 ± 2.239 mgL^{-1}, after 2h of reaction (Table 1). Further increase in reaction time (120 min), did not affect final glucose concentration. This finding could be possibly attributed to parallel reactions that took place throughout cheese whey hydrolysis, such as Maillard and glucose degradation reactions.

Table.1: Acid hydrolysis of cheese whey: Glucose concentration throughout acid hydrolysis process at variant pH values (1-5) and reaction times (10-120 min), at 100 ^oC.

Time of reaction (min)	pH	Final glucose concentration* (mgL^{-1})
10	1	336.660 ± 0.778
10	2	235.780 ± 6.985
10	3	103.420 ± 3.549
10	4	092.540 ± 0.603
10	5	078.210 ± 1.983
30	1	726.620 ± 6.481
30	2	642.860 ± 1.801
30	3	378.730 ± 3.993
30	4	220.920 ± 1.604
30	5	180.820 ± 3.681
50	1	1900.28 ± 1.108
50	2	1370.67 ± 4.441
50	3	810.930 ± 4.147
50	4	412.020 ± 5.840
50	5	309.540 ± 2.033
80	1	3283.34 ± 1.230
80	2	2201.72 ± 1.730
80	3	1278.34 ± 9.575
80	4	641.270 ± 2.351
80	5	314.670 ± 2.534
120	1	3873.66 ± 2.239
120	2	2467.78 ± 6.179
120	3	1330.24 ± 2.788
120	4	683.670 ± 2.497
120	5	378.280 ± 3.613

*The results are expressed as the mean values of three replicates (±SD)

Glucose production throughout 120 min of cheese whey hydrolysis at optimum pH=1, at varying temperatures ranging from 50 ^oC to 120 ^oC are presented in Fig-1. It can be easily observed that increasing temperature of acid-catalyzed hydrolysis process from 50 ^oC to 100 ^oC, (maximum glucose produced after 120min of reaction Table-1) also final glucose concentration increased from 0.97 gL^{-1} to 3.872 gL^{-1}, implying that temperature is a very crucial parameter that affects lactose to glucose hydrolysis efficiency.

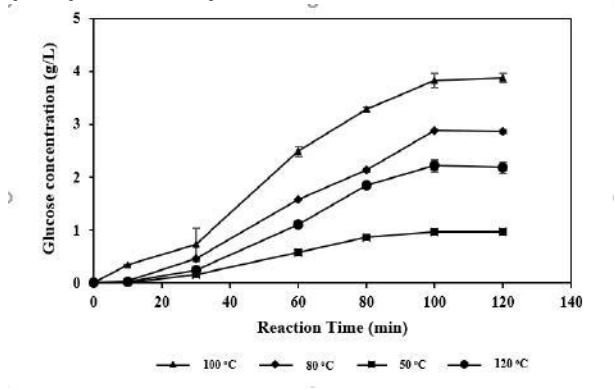

Fig.1: Acid hydrolysis of cheese whey during 120h of acid catalyzed process at 50 ^oC; 80 ^oC; 100 ^oC; 120 ^oC at optimum pH=1

*The results are expressed as the mean values of three replicates (±SD)

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On the other hand, further increase of the reaction temperature, above 100°C, did not positively affect glucose production in the final produced hydrolysate (Fig.1). It seems that during sugar acid hydrolysis at pH values lower than 2 and temperature above 100°C, furfural, formic acid, acetic acid and 5-hydroxymethylfurfural are produced, hindering hydrolysis efficiency. To the best of our knowledge, such an investigation, evaluating potential production of acid catalyzed cheese whey (derived from "Kalathaki", "Anthotiro", "Feta'' and "Melixloro" cheese production process, from Aegean islands) hydrolysates as well as optimization of this process has never before been published.

3.2 Hydrolysis of cheese whey via enzymatic hydrolysis

Hydrolysis of cheese whey using *Aspergillus oryzae* β-galactosidase, aiming at producing glucose rich streams as well as optimization of enzymatic process was studied. The effect of several parameters, affecting enzymatic process, : a) temperature (10 °C and 55 °C), b) reaction time (0h-12 h), c) initial enzymatic activity (3 UmL^{-1}, 6 UmL^{-1}, and 9 UmL^{-1}), d) initial cheese whey concentration (500 gL^{-1}, 800 gL^{-1}, 1200 gL^{-1}) and e) pH (1-7) were evaluated.

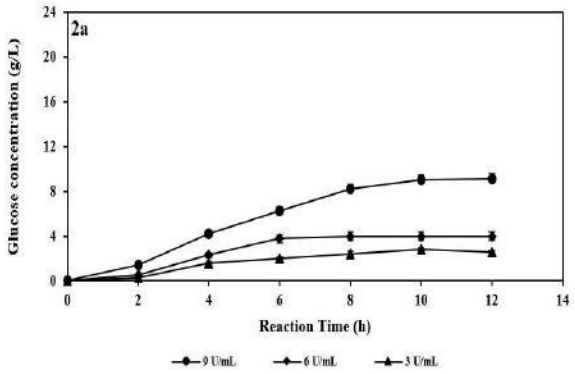

Fig.2: Enzymatic hydrolysis of cheese whey throughout 12h of reaction bioprocess, using A. oryzae derived β-galactosidase preparations of 3 UmL^{-1}, 6 UmL^{-1}, 9 UmL^{-1} at optimum pH (of 5) and initial cheese whey concentration (1200 gL^{-1}). Glucose production at (2a) 10°C (2b) 55°C.

*1 one unit of β-galactosidases activity (U) was defined as the amount of enzyme that liberates 1.0 μmole of o-nitrophenol per minute under assay conditions

*2: The results are expressed as the mean values of three replicates (±SD)

The effect of the temperature (10°C and 55°C), reaction time (0-12h), initial enzymatic activity of *A. oryzae* β-galactosidase (3 UmL^{-1}, 6 UmL^{-1}, and 9 UmL^{-1}), at optimum pH (approximately equal to 5) and initial cheese whey concentration (of 1200 gL^{-1}), are shown in Fig. 2. The main purpose regarding cheese whey hydrolysis experiments performed at low temperature (10°C), was the production of a novel glucose-rich stream using cheese whey as raw material without affecting its sensorial and nutritional characteristics. According to the results presented in Fig. 2 all the studied parameters affected the enzymatic process and thus, final glucose production and hydrolysis degree. Maximum production of (approximately) 18.78 gL^{-1} glucose (more specifically 18.777 ± 0.007 gL^{-1}), achieved at 55°C, pH around 5, after 12 hours of enzymatic hydrolysis of 1200 gL^{-1} cheese whey when the initial enzymatic activity of β-galactosidase was 9 UmL^{-1} (Fig-2b). It has been reported, that the enzymatic activity of *A. oryzae* enzymes (including β-galactosidase) may be influenced by multiple environmental factors, among which the most important are temperature, pH and initial enzymatic activity (Dimou *et al*., 2015). It seems that these factors possibly affect the tridimensional structure or the remaining protein conformation in the medium, affecting hydrolysis efficiency (Dimou *et al*., 2016; Jurado *et al*. 2004). Additionally, it could be concluded that the enzymatic activity directly affects lactose hydrolysis, throughout hydrolysis process. Increasing initial β-galactosidase enzymatic activity from 3 UmL^{-1} to 9 UmL^{-1}, final glucose concentration presented a two point four-fold increase, approximately (Fig.2b).

The effect of concentration on cheese whey hydrolysis yield or efficiency, at optimum conditions (initial enzymatic activity=9UmL^{-1}; T=55°C, pH=5, time=12hours), has been also studied.

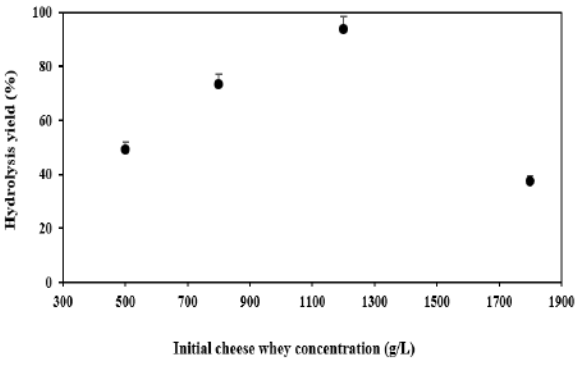

Fig.3: Effect of initial cheese whey concentration on hydrolysis yield (lactose to glucose conversion), at optimum conditions (9 UmL^{-1} enzymatic activity, reaction time=12h; T=55°C)

Fig.3 shows that the hydrolysis efficiency was maintained above 73% at initial cheese whey concentrations at the range of 800-1200 gL^{-1}, while it decreased significantly when using initial cheese whey concentration higher than 1200 gL^{-1} (e.g 37 % when initial cheese whey concentration was 1800 gL^{-1}). Similar trend has been

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previously, reported by Dimou *et al*. (2015), in the case of wine lees hydrolysis. The effect of pH value on cheese whey hydrolysis yield was evaluated by conducting experiments within the range of 1 to 7, as it can be seen in Fig.4).

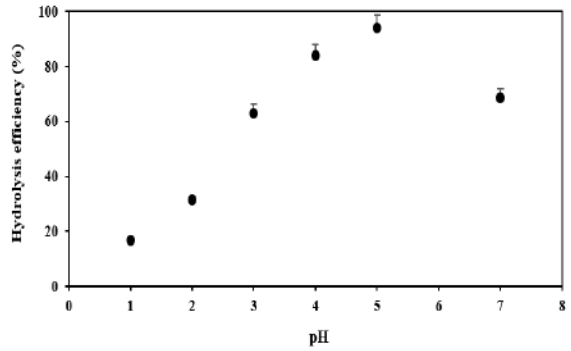

Fig.4: Effect of pH value on hydrolysis efficiency (percentage of total lactose concentration to glucose conversion) of cheese whey at optimum conditions (9 U mL^{-1} enzymatic activity, reaction time=12h; T=55°C and 1200 gL^{-1} initial cheese whey concentration)

Comparable lactose to glucose conversion yield were achieved at pH values in the range of 4.5 to 5, while hydrolysis yield was decreased at higher values. The pH value of 5M was determined as the optimum for cheese whey hydrolysis, using *A. oryzae* β-galactosidase, as an overall lactose to glucose yield of 93.98 % was achieved. To our knowledge there is no literature regarding unique cheese whey, delivered from Aegean islands, hydrolysis, although several researchers have studied hydrolyses of agroindustrial and industrial food processing by-product streams, using commercial or crude enzymes (Souza Moreira *et al*., 2012; Dimou *et al*.; 2015; 2016a; Lapena et al., 2018). So, in this study the highest total lactose hydrolysis of unique indigenous "Aegean" delivered mixture of Greek cheese whey, achieved at 55°C after 12h of enzymatic optimized process. Initial concentration of β-galactosidase preparation, highly affected final glucose production as well as lactose hydrolysis yields. Higher initial concentration of β-galactosidase preparation (for instance 12 gL^{-1}) did not further increased cheese whey hydrolysis efficiency. Dimou *et al*. (2015), studying enzymatic hydrolysis of wine lees, concluded that the initial enzymatic activity of crude *A. oryzae* enzymes highly affects hydrolysis yield. In this study, the maximum degree of hydrolysis achieved was approximately 94 %, higher than that observed by Haider *et al*. (2009), who studied whey lactose hydrolysis process, using β-galactosidase, of 0.88 UmL^{-1} and 0.44 UmL^{-1} concentrations, at 37°C, respectively. The authors reached a maximum degree of hydrolysis of 70%, at the end of enzymatic activity reaction (after 12h). The results obtained regarding unique cheese whey hydrolysis of Greek Aegean islands, are very satisfactory.

3.3 Development of industrially feasible process for the production of innovative glucose rich streams, suitable for novel food applications

The optimized enzymatic as well as acid catalyzed hydrolysis of unique Aegean delivered cheese whey was evaluated in a short industrially feasible 6 h reaction process, aiming at producing a glucose-rich hydrolysate that could find possible application, towards the production of "novel" functional foods with nutritional value, addressed to those who suffer from lactose intolerance. Glucose production, throughout acid as well as enzymatically catalyzed reaction, under optimum conditions in a 6-h process, is presented in Fig.5.

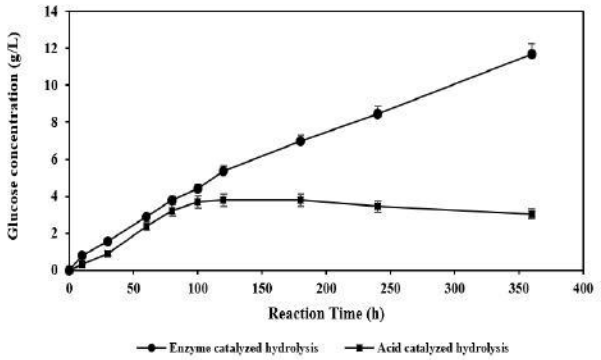

*Fig.5: Glucose concentration from cheese whey hydrolysis catalyzed either by acids[*1,*3] or β-galactosidase[*2,*3] after 6h of reaction, at optimum conditions for each process*

*[1]optimum conditions for acid hydrolysis of cheese whey: temperature=100 °C; pH=1; *[2]optimum conditions for enzymatic hydrolysis of cheese whey: temperature=55 °C; pH=5; initial enzymatic activity=9 U/mL *[3] The results are expressed as the mean values of three replicates (±SD)

After 6 h of cheese whey hydrolysis, hydrolysis yield is approximately 74 %, regarding enzymatically conducted process, while hydrolysis yield achieved through the acid catalyzed process is almost 18%. Thus, enzymatically derived hydrolysates could be possibly used for the production of novel lactose free products or low lactose products substituting other types of common commercial sweeteners. These results are very promising for further utilization of enzymatically catalyzed cheese whey in industrial food sector either substituting commercial nutrient sources such as glucose syrups and other saccharides. Lactose intolerance refers to syndromes such as diarrhea, abdominal pain, flatulence, and/or bloating that occur after ingestion of lactose containing food products. In fact an individual who suffer from lactose intolerance cannot digest or absorb lactose owning to a genetically programmed decrease in intestinal galactosidase (lactase) that occurs after weaning. This is a

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condition well known as resistance lactase pathophysiology or attributed to the damage caused to the lining of the epithelial cells in the digestive tract (Tomar *et al*., 2014). Lactose intolerance may be troublesome but in fact is not considered a condition that needs medical treatment, when individuals follow a diet that contains food products free or almost free of lactose. Thinking that 75% of the world's adult population is lactose or almost lactose intolerant the production of novel food free of lactose is of high significance. Besides, it has been reported that reduction of lactose concentrations from 70 to 80% in food products is enough for the vast majority of those who suffer from lactose intolerance (Szilagyi *et al*., 2015). So, enzymatic hydrolysis, if considered a treatment to cheese whey, prior to its industrial application for the designing and production of novel functional foods, could possibly improve the sensorial and technological properties of novel bio-produced functional products, addressed to those who suffer from lactose intolerance. Furthermore, it has been reported that *β*-galactosidase treatment of milk, could be possibly applied, as a bioprocessing stage, toward the production of sweeteners (Panesar *et al*., 2010).

3.4 Biocarotenoids and ergosterol production by *R. glutinis* cultivations, using cheese whey hydrolysates as nutrient supplements

Enzymatically derived cheese whey hydrolysates used as feedstocks, for the production of carotenoids and ergosterol, using *Rhodotorula glutinis* CCY 20-2-26.

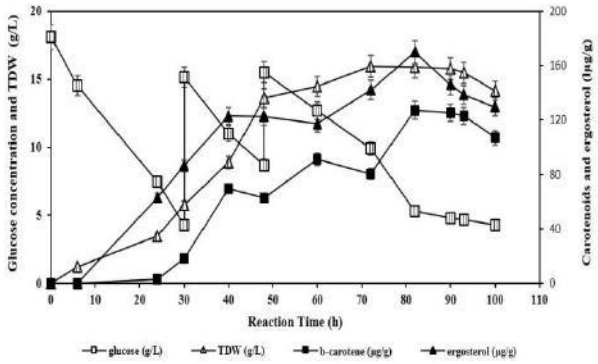

Fig. 6: Consumption of glucose as well as production of total dry weight (TDW*), carotenoids (b-carotene*) and ergosterol* using enzymatically derived cheese whey hydrolysate as nutrient supplements, during R glutinis fed batch fermentation*

* The results are expressed as the mean values of three replicates (±SD)

As it can be seen in Fig.6, *R. glutinis*, displayed, a two-phase growth character, characterized by a prolonged stationary phase. This could be probably attributed, to the ability of yeast cells to utilize supplementary energy resources such as lipids formed during yeast growth period. Furthermore, carotenoids as well as ergosterol production, during growth of *Rhodotorula glutinis* presented some fluctuations, with some local major and minor, as it can be seen in Fig.6. The major carotenoids produced by *R. glutinis* cells, under optimized conditions (T=26ºC; agitation speed=200-500rpm, pH=6.2-6.5) in bioreactor fed batch fermentations as well as preliminary shake flask cultivations (data not shown), using cheese whey hydrolysates as nutrient supplements was b-carotene (almost 0.95 g b-carotene/g total carotenoids). The maximum carotenoid production (carotenoids per dry weight) as well as ergosterol production (ergosterol per dry weight), using cheese whey hydrolysates (enzymatically derived), as bioprocessing feedstock achieved when the initial glucose concentration of enzymatically derived cheese whey hydrolysates was 18 gL^{-1} after 82 h of cultivation *R. glutinis* (while *R. glutinis* growth has already reached stationary phase) as it can be seen in Fig. 6. Ergosterol observed partly as the additional parameter of biomass quality, while monitoring competition of two specialized branches of isoprenoid pathway, which is used for the biosynthesis of both carotenoids and sterols (Britton *et al*., 1995). The production of ergosterol was very similar to the production of b-carotene (Fig.6), even though these metabolites are formed in competitive branches of isoprenoid metabolic pathway. More specifically, the highest production of 127.3 ± 0.41 $\mu g g^{-1}$ (or 2023.03 ± 0.41 $\mu g L^{-1}$), 170.78 ± 0.38 μg g-1 (or 2703.917 ± 4.37μg L^{-1}) and 15.89 ± 0.05 gL^{-1}, of total carotenoids, ergosterol and total dry weight, respectively achieved, using as feedstock cheese whey hydrolysates of 18 gL^{-1} glucose, enriched with 0.15 gL^{-1} $MgSO_4 \cdot 7H_2O$, 5 gL^{-1} K_2HPO_4, 5 gL_{-1} yeast extract and 5 % (wv^{-1}) NaCl, while pH was adjusted to the range of 6.2-6.5.

Under the same conditions, when deproteinized cheese whey prepared by acid hydrolysis process, used as fermentation feedstock, 59.54 ± 0.73 $\mu g g^{-1}$ (or 210.57 ± 1.12 $\mu g L^{-1}$) carotenoids, 53.49 ± 1.16 $\mu g g^{-1}$ (or 189.16 ±1.5 $\mu g L^{-1}$) ergosterol and 3.53 ± 0.05 gL^{-1} total dry weight produced, after 60h of fermentation. This low metabolite production during *R. glutinins* growth, using acid hydrolysates could be possibly attributed to inhibitory by-products produced during acid catalyzed cheese hydrolysis process (Baek *et al*. 2008). Preliminary experiments conducted, using crude cheese whey hydrolysates as nutrient supplements, led to approximately, under the same conditions led to 87 % less carotenoids and ergosterol production, highlighting the incapability of the studied strain to consume lactose. Taking into consideration all the above, is obvious that enzymatic derived cheese whey hydrolysates, form a better nutrient supplement for *R. glutinis* CCY 20-2-26 growth and concurrent metabolite production.

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Very few studies have been published, using cheese whey as bioprocessing feedstock for concurrent carotenoid and ergosterol production by *Rhodotorula glutinis* strains. Kanzy *et al*. (2015), studying potential production of carotenoids using cheese whey as nutrient supplement reported that the maximum biomass (13.95 gl^{-1}) and volumetric carotenoid production (6.544 mgL^{-1}) were scored by an isolated *R. glutinis* strain after 120 h incubation at 30 °C and pH 6.6 in a medium containing 3% NaCl. Aksu *et al*. (2005) studied the production of carotenoids by *R.glutinis*, evaluating potential usage of low-cost substrates (glucose, sucrose from molasses and lactose from cheese whey) as fermentation media. The researchers concluded that the highest concentration of total carotenoids was obtained in a medium containing 20 gL^{-1} molasses sucrose. The highest specific carotenoid yield (35.5 mgg^{-1}) was reached when initial cheese whey lactose was equal to 13.2 gL^{-1}. To the best of our knowledge the results presented regarding concurrent production of carotenoids and ergosterol, are among the highest in literature cited publications using *R. glutinis* as microbial strain. Furthermore, it is of high significance to mention the genuity of this research regarding the revalorization of a unique by-product stream to value-added biomolecules with nutritional interest and several industrial food applications.

IV. CONCLUSION

In this study potential biocarotenoid production as well as other metabolite production such as ergosterol, using cheese whey derived from Aegean islands, as generic feedstock though the designing, development and optimization of a series of biochemical, chemical and biotechnological processes was evaluated and verified. Enzymatically derived cheese whey hydrolysate seems to be a better source for both bio-carotenoid and ergosterol production, compared to either untreated cheese whey or acid catalyzed cheese whey hydrolysates, under optimized conditions. Cheese whey hydrolysates produced via optimized enzymatic hydrolysis could possible find several applications for the production of carotenoids, ergosterol and other novel functional products such as novel lactose free food preparations or novel functional foods enriched with bio-carotenoids and/or bio-ergosterol. Integration of cheese whey, which is a low-cost substrate to nutritionally value-added products such as biocarotenoids, ergosterol and glucose rich streams free or semi-free of lactose is of great academic and industrial interest, while satisfying the principles of sustainable development and opening new areas in revalorizing by-product streams to value added products. These value-added products could find several applications in industrial food sector toward the production of novel enriched with bio-carotenoids and ergosterol products as well as innovative food products free or semi free of lactose, using enzymatically derived cheese whey as alternative source of sweetener.

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CONFLICTS OF INTEREST

The authors confirm that have no conflict of interest.

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Assessment of Crushed Glass as a Medium for Rapid Rate Filtration

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Abstract**—** *This study was carried out to assess the potential of crushed glass as a filter medium in high rate filtration. Waste glass collected from a glass manufacturing industry was crushed using hammer mill and sorted into different particle sizes (0.300 mm, 0.425 mm, 0.550 mm, 1.18 mm and 2.36 mm). The gravel layer was between 2.36 mm to 4.75 mm. The glass were arranged in layers and used as the filter bed for the water filtration process. The depth of each medium was set at 800 mm with a constant flow rate of 0.0275 l/s, and the experimental run of 4 hours. The flow rate, turbidity and head at the ports were monitored at every 30 minutes interval. Glass shows a tremendous decrease in filtration rate with time compared to sand and also a higher headloss developed. At 2.5 hours, glass had attained a turbidity of 0 NTU while sand at 3.5 hours. The results show an increase in filtration efficiency in glass compared to sand which suggests that crushed glass could be used conveniently as a medium for rate of filtration.*

Keywords**— Crushed glass, high rate filtration, filter media, surface water and hammer mill.**

I. INTRODUCTION

Water is one of the most abundant compounds found in nature, covering approximately three-fourths of the earth. In spite of this apparent abundance, several factors serve to limit the amount of water available for human use (Bove*et al*., 2015). Though, water occupies about 71% of the earth's surface over 97% of the total water supply is contained in the oceans and other saline bodies of water and is not readily useable for most purposes. Thus for general livelihood and the support of their varied technical and agricultural activities, humans must depend on the remaining 0.62% found in fresh water lakes, rivers and ground water supplies (Todd, 2011;Huck*et al*.,2010).

The World Health Organization (WHO) has however set up standards for potable water. To meet these standards, the raw water gotten from nature will have to undergo a series of treatment processes. A typical treatment plant for surface water is made up of aeration unit, coagulation unit, sedimentation unit, filtration unit, disinfection and then distribution unit. Water which has passed through the coagulation unit and then settled, is invariably passed through a filter before it is disinfected and supplied to users. The most common filter used in conventional treatment plants all over the world is made up of silica sand underlain by gravel, and it filters water at rates ranging from 80 to 250 litres per minute per square metre of the surface area of the filter bed (Ogedengbe, 1985).

Rapid filtration generally implies a process, which includes coagulation, flocculation, clarification and disinfection (McGhee, 1991). The rapid filters are usually constructed as open-top, free-surface units and less frequently as in-line pressure units(Matilainenet al., 2010). The rapid sand filters utilize a bed of silica sand ranging from 0.6 to 0.75 m in depth. Sizes may range from 0.35 mm or even larger, with effective sizes of 0.45 to 0.55 mm. a uniformity coefficient of 1.65 is commonly specified (Korkosz et al., 2012).

Filtration rates following flocculation and sedimentation are in range of 1.4 to 6.8 l/m^2s, with 3.4 l/m^2s normally the maximum design value(Bove*et al*., 2015). Because of the higher filtration rates, the area requirement of a rapid filtration plant is about 20% of that required for the slow sand filters, although the latter usually do not require pre-treatment of the raw water (Korkosz *et al*., 2012).

The idea of using crushed glass as filter medium is a new development. Glass scraps which are more are less "wastes" from glass industries are available in large quantities and can be processed to serve a useful purpose in water filtration. Bryant et al. (2011) has established that with the use of recycled crushed glass, the quality of water was improved. Hence in furtherance of research using this filter medium, a confirmation on whether these qualities will be achieved with glass from our local industries was made the subject of this study.

1.1 Description of the Study Area

Effluent from the sedimentation tanks of the Opa Water treatment plant in Obafemi Awolowo University, Ile –Ife was siphoned and used as the influent water sample. It was ensured that the water was not contaminated or polluted with any other materials other than the state it was before being collected from the weir.

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II. MATERIALS AND METHODS

The materials that were used for the project include the filter media (crushed glass and silica sand), Polyvinyl chloride (PVC) pipes of 4 inches diameter for the construction of the pilot filter unit, pipes and appurtenances of ¾ inches and water (effluent from sedimentation tank).

The silica sand used for the filtration process was obtained from the Opa Water treatment plant. This particular sand material is used as the filter bed in treating water supplied to the university community. The silica sand obtained was in three batches and arranged in increasing order of coarseness and sizes.

Crushed glass materialwas obtained from TSG industry Olodo, Ibadan. The glass obtained was post-industrial plate glass scrap. This was chosen because it is completely free of the potential organic and inorganic contamination that can be present in post-consumer container glass.

The following equipment and apparatus were procured for the filtration process colorimeter, water sample bottles, measuring cylinder, stop watch and manometer.

The turbidity of the influent and effluent water samples was measured using a colorimeter (JENWAY, 6501).

According to Matilainen*et al*.(2010), the values obtained from the colorimeter used can be converted to NTU readings using the equation

$$Y = 275.5 + 2.79x \qquad (1)$$

where x = colorimeter reading; Y = turbidity in NTU

In the absence of a pressure gauge for taking pressure reading a manometer board was constructed. The manometer board was constructed from plywood, with supports made from wooden members. It consisted of three manometer tubes, the tubes were not more than 2.0 mm in diameter and about 1.5 m long. The manometer board was provided with graduated scale for monitoring the headloss developed in the filter bed.

Pilot filter unit made from a circular cross polyvinyl chloride pipe with 4 inches diameter and cross-sectional area of 8,108 mm^2 was used. The head of water above the filter bed was kept constant by the overflow mechanism. This was done by making a hole of about 10.0 mm in the pipe which prevented water rising beyond a particular level. An underdrain was provided at the bottom of the pilot unit to support the medium and to allow the effluent from the unit pass to the effluent storage unit. The filter unit was supported at the base and braced properly for stability and possible leakage. Along the height of the pipe were installed three manometer ports to record the headloss at different level of the bed (Bove*et al*., 2015).

The main activities or operation include sieve analysis, determination of specific gravity of the materials, arrangement of the media into the unit, charging of filter, experimental procedure and data collection.

The American Standard for Testing and Materials (ASTM) sieve sizes were used. The following sieve sizes were used for the analysis, sieve numbers 8.0, 16.0, 20.0, 40.0 and 50.0 with opening diameters 2.36 mm, 1.18 mm, 0.85 mm, 0.425 mm and 0.30 mm respectively.

Horan and Lowe (2007) states that for the determination of oversize and undersize aggregates and especially for research work on aggregate grading, additional sieve sizes are required. The results of the sieve analysis were used to determine the effective size and uniformity coefficient of the materials.

The glass material procured from the glass industry was crushed, screened and sieved to meet the specification required for the filter media. The glass material was crushed using a crushing machine into different particle sizes. Glass particles extremely larger than the required size were re-crushed. The sand procured was washed thoroughly and dried before sieving.

The system was set-up as shown systematically in Figure 1, and was tested for leakages. The crushed glass particles were arranged into the vertical circular pipe with large particle sizes at the bottom (1.18 – 2.36 mm), followed by 0.425 – 0.850 mm sizes and then 0.30 mm at the uppermost layer. Particle sizes ranging from 2.36 mm – 4.75 mm was used as the gravel layer just above the underdrain (Soyer*et al*., 2010).

Clean water was then introduced from the top to remove all air bubbles in the medium and ensure proper gradation. The same procedure was carried out for sand. This was done in preparation of experimental runs.

Experimental procedure was done by opening the influent valve to allow the influent to be at the desired and predetermined flow rate. The effluent control valve was then opened to allow flow from the filter bed. Water level above the medium was maintained to avoid overflow which was done by the provision of holes at the side of the pipe. The filtration rate, turbidity and head at the ports were monitored at 30 minutes interval, with the runs lasting for 4 hours using a constant flow and variable head method (Lee, 2014).

The same procedure was carried out for sand with the depth of each medium set at 800 mm including the gravel layer. The effluent valve was shut at the end of the experimental runs and then the influent, leaving the filter under a full head of water.

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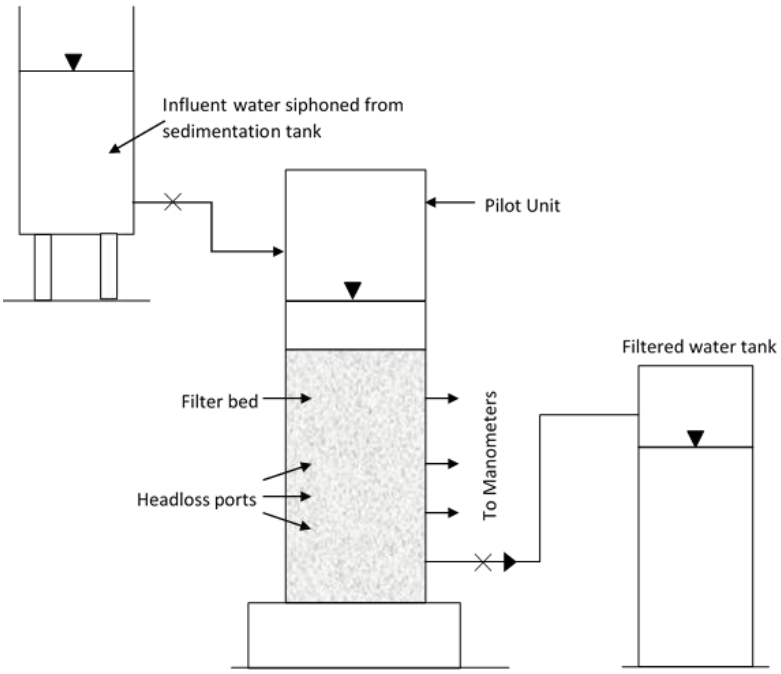

Fig.2.1: Schematic layout of filter unit

III. RESULTS AND DISCUSSION

The physical properties of the filter media are shown in Table 1. The grading curve of the crushed glass and sand media extends over a limited range. This shows that the crushed glass is less dense than sand but denser than water, therefore will not float in water.

Table.1: Physical properties of filter media

Physical properties	Sand	Glass
Effective size (D_{10}) (mm)	0.357	0.671
Uniformity coefficient (U_c)	1.5	1.906
Specific gravity (G_s)	2.61	2.53

3.1 Headloss and Filtration Rate

The headloss build-up across the filter beds was measured in centimetres of water developed above the bed. The variation of headloss with time between the two media is shown in Table 2. The effluent from the underdrain was noticed to start flowing at 2 minutes and 2.57 minutes for sand and glass respectively after the influent value was opened. The initial headloss developed at this time in each case was also recorded to be 8.5 cm and 11.5 cm respectively. The variation of headloss between the two media with time is shown in Figure 2, there is an increase in water headloss developed across the bed with time and it is fairly uniform. It can be seen from this figure that glass developed a higher headloss than sand within the same filtration time. This was due probably to the rapid clogging of the glass medium which can be seen as a result of relatively smaller interstitial spaces in comparison with sand (Horan and Lowe, 2007).

The flow rate from the influent valve was set at a constant value of 0.0275 l/s for the two filter media throughout the experimental run. The variation in filtration rate with time is shown in Table 2 and Figure 3. The filtration rate of both media decreases rapidly with time. The deciding factor for such behaviour is the particle size of the glass medium which on average has less rounded edges than sand (Horan and Lowe, 2007). Hence, it can be declared that the reason for filtration rate decline in the glass medium being higher was due to its more efficient trapping of smaller turbid particles (Lee,2014 ;Bryant et al.,2011).

Table.2: Headloss and filtration rate across the bed with time

	Time (hrs)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Headloss (cm)	**Glass**	12.8	14..2	15.5	17.2	19.0	21.0	22.2	23.5
	Sand	9.6	10.9	11.8	13.0	14.3	15.3	16.5	18.0
Filtration rate ($l/m^2.s$)	**Glass**	2.812	2.603	2.43	2.22	2.109	1.974	1.739	1.517
	Sand	3.195	2.973	2.837	2.825	2.738	2.677	2.541	2.442

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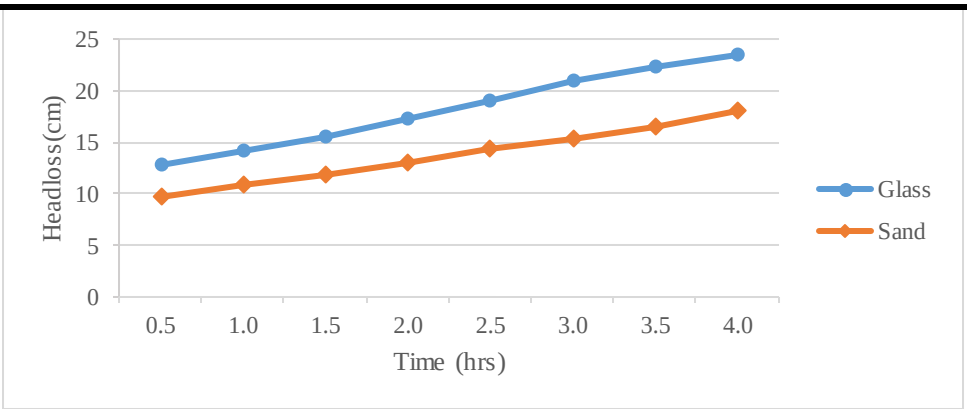

Fig.3.2: Variation of headloss across the beds with time

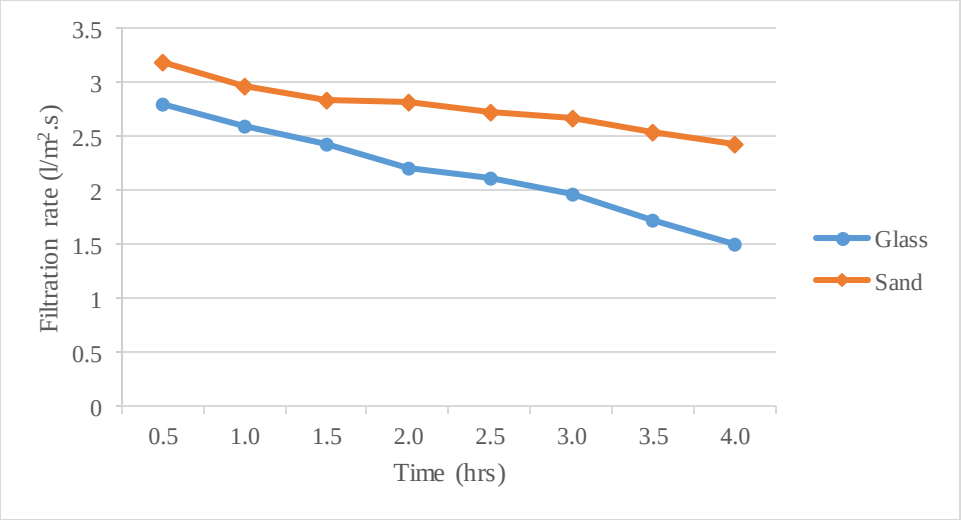

Fig.3.3: Variation of filtration rate with time between the filter beds

3.2 Turbidity

According to Ogedengbe (1985), a filtration run would normally be terminated when, either the headloss across the bed becomes excessive or the quality of the water produced declines to an unacceptable level. Either of these two conditions could occur first.

The results of turbidity readings in NTU are shown in Table 3 and the plot in Figure 4. This shows correlation and variation in turbidity for the two media (glass and sand) at different points in time. The values gotten from the colorimeter were converted to NTU using the standard expression in Equation (1). It was observed to be equal for the first hour of filtration after which a sharp decline was observed in glass turbidity. At exactly 2 hrs filtration time both media attain equal value with sand afterwards having a constant value up to the 3 hour. Finally, at 3.5 – 4 hours filtration time both media recorded 0 NTU. The result shows that finer particles were removed in the glass filter more efficiently, reflected by decrease in NTU.

Table.3: Converted colorimeter readings

	Time (hrs)	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Colorimeter readings	Glass	96	97	97	98	98	99	99	100	100
	Sand	96	97	97	97	98	98	98	99	99
Turbidity (NTU)	Glass	7.66	4.87	4.87	2.08	2.08	0.00	0.00	0.00	0.00
	Sand	7.66	4.87	4.87	4.87	2.08	2.08	2.08	0.00	0.00

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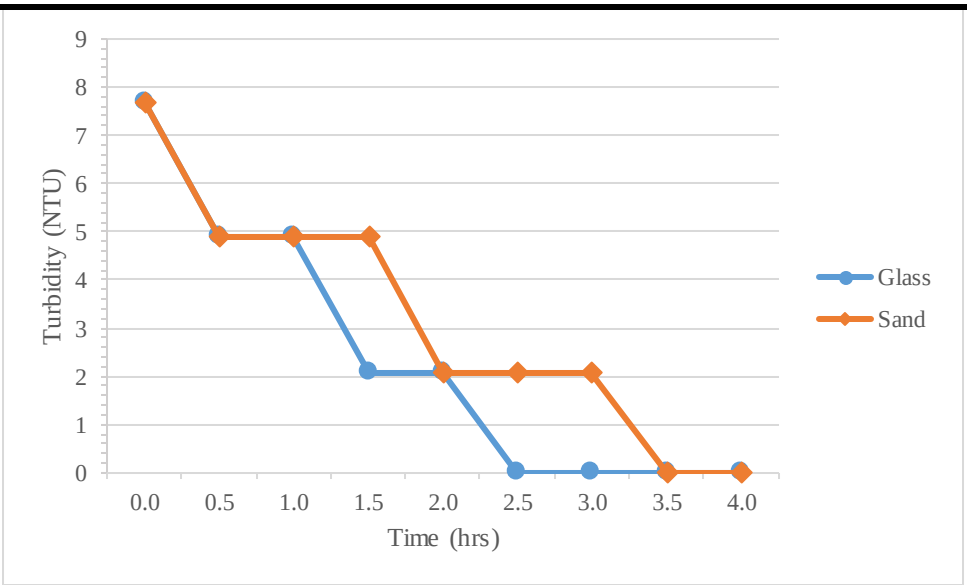

Fig.3.4: Variation of turbidity with time between the filter effluents

IV. CONCLUSION

Based on the results of the study, it can be stated that crushed glass is capable of being used as a medium for water filtration. The following conclusions were made: glass is potentially useful for water filtration, with improved water quality through longer filtration time, filtration rate is les with a higher head loss build up with glass, due to the crystal clear nature of glass, less water was required to clean the filter bed before the start of experimental run and glass is less dense than sand, therefore, less medium by weight is required when using crushed glass as filter medium.

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A Study on Diversity of Spiders at Malavagoppa Village, in Shimoga District, Karnataka

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Abstract**—** *Spiders belong to the order Araneae which is the largest order among the class Arachnida. Spiders are considered as indicators of overall species richness and the health of terrestrial communities. The village with different plantations harbors a good number of spiders. The survey was carried out from February 2018 to January 2019 by using active searching, beating, leaf sampling and net sweeping techniques. During the study, a total number of 51 species belonging to 42 genera of 16 families were documented. In the present study it was found that the family Araneidae was dominated by having 14 species followed by the family Salticidae having 12 species and the least were from Clubionidae, Corinnidae, Eresidae, Gnaphosidae, Hersiliidae, Linyphiidae, Sparassidae, Theridiidae and Uloboridae with a single species from each family.*

Keywords**— *Araneidae, Malavagoppa Village, Spiders Diversity.***

I. INTRODUCTION

Spiders form one of the most ubiquitous groups of predaceous organisms in the animal kingdom (Riechert and Lockley 1984). Among all organisms, spiders (Order: Araneae) form the seventh largest order in terms of number of known species (Sebastian and Peter, 2009). They belong to the class Arachnida of the Phylum Arthropoda that possesses jointed appendages with a chitinous exoskeleton system. They are characterized by two body parts, the cephalothorax having 4 pairs of segmented legs and the abdomen. They have simple eyes, no antenna and no wings, which differentiate them from insects.

Spiders are the key components of all ecosystems in which they live. They are the ancient animals with a history going back over 350 million years (Sebestian and Peter, 2010). This is the most diverse, female dominated and entirely predatory order in the Arthropod world. Globally, spiders include about 48,147 described species in 4131 genera and 117 families. (World Spider Catalog, 2019). They are distributed on every continent except Antarctica and have adapted to all known ecological environments except air and the open sea (Foelix, 1996). Spiders of the Western Ghats are poorly studied compared to other parts of the country. With respect to its geographical, climatic and ecological features, the Western Ghats harbors a rich amount of arachnids out of which spiders have a huge share. Studies on Indian spiders were initiated by Blackwall (1850). Different studies have shown that spiders regulate prey populations depending on their density. Recent studies have shown that the spiders act as ecological indicators and early warning signs of environmental changes (Kremen et. al., 1993).

II. MATERIALS AND METHOD

Study site:

Malavagoppa village, located at 13.8998°N 75.6194°E Shimoga district, Karnataka. This village is an urban area with good number of vegetation.

Methods:

Study sites included indoor habitats such as inside houses and other buildings, surroundings of human dwellings; outdoor habitats included were gardens, areca plantations, and open field.

The study was carried out from February 2018 to January 2019 covering all three seasons.

Survey was done 2 times in a week. The techniques involved were all out active searching, beating, visual observation and photographic methods. Collections were done by hand picking, gentle beating on surroundings so as to make the individual pass into the collection box. Detailed morphological study was done using Stereo-zoom and compound microscope. Identifications were done by referring 'Spiders of India' book, literatures and other scientific papers.

Statistical analysis:

Calculations were done by using Microsoft excel.

Formulae used:

$$\text{Frequency} = \frac{\text{Number of seasons studied}}{\text{Total number of seasons studied}}$$

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$$\text{Density} = \frac{\text{Total number of individuals of a species}}{\text{Number of seasons studied}}$$

$$\text{Abundance} = \frac{\text{Total number of individuals of a species}}{\text{Number of seasons in which occured } 2a}$$

Shannon's diversity index, $H = - \sum Pi \ (\ln Pi)$

Simpson's value, $D = \sum ni \ (ni-1) \ / N \ (N-1)$

Where, ni = Number of individuals of a species

N = Total number of all individuals

III. RESULTS

A. Species diversity of spiders

The study site has a great spider diversity of 51 species belonging to 42 genera of 16 families.

In the present study site, the family Araneidae is dominated by having 14 species (9 genera) followed by the family Salticidae having 12 species (12 genera), the Tetragnathidae with 6 species (3 genera), Lycosidae with 3 species (3 genera), Oxyopidae with 3 species (2 genera), Thomisidae and Pholcidae with 2 species (2 genera) in each and the Clubionidae, Corinnidae, Eresidae, Gnaphosidae, Hersiliidae, Linyphiidae, Sparassidae, Theridiidae and Uloboridae were identified with single species in each (Table.1).

Table.1: Spiders recorded in Malavagoppa village during Feb 2018- Jan 2019

Sl. No	Family	Scientific name	Common name	Species count
1	Araneidae	*Araneus spp* (Clerck, 1757)	Angulated Orb Weaver	14
		Argiope anasuja (Throell,1887)	Signature Spider	
		Argiope pulchella (Throell,1881)	Garden Cross Spider	
		Argiope spp.1 (Audouin, 1826)	Signature Spider	
		Cyclosa bifida (Doleschall, 1859)	Long-bellied Cyclosa	
		Cyrtophora cicatrosa (Stoliczka, 1869)	Tent-web Spider	
		Cyrtophora citricola (Forsskal, 1775)	Tropical Tent-web Spider	
		Eriovixia spp.(Archer, 1951)		
		Gasteracantha geminata (Fabricius, 1798)	Oriental Spiny Orb-weaver	
		Gasteracantha spp.1 (Sundevall, 1833)	Spiny Orb-weaver	
		Neoscona crucifera (Lucas, 1838)	Common Garden Spider	
		Neoscona nautica (L. Koch, 1875)	Grey Sphere Spider	
		Parawixia dehaani (Doleschall, 1859)	Abandoned Orb-weaver	
		Thelacantha brevispina (Doleschall, 1857)	False Gasteracantha (Double Spotted Spiny Spider)	
2	Clubionidae	*Clubiona spp.* (Latreille, 1804)	Leaf Curling Sac Spiders	1
3	Corinnidae	*Castianeira zetes* (Simon, 1897)	Black Ant-mimicking Spider	1
4	Eresidae	*Stegodyphus sarasinorum (*Karsch,1892)	Indian Social Spider	1
5	Gnaphosidae	*Gnaphosa spp.* (Latreille, 1804)	Ground Dwelling Spider	1
6	Hersiliidae	*Hersilia savignyi* (Lucas, 1836)	Two Tailed Spider	1
7	Linyphiidae	*Neriene sundaica* (Simon, 1905)	Black Dwarf Spider	1
8	Lycosidae	*Geolycosa spp.*(Montgomery, 1904)	Wolf spider	3
		Hippasa agelenoides(Simon,1884)	Funnel Web Spider	
		Pardosa spp. (C. L. Koch,1847)	Wolf Spider	
9	Oxyopidae	*Oxyopes javanus* (Throell,1887)	Striped Lynx Spider	3
		Oxyopes sunandae (Tikader, 1970)	Orange Lynx Spider	
		Peucetia viridians (Hentz,1832)	Green Lynx Spider	
10	Pholcidae	*Crossopriza lyoni* (Blackwall, 1867)	Tailed Cellar Spider	2
		Pholcus spp. (Walckenaer, 1805)	Daddy long leg spider	
11	Salticidae	*Asemonea spp.* (O. Pickard-Cambridge,1869)	Green jumping spider	12
		Bavia kairali (Samson & Sebastian,2002)	Scorpion Spider	
		Carrhotus viduus (C. L. Koch,1847)	Double Striped Carrhotus	

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		Chrysilla volupe (Karsch,1879)	Colored Jumping Spider	
		Epeus indicus (Proszynski,1992)	White Spotted Green Jumper	
		Hyllus semicupreus (Simon, 1885)	Heavy-bodied Jumping Spider	
		Menemerus bivittatus (Dufour, 1831)	Gray Wall Jumper	
		Myrmarachne plataleoides (O. Pickard-Cambridge,1869)	Kerengga Ant-like Jumper	
		Phintella vittata (C. L. Koch,1846)	Banded Phintella	
		Plexippus paykulli (Audouin, 1826)	Pantropical Jumping Spider	
		Portia albimana (Simon, 1900)	Fringed Jumping Spider	
		Telamonia dimidiata (Simon, 1899)	Two-striped Jumping Spider	
12	Sparassidae	*Heteropoda venatoria* (Linnaeus, 1767)	Huntsman Spider	1
13	Tetragnathidae	*Leucauge decorata* (Blackwall, 1864)	Decorative silver orb weaver	6
		Leucauge tessellata (Throell,1887)	Long jawed orb weaver	
		Leucauge spp.1 (White, 1841)	Decorative spider	
		Opadometa fastigata (Simon, 1877)	Pear Shaped Leucauge	
		Tetragnatha mandibulata (Walckenaer, 1841)	Long-jawed Orb-weaver	
		Tetragnatha spp.1 (Latreille, 1804)	Long jawed Spider	
14	Theridiidae	*Theridion spp.* (Walckenaer, 1805)	Comb-footed Spider	1
15	Thomisidae	*Misumenops rubrodecoratus* (Millot,1942)	Green Crab Spider	2
		Thomisus spectabilis (Doleschall, 1859)	White Crab spider	
16	Uloboridae	*Uloborus spp.* (Latreille, 1806)	Feather-footed Spider	1

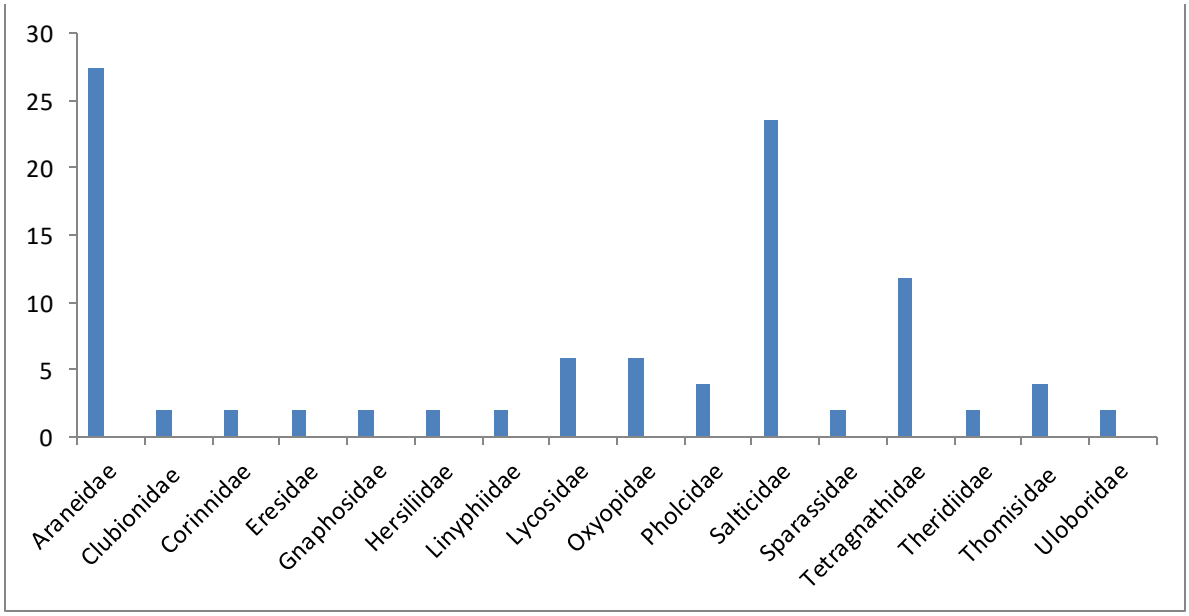

Fig.1: Graph of species percentage under each family

B. Diversity richness and seasonal abundance

Three main seasons were studied viz., rainy, winter and summer. Most spider species were found throughout the study period and few were found only in two seasons. But, only one species (*Oxyopes sunandae*) has occurred in one season (rainy). (Table 2).

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Table.2. Shows individual counts of each species during different seasons.

Sl No.	Spider Species	Individual counts of spider in different seasons			Total count
		Rainy	Winter	Summer	
1	*Araneus spp*	11	9	3	23
2	*Argiope anasuja*	12	13	9	34
3	*Argiope pulchella*	13	14	8	36
4	*Argiope spp.1*	6	5	3	14
5	*Asemonea spp*	8	9	6	23
6	*Bavia kairali*	2	1	0	3
7	*Carrhotus viduus*	4	4	3	11
8	*Castianeira zetes*	15	16	11	42
9	*Chrysilla volupe*	12	13	16	41
10	*Clubiona spp*	14	8	6	28
11	*Crossopriza lyoni*	50	55	50	155
12	*Cyclosa bifida*	25	23	15	63
13	*Cyrtophora cicatrosa*	40	44	25	109
14	*Cyrtophora citricola*	20	20	12	52
15	*Epeus indicus*	4	4	3	11
16	*Eriovixia spp.*	4	3	3	10
17	*Gasteracantha geminata*	12	12	0	24
18	*Gasteracantha spp.1*	2	1	0	3
19	*Geolycosa spp.*	40	45	50	135
20	*Gnaphosa spp*	8	7	2	17
21	*Heteropoda venatoria*	6	4	4	14
22	*Hersilia savignyi*	10	11	11	32
23	*Hippasa agelenoides*	30	31	29	90
24	*Hyllus semicupreus*	4	3	2	9
25	*Leucauge decorata*	12	15	7	34
26	*Leucauge tesselata*	4	2	0	6
27	*Leucauge spp.1*	2	1	0	3
28	*Menemerus bivittatus*	10	8	9	27
29	*Misumenops rubrodecoratus*	1	2	0	3
30	*Myrmarachne plataleoides*	16	17	15	48
31	*Neoscona crucifera*	32	34	33	81
32	*Neoscona nautica*	4	3	2	9
33	*Neriene sundaica*	30	34	15	79
34	*Oxyopes javanus*	45	50	50	145
35	*Oxyopes sunandae*	2	0	0	2
36	*Opadometa fastigata*	2	2	0	4
37	*Parawixia dehaani*	1	1	0	2
38	*Pardosa spp*	45	56	60	**161**
39	*Peucetia viridans*	20	25	16	61
40	*Phintella vittata*	2	4	8	14
41	*Pholcus spp*	55	50	55	160
42	*Plexippus paykulli*	62	65	64	191

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43	*Portia albimana*	0	2	2	4
44	*Stegodyphus sarasinorum*	7	8	14	29
45	*Telamonia dimidiata*	13	12	10	35
46	*Tetragnatha mandibulata*	9	11	8	28
47	*Tetragnatha spp.1*	1	2	0	3
48	*Thelacantha brevispina*	9	9	0	18
49.	*Theridion spp.*	9	10	9	28
49	*Thomisus spectabilis*	4	5	0	9
51	*Uloborus spp*	4	5	2	11
Total count during each season		753	**788**	650	2174

Table.3: Frequency, density, abundance and species importance value of each species

Sl No.	Species	F	D	A	SIV
1	*Araneus spp*	1.00	7.67	7.67	3.21
2	*Argiope anasuja*	1.00	11.33	11.33	3.76
3	*Argiope pulchella*	1.00	11.67	11.67	3.71
4	*Argiope spp.1*	1.00	4.67	4.67	2.80
5	*Asemonea spp*	1.00	7.67	7.67	3.21
6	*Bavia kairali*	0.67	1.00	1.50	1.58
7	*Carrhotus viduus*	1.00	3.67	3.67	2.66
8	*Castianeira zetes*	1.00	14.00	14.00	4.08
9	*Chrysilla volupe*	1.00	13.67	13.67	4.03
10	*Clubiona spp*	1.00	9.33	9.33	3.44
11	*Crossopriza lyoni*	1.00	51.67	51.67	9.23
12	*Cyclosa bifida*	1.00	21.00	21.00	5.03
13	*Cyrtophora cicatrosa*	1.00	36.33	36.33	7.13
14	*Cyrtophora citricola*	1.00	17.33	17.33	4.53
15	*Epeus indicus*	1.00	3.67	3.67	2.66
16	*Eriovixia spp.*	1.00	3.33	3.33	2.61
17	*Gasteracantha geminata*	0.67	8.00	12.00	2.53
18	*Gasteracantha spp.1*	0.67	1.00	1.50	1.58
19	*Geolycosa spp.*	1.00	45.00	45.00	8.32
20	*Gnaphosa spp*	1.00	5.67	5.67	2.93
21	*Heteropoda venatoria*	1.00	4.67	4.67	2.80
22	*Hersilia savignyi*	1.00	10.67	10.67	3.62
23	*Hippasa agelenoides*	1.00	30.00	30.00	6.27
24	*Hyllus semicupreus*	1.00	3.00	3.00	2.57
25	*Leucauge decorata*	1.00	11.33	11.33	3.71
26	*Leucauge tessellata*	0.67	2.00	3.00	1.71
27	*Leucauge spp.1*	0.67	1.00	1.50	1.58
28	*Menemerus bivittatus*	1.00	9.00	9.00	3.39
29	*Misumenops rubrodecoratus*	0.67	1.00	1.50	1.58
30	*Myrmarachne plataleoides*	1.00	16.00	16.00	4.35
31	*Neoscona crucifera*	1.00	33.00	33.00	6.68
32	*Neoscona nautica*	1.00	3.00	3.00	2.57
33	*Neriene sundaica*	1.00	26.33	26.33	5.76

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34	*Oxyopes javanus*	1.00	48.33	48.33	8.78
35	*Oxyopes sunandae*	0.33	0.67	2.00	0.81
36	*Opadometa fastigata*	0.67	1.33	2.00	1.62
37	*Parawixia dehaani*	0.67	0.67	1.00	1.53
38	*Pardosa spp*	1.00	53.67	53.67	9.51
39	*Peucetia viridans*	1.00	20.33	20.33	4.94
40	*Phintella vittata*	1.00	4.67	4.67	2.80
41	*Pholcus spp*	1.00	53.33	53.33	9.46
42	*Plexippus paykulli*	1.00	**63.67**	**63.67**	**10.88**
43	*Portia albimana*	0.67	1.33	2.00	1.62
44	*Stegodyphus sarasinorum*	1.00	9.67	9.67	3.48
45	*Telamonia dimidiata*	1.00	11.67	11.67	3.76
46	*Tetragnatha mandibulata*	0.67	1.00	1.50	1.58
47	*Tetragnatha spp.1*	1.00	9.33	9.33	3.44
48	*Thelacantha brevispina*	0.67	6.00	9.00	2.26
49.	*Theridion spp.*	1.00	9.33	9.33	3.44
49	*Thomisus spectabilis*	0.67	3.00	4.50	1.85
51	*Uloborus spp*	1.00	3.67	3.67	2.66

F- Frequency; D- Density; A- Abundance; SIV- Species Importance Value

Frequency value is 1 for those species which found throughout all seasons, 0.67 for which found in only two seasons and 0.33 tells it was found only in one season. Highest density is 63.67 for *Plexippus paykulli*, followed by 53.67 of *Pardosa spp*. The least density is of *Parawixia dehaani* which is 0.67. Abundant species was *Plexippus paykulli* showing the abundant value 63.67 and the least abundant was *Parawixia dehaani*, having the abundant value 1.00. SIV is highest for *Plexippus paykulli* i.e. 10.88 shows the greater dominancy of the species followed by *Pardosa spp*. with 9.51 and least was for *Parawixia dehaani*.

Shannon index is 3.39 and Simpson value is 0.447 which indicates greater biodiversity of spiders in the study area (Table.3).

IV. CONCLUSION

From the present study it is evident that spiders can survive in different habitats as Ground runners (Lycosidae, Salticidae, Corinnidae, Gnaphosidae, and Sparassidae) Foliage runners (Clubionidae, Hersiliidae) Stackers (Oxyopidae and Salticidae), Ambushers (Thomisidae), and Web-builders. The types of vegetation greatly affect the population and diversity of spiders. The study area being an urban region, provide a good environment for the survival of spiders. Rainy season and winter season provide favourable condition for these creatures by supplying a large population of prey insects. Shannon index being 3.39 indicates good species diversity in all three main seasons. The individual count was highest during winter season and lowest during summer season. This indicates overall diversity richness in the study area as they are both good predator and prey. Species occurrence and abundance is also related to habitat preference.

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Study sites

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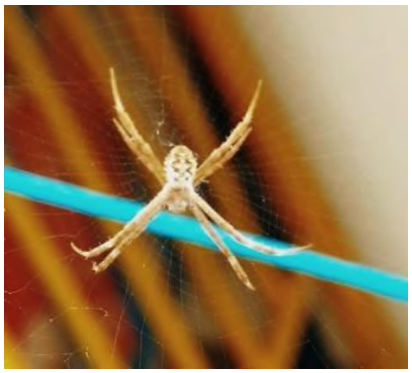
Species Plates


Araneus spp *Argiope spp.1* *Argiope pulchella*



Argiope anasuja *Cyclosa bifida* *Cyrtophora citricola*

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Cyrtophora citricola


Eriovixia spp


Gasteracantha geminata


Gasteracantha spp.1


Neoscona crucifera


Neoscona nautica


Parawixia dehaani


Thelacantha brevispina


Clubiona spp


Castianeira zetes


Stegodyphus sarasinorum


Gnaphosa spp.

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Hersilia savignyi


Neriene sundaica


Geolycosa spp


Hippasa agelenoides


P ardosa spp.


Oxyopes javanus


Oxyopes sunandae


Peucetia viridians


Crossopriza lyoni


Pholcus spp.

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Asemonea spp


Bavia kairali


Carrhotus viduus


Chrysilla volupe


Epeus indicus


Hyllus semicupreus


Menemerus bivittatus


Myrmarachne plataleoides


Phintella vittata


Plexippus paykulli

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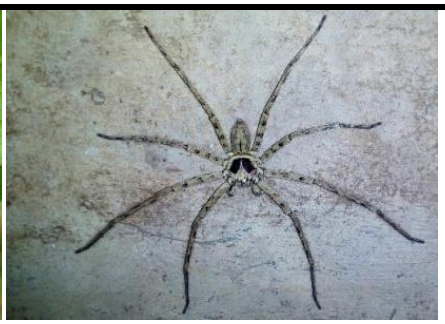


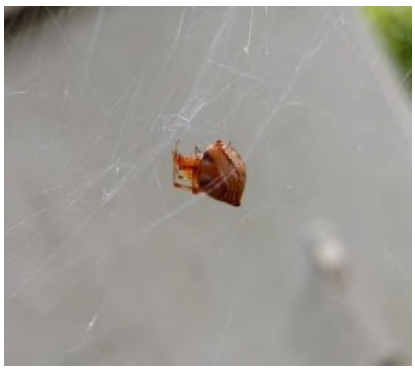
Portia albimana *Telamonia dimidiata* *Heteropoda venatoria*



Leucauge decorata *Leucauge tessellata* *Leucauge spp.1*



Opadometa fastigata *Tetragnatha spp.1* *Tetragnatha mandibulata*



Theridion spp *Misumenops rubrodecoratus* *Thomisus spectabilis*

Uloborus spp

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Bangladeshi Honey: A Possible source of beneficial Aerobic Bacteria

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***Abstract*—** *Honey is known to have many health benefits and hence had been used as natural medicine for ages. Literature suggests that bioactive agents can come from the secondary metabolites produced by the bacteria present in honey.Existenceof several bacteria has been reported in European and Australian honeys. Therefore, the aim of this study was to isolate and identify the bacteria present in honeysavailable in Bangladesh. Honey samples from seven types of natural flowers and six commercial kinds of honeyof Bangladesh were tested in this study. Prevalence of bacteria in each sample was checked through serial dilution method. Then different types of bacteria were identified by culturing them on selective agars and biochemical tests. Presence of Micrococcus luteus(100%), Streptococcus (92%), Staphylococcus aureus(77%), Bacillus (23%), Lactobacillus (15%), Klebsiella (8%) and Escherichia coli(8%) were detected.The secondary metabolites of these bacteria can be extracted and checked for potential development as medicines from nature*

***Keywords*— Honey, Bacteria, Natural Products, Bangladesh.**

I. INTRODUCTION

Honey is a natural sweetening agent that has been used as a sweetener in place of sugar for centuries.Apart from this, antibacterial agents, vitamins and antioxidants were reported to be present in honey. Therefore it is known to have numerous health benefits and is often used to treatcolds, cough, sore throats, dysentery, and many other infections[1]. Most bacteria and other microbes cannot grow or reproduce in honey due to the antibacterial activity of honey. It has been reported that Manuka honey has exhibited antimicrobial activity against a few pathogenic bacteria such as *Helicobacter Pylori* (*H. Pylori*).This is due to the secondary metabolites found in it and thus this honey can be used for the treatment of wounds and stomach ulcers[2–4].Honey also inhibits the growth of quorum sensing bacteria [2]. The bees produce an enzyme (invertase) for processing honey inside their body. Honey is too concentrated for microorganisms to grow[5].In our previous research, we have shown that multidrug-resistant bacteria were found to be sensitive against Bangladeshi raw and commercial honeys. Also, the sensitivity of bacteria to antibiotic increases when honey is used alongside the antibiotics [6].

However, honey contains several microorganisms. Microorganisms which survive in honey are those that withstand the concentrated sugar, acidity and other antimicrobial characters of honey. Diluted honey inhibits the growth of pathogenic bacteria whereas they support the growth of non-pathogenic bacteria that can be useful for human health [7]. It has been observed that *Bacillus*, *Micrococcus* and *Saccharomyces* species could be readily isolated from honeycombs and adult bees[8]. Aerobic spore-forming *Bacillus*is the most frequently encountered microbes on the external surface and intestine of the honey bees [9–12]. A number of microbial species that have been isolated from the feces of bee larvae are *Enterobacteriaceae*, *Penicillium spp*., *Aspergillus spp*., and *Torulopsis spp*.[13]. These bacteria produce various antibiotics, other antimicrobial agents and secondary metabolites which can be beneficial to human.

Therefore, the aim of this study was to identify the bacteria in the raw and commercial honeys available in Bangladesh. And shed some light on the bioactive products that they can produce.

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II. METHODOLOGY

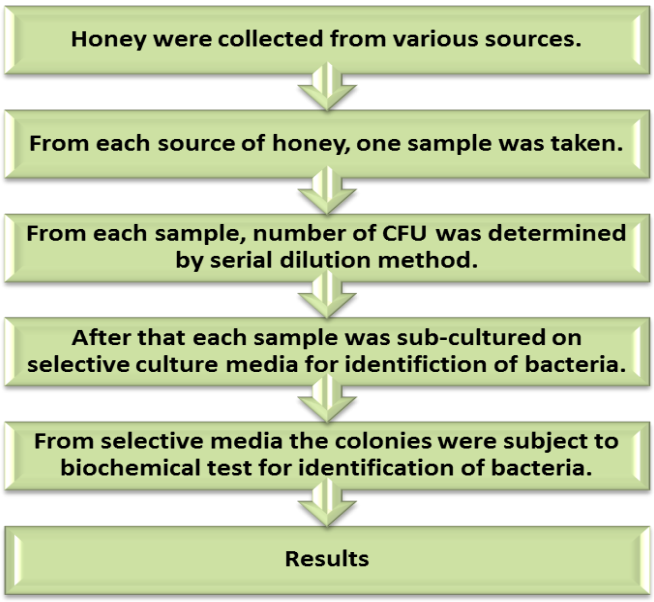

Fig.1: Workflow of the research.

Seven raw honey samples were collected from different locations of Bangladesh, and six commercial honey (C1-C6) samples were taken. For each sample, serial dilutions were prepared. An aliquot of 0.1 ml from each dilution was transferred on to nutrient agar (HiMedia®, M001) and cultured through spread plate technique. After incubation on the next day colonies were counted on each plate, and thus the number of CFU (Colony Forming Unit) was determined for all the honey samples. Then from nutrient agar, the colonies were subcultured on Mannitol Salt Agar, Blood Agar (OxoidTM, CM0055) and MacConkey Agar (OxoidTM, CM0007) for growing selectively. Finally, those were subject to different biochemical tests for identification. The biochemical tests were Catalase test, Oxidase test, Coagulase test, Motility test, Indole test, MRVP, Citrate utilization, and Urease test. All these biochemical tests were done according to the standard methods. After all the biochemical tests, results were observed and interpreted. The workflow has been summarized in Figure-1.

III. RESULTS

The results of both raw and commercial honeys are described in Table-1 and Table-2. Also, the number of identified species in each sample source and prevalence of different bacterial species are represented graphically in Figure-2 (a), (b) & (c).

Table.1: Bacteria identified in raw honeys

Location and Type of flower	10^{-1} CFU/ml	Bacteria identified
Jessore mixed honey	3	*Staphylococcus aureus*, *Streptococci* and *Micrococcus luteus*
Rajshahi Lychee honey	>800	*Bacillus cereus* and *Micrococcus luteus*
Rajshahi Mustard honey	2	*Staphylococcus aureus*, *Streptococci*, *Bacilli* and *Micrococci*
Khulna Khalsi flower honey	8	*Staphylococci*, *Streptococci*, *Lactobacilli* and *Micrococci*
Khulna Goran flower honey	18	*Staphylococcus aureus*, *Streptococci*, *Lactobacilli* and *Micrococci*
Chapainawabganj Kul flower honey	9	*Streptococci* and *Micrococci*
Chapainawabganj black cumin flower honey	4	*Streptococci*, *E. coli* and *Micrococci*

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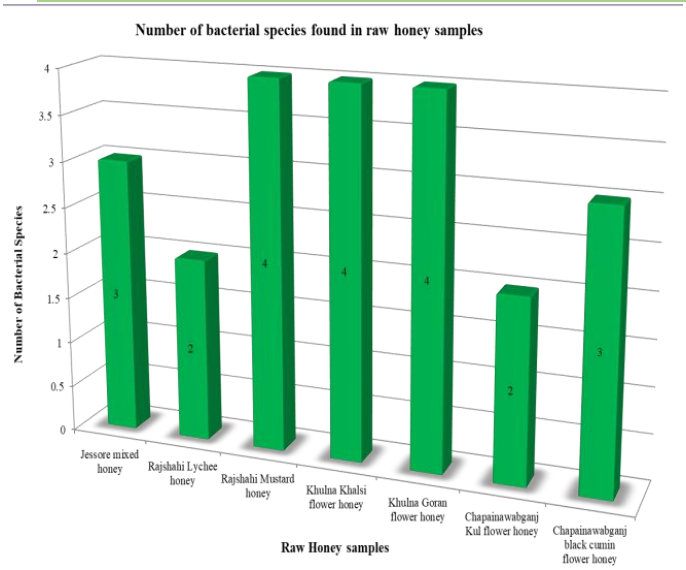
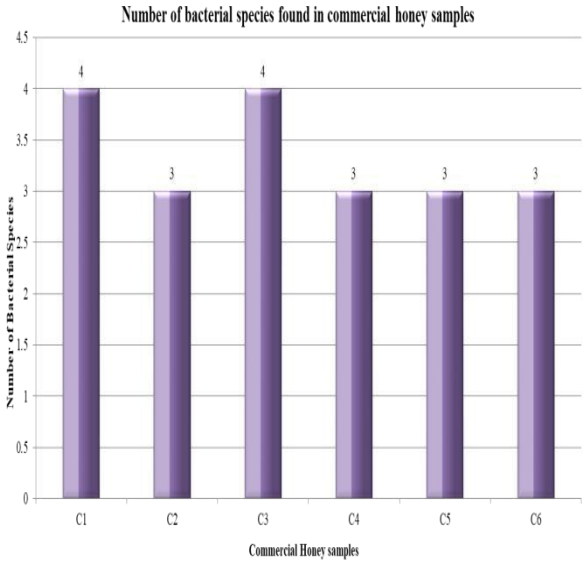
Table.2: Bacteria identified in commercial honeys

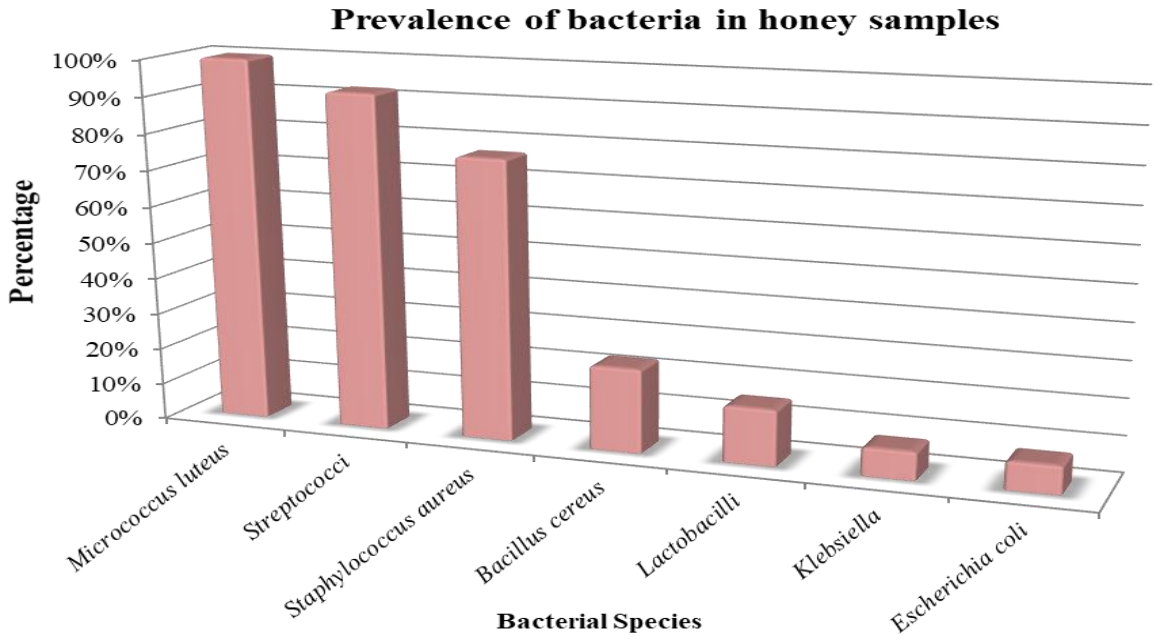
Product name	10^{-1} CFU/ml	Bacteria identified
C1	5	*Staphylococcus aureus*, *Streptococci*, *Bacilli* and *Micrococci*
C2	100	*Staphylococcus aureus*, *Streptococci* and *Micrococci*
C3	4	*Klebsiella*, *Staphylococcus aureus*, *Streptococci* and *Micrococci*
C4	79	*Staphylococcus aureus*, *Streptococci* and *Micrococci*
C5	17	*Staphylococcus aureus*, *Streptococci* and *Micrococci*
C6	>200	*Staphylococcus aureus*, *Streptococci* and *Micrococci*



(a)



(b)



(c)

Fig.2:(a)Number of bacterial species found in raw honeys. (b) Number of bacterial species found in commercial honeys.(c) Prevalence of different bacterial species in percentage (%).

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IV. DISCUSSION

Most of the studies conducted on honey had been focused on the antibacterial properties of honey. However, in two of the similar studies, the presence of *Bacilli, Staphylococcus aureus, Klebsiella and E.coli* were determined. But there was also the presence of *Pseudomonasaeruginosa*, *Shigella spp*. and *Enterobacter*[14,15].

Herein we showed, the bacteria isolated and identified in both raw and commercial honey; which were*Micrococcus luteus*(100%), *Streptococcus* (92%), *Staphylococcus aureus* (77%), *Bacillus* (23%), *Lactobacillus* (15%), *Klebsiella* (8%) *and Escherichia coli* (8%). It can be noticed that there are similarities between the species of bacteria which were found in all of the thirteen honey samples. The variety of species were found to be high in local raw honey samples rather than in commercial honeys. But the number of bacteria were found to be significantly high in the commercial honeys. Although the bacterial content of two of the commercial honeys were high in amount, as these honeys were found to be highly diluted.In a similar study conducted in Pakistan, it was shown that the quantity of colony forming unit was high; as found in this study[16]. In another research conducted by Amy K. Breslin et al. (2011), the local honeys were found to be more contaminated than the commercial honeys[17].Similarly, Snowdon and Cliver (1996) showed that different microbial species in honey may reach a concentration of some thousands colony forming unit (CFU) per gram [11]. It can be seen that the findings in these studies conducted in other countries support the existence of microorganisms in honey samples as done in our study.But surprisingly in a study conducted by Peter B. Olaitan et al. (2007), Yeast, *Streptomyces*, Mould, *Actinomycetes* were detected unlike the other researchers [5]. So if further researches are done with broader sample sources, there are possibilities that more different species might be found to be present in honeys. It is to be noted that 16s RNA sequencing could be done in future researches to identify the bacteria specifically.

The bacteria that were identified in these honeys have numerous good effects on human health. It was found that *Bacillus* species produces bacitracin, a peptide antibiotic that is effective against other gram-positive bacteria [18]. This bacitracin functions by inhibiting the cell wall. *Bacillus* also produces some other important antibiotics such as subtilin, surfactin and mycobacillin [18–20]. *Micrococcus luteus* produces an antibiotic called neoberninamycin which is effective against gram-positive and anaerobic bacteria [21].A species of *Streptococcus* called*Streptococcus thermophilus* was found to be beneficial for the patients with lactose intolerance disorder as this species produces the enzyme lactase[22]. This bacterium also produces antibiotic-like substances and bactericidal proteins called bacteriocins[22]. Also *Staphylococcus aureus* was found to show inhibitory effect on autoimmune inflammation of the central nervous system and prevent the development of clinical experimental autoimmune encephalomyelitis [23].

V. CONCLUSION

Though honey is widely used as a sweetener andit has good effects on health, it was necessary to determine the microbial quality of Bangladeshi honey. From this study it was found that commercial honeys and natural raw honey from flowers in different areas of Bangladesh contains various bacteria.These bacteria are known to produce some secondary metabolites which can be used as bioactive agents in ailment of various diseases.

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The Accessibility Level of Cigudeg Village as Prospective Capital City of West Bogor Regency

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Abstract**—** *This article contains a spatial review concerning the appointment of Cigudeg Village as a candidate of the capital city of Bogor Barat Regency. Bogor Barat Regency is one the regencies are being proposed to be a new regency in West Java Province. This research aimed to determine the feasibility of Cigudeg Village as the capital city candidate from the spatial accessibility point of view. The research begun by proposing other locations instead of Cigudeg Village as a comparison. The selection of village candidates was based on 3 scenarios: (1) all villages in Cigudeg District were deserved to be choosen as the capital city candidates; (2) all villages within the Bogor Barat Regency were deserved to be selected as the capital city candidates; (3) a center geographical location of Bogor Barat Regency was deserved to be included in the selection of the capital city of Bogor Barat Regency. The analysis of location-allocation weighted by the total population per district, was used to obtain the chosen village based on the above scenarios. The analysis resulted in three different chosen villages, that are Cigudeg (scenario 1), Leuwiliang (scenario 2) and Bayuresmi 2 (scenario 3). A comparative analysis then carried out to choose which village among those three that actually has a highest value of accessibility. Travel distance, travel time, and public transportation cost used a base of comparative analysis. The result of comparative analysis shows that Leuwiliang Village has the highest accessibility level, followed by Cigudeg and Bayuresmi 2 on the third place.*

Keywords**— Accessibility, Network Analyst, Cigudeg, Location-allocation, Google Maps, Grab.**

I. INTRODUCTION

The choice of geographical location of the central government is very important for regional development. The central government is expected to be able to encourage regional development, encourage the spread of development and increase equity in development [14]. The district capital with its function as the center of government administration is also closely related as a service center for the community. The government sector here must be able to predominantly provide services to the community.

West Bogor Regency is a candidate for a new autonomous region that proposed by the government of West Java Province as a fraction of Bogor Regency. The expansion of West Bogor Regency is a priority for West Java Province considering that the Bogor Regency region is currently too broad with a number of districts around 40 districts. Assistant District Government of Bogor said that a region can do maximum service if it has a population of around 3 million people. At present Bogor Regency has 5.7 million inhabitants and this makes the range of control of public services and government supervision increasingly heavy [15].

According to information from the Cigudeg District office, West Bogor Regency candidate has determined Cigudeg Village of Cigudeg District as a candidate for the West Bogor Regency government center [6]. Determination of Cigudeg Village of as a candidate for the capital was a result of a study conducted by a team of regional planning consultant in 2008 [24].

In the selection of the location of the district capital, it is necessary to consider the technical aspects, strategic aspects and administrative aspects [19]. From this administrative aspect, one thing that needs to be considered is that the chosen capital or location should has the smallest total physical distance so that it is easily accessible from all regions for smooth operation in government services. Similar considerations were also applied by the provincial government of The Eastern Cape, South Africa in 1994 [28]. Two of the 6 considerations used by The Eastern Cape Provincial Government are (a) Centrality, which considers the geographical location of the capital in relation to the provincial population and (b) the accessibility of the provincial capital in regard to geographical accessibility which includes communication and infrastructure connections.

A research concerning the multi-factor consideration in selection of a capital for a country [18] even reported that the consideration of the central geographical location (centroid), that put the capital city in the middle of the

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administrative area has encouraged a number of countries to move the capital city of their country. Italy chose the city of Rome on the basis of consideration of its central position to connect south and north. The French capital, Paris, is also a central city that connects various cultural regions in the country, such as the Rhine culture, Frank culture, Roman culture, and so on. Kazakhstan moved its capital from Alma-Ata located in the southwest corner of the country to Akmola in the centre. Nigeria moved its capital from Lagos in the western coastal region to downtown Abujia. Tanzania moved its capital from Dar es Salaam to Dodoma in the centre of the country, which is almost the same distance from all administrative districts in the country. Belgrade, which was once the capital of Yugoslavia, and which is currently the capital of the Republic of Serbia, is located in the central position of the Balkan Peninsula. From the description above it appears that the aspect of geographical location in relation to accessibility and the balance of the distance between the capital and all administrative centers at a lower level are factors that must be considered in the selection of the capital city or government centre. The choice of location based on the midpoint of the administrative (centroid) area basically also applies to the level of government below it, such as provinces, regencies and districts.

The Cigudeg District area, geographically, stretches from the centroid of West Bogor Regency to the north and west part of West Bogor Regency. Cigudeg Village itself is located in the western part of approximately 10 km from the centroid of West Bogor Regency. The location of Cigudeg Village, geographically, is suspected will create a wide disparity in distance between 14 districts within West Bogor Regency. To what extent this disparity occurs, it is necessary to conduct a spatial study related to travel distance, travel time and travel cost from each district to Cigudeg Village. In other word, it is necessary to evaluate spatially the accessibility level of Cigudeg Village.

There are several methods actually for measuring the accessibility level. However, for the case of measuring the accessibility level of Cigudeg Village, a comparative analysis by proposing other locations as a comparison is considered the most suitable. This method is expected to be able to answer whether Cigudeg Village is feasible to be the capital of West Bogor Regency or otherwise another village. The use of comparative analysis by proposing other villages as a comparison is considered also to be at the right moment, since the determination letter of Cigudeg Village as the capital of West Bogor Regency has not been issued.

This study is intended to conduct spatial based analysis with the aim to determine the accessibility level of Cigudeg Village as a candidate for government centre of West Bogor Regency.

II. EXPECTED BENEFITS

The comparative analysis in this case could end up with the determination of Cigudeg Village or another village as a village that has the highest level of accessibility. The result will at least provide a second opinion for the West Java provincial government in determining the most suitable village to be designated as the capital of the West Bogor Regency.

The use of network analyst combined with the Google Maps and online transportation services in this study is expected to enable motivate those who take part in the geospatial field to further develop this combination of applications as part of the development of science and knowledge, in particular the development of network analyst.

As a person who is engaged in the field of education and training, the results of this study are expected to be able to encourage coaches involved in GIS training, particularly in the network analyst training to do the same research but in different cases. The series of studies in different cases is expected to enrich the experience and improve the knowledge and skills of the coaches that can finally be transferred to the participants.

This research is also expected to provide benefits to the communities in saving travel time and costs, especially for people who have high mobility, and the business actors in goods and services distribution.

III. LITERATURE REVIEW

3.1 Accessibility and accessibility parameters

The concept of accessibility has been used in a number of scientific fields over the past few decades such as transportation planning, urban planning and geography, and plays an important role in policy making. Accessibility is generally used to refer to efforts, means, or modes, by which a goal can be achieved [4]. In general accessibility is defined as the ease (or difficulty) of services that can be achieved from a location [11]. Others define it as the ability to reach a place in relation to another place [25]. In this context, accessibility refers to the ease of achieving goals. The closer the origin and destination to the transportation system the higher the level of accessibility. The more variations in modes of transportation to travel from the place of origin to a particular destination, the better the accessibility. The less time and money spent on the trip, the more that can be achieved in a particular budget, the greater the accessibility [17]. Furthermore [25] said that accessibility determines equal access and opportunities, and

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accessibility related to geography is an important element in the mobility of people, goods, or information.

The component of accessibility can be identified from various aspects and practical values which are theoretically important in measuring accessibility [12]. These aspects are land use, transportation, time and individuals. Two main components of accessibility in transportation and geography are location and distance [25]. A more detailed explanation states [16], that accessibility in transportation systems includes components of distance, time taken and costs to reach each destination with different modes of transportation, while accessibility of location usually consists of two elements: (a) transportation elements (resistance or impedance) and (b) elements of activity (motivation or attraction or utility). Measurement of accessibility generally use impedance, time, or public transportation costs, and urban spatial distributions to produce numerical accessibility indexes for each location in the study area [16].

3.2 Travel distance parameter

The distance between two or more locations in the transportation system and geography can be interpreted as spatial distance. In general there are 3 methods of calculating spatial distance, that are: (a) Euclidean distances; (b) Vector-based road network and (c) Raster-based cost-weighted distances [27]. The distance of actual vector-based road networks [8] is considered as the best alternative and more in-line with human perceptions of access to open space. Actual travel distance based on road networks basically measures the distance between the parcel and the closest open space that is in demand [27]. Regarding to the measurement of spatial distance, geographic information systems (GIS) are the only technology that capable of conducting the spatial distance analysis. The Esri Network Analyst tools have been widely used by researchers or practitioners in vector-based road-network actual calculation. [10]. [35], [37], [1], [3], and [2] are 5 researchers of many researchers who have used Esri Network Analyst tools in their researchs.

Besides ESRI Network Analyst, several web/ online GIS applications such as Google Maps, Mapquest, Bing and Rand McNally also offer accurate driving directions in almost all locations in the world [8]. This web / online GIS application is even able to eliminate the cost of road network-based distance analysis caused by purchasing a software and procurement history of travel data as happens when using the Desktop Esri Network Analyst [8]. A comparative test on the distance between Google Maps and Network analyst showed that results were not significantly different [36].

3.3 Travel time parameter

Associated with the calculation of travel time, in the 1970s research mostly focused on understanding physical distance between points [22]. The focus on time perception begins later with the understanding that travel time turns out to be far more important than actual physical distance [23]. Furthermore, insofar as travel time by car and transit varies by time of day, this means accessibility also varies in and across days [20]. Travel time is broadly defined as the time necessary to traverse a route between any two points of interest. Travel time can be directly measured by traversing the route(s) that connects any two or more points of interest. Travel time is composed of running time, or time in which the mode of transport is in motion, and stopped delay time, or time in which the mode of transport is stopped (or moving sufficiently slow as to be stopped) [31]. The use of ArcGIS 10 software has been carried out in order to conduct travel time modeling through an approach: (a). Travel time by private car; (b) Travel time by public transportation; and (c) Door-to-door approach. Their modeling concluded that travel time calculations require a software with capabilities more than standard GIS software [26].

The estimation of actual travel time is also offered by Web- GIS based applications, such as Google Maps [8]. A research aimed to get an estimation of the O-D trip time matrix with the Google Maps approach, found at least 4 advantages of the Google Maps API compared to the ArcGIS Network Analyst approach. These advantages are: (a) no need to prepare a network dataset; (b) use more updated road data; (c) take into account road congestion and (d) consider the difference between rush hour and non-busy hours [34]. The use of Google Maps based data for analysis of travel time is also carried out in the city of Kaunas [9]. In this analysis they concluded that Google Maps provides a variety of data about car travel times that can be accessed through the Google Matrix Distance. With these data they were able to make an average travel time matrix that can be used as a skim matrix to validate the macro model of the city of Kaunas.

3.4 Public transportation cost parameter

Accessibility is basically the cost of travel between the place of origin and destination [11]. A research about applying time-varying travel-time costs for emergency response vehicle routing in Davis County, Utah, concluded that the latest technological developments with electronic tariff payment systems and the use of GPS devices created favorable conditions for implementing a distance-based tariff structure [35]. This model was explicitly able to track passenger routes so that allowing to accurately calculate the distance-based rates. The distance-based tariff calculation system is now the basis

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for determining travel fares or fees from online-based public transportation services such as Grab, Go-jek, and Uber [32].

Online transportation is one of the latest service innovations in m-commerce. Online transportation services or travel sharing are individual transportation services where customers can book trips (cars, motorbikes, etc.) through cellular applications and drivers can respond to orders through applications [33]. This service provides several benefits such as the driver and the customer can find out the location of each other accurately, customers can see driver and vehicle information, and customers can easily find transportation to go to another place (time efficiency). The transportation services combined with the sophistication of internet technology made it easier for people to place an order, know the rate of transportation costs, location of destination and driver's identity [5]. This benefit makes travel sharing increasingly popular among urban communities.

Online transportation services or sharing trips are now a common means for people to fulfill their travel needs [29]., The popular online transportation services for Indonesia currently are Go-Jek and Grab which hold the largest market share and compete each other. The popularity of Grab and Go-Jek as an online means of transportation implicitly shows that the rates offered by these two transportation services can be considered as representing the rates of travel by public transportation. On the basis of the above arguments, the accessibility measurement of prospective capital of West Bogor Regency on the parameter of public transportation cost, will likely use the estimated travel fares provided by Grab application.

3.5 Network Analyst

The Network Analyst application is GIS software that utilizes topology point and line features that are packaged in network data sets. Network analyst has 6 functions of analysis, namely: (a) New Route to calculate the best travel route according to distance and travel time from the point of origin to the destination point; (b) New Service Area to calculate the service range of service centers based on distance or travel time; (c) New Closest Facility to calculate the nearest service center from the incident; (d) New O - D matrix to calculate distance or travel time from the origin to various destination points; (e) New Vehicle Routing Problem to calculate the best travel routes in serving a number of consumers; (f) New Location-Allocation to select service centers from a number of proposed service centers [10].

IV. RESEARCH METHODS

4.1 Research Approach

This study uses a spatial approach by utilizing geospatial data and Geographic Information System (SIG) technology - Network Analyst, assisted with Google Maps application, and the online based transportation service application (Grab).

4.2 Research Sites

The research area is the administrative area of the West Bogor Regency candidate with the coverage area as shown in Fig. 1. The prospective West Bogor Regency is geographically located between 106º24'0 "E to 106º46'0"E and between 6º18'0"S to 6º46'0"S. This prospective regency is bordered by Bogor City and Bogor Regency on the East side, Sukabumi Regency on the South side, Lebak Regency on the West side, and Tanggerang Regency and Tangerang Selatan City on the North side. It has an area of approximately 131,761.94 ha with a population in 2017 of around 1,514,577 people [7].

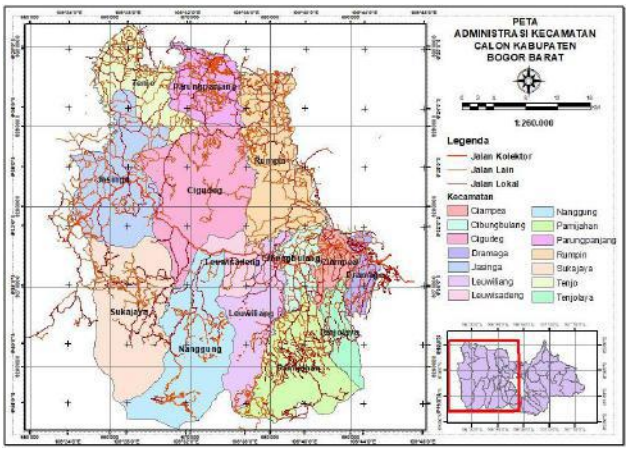

Fig 1. Map of prospective West Bogor Regency

The West Bogor Regency candidate is planned to consist of 14 districts and 166 villages. The 14 districts are Leuwiliang, Ciampea, Cibungbulang, Pamijahan, Rumpin, Jasinga, Parung Panjang, Nanggung, Cigudeg, Tenjo, Dramaga, Sukajaya, Leuwisadeng and Tenjolaya Districts. Cigudeg district itself as a candidate for the central government consists of 15 villages, namely: Argapura, Bangunjaya, Banyuasih, Banyuresmi, Banyuwangi, Batujajar, Cintamanik, Mekarjaya, Rengasjajar, Sukamaju, Sukaraksa, Tegarlega, Wargajaya and Cigudeg villages [6]. All villages and districts are connected to one another through road infrastructure which is dominated by other roads and local roads. The only collector road that crosses the West Bogor Regency candidate is a road that connects Bogor City with Rangkasbitung, Lebak Regency. Henceforth, the prospective West Bogor Regency will be referred to as West Bogor Regency.

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4.3 Analysis Method

This research was conducted in stages using 3 (three) applications, that were Network Analyst, Google Maps and Grab. The first stage was to determine the selected village, the second stage was to calculate the distance to the selected village. The third stage estimated the travel time to the selected village, the fourth stage estimated the cost of public transportation to the selected village, and the final stage determined the level of accessibility among the selected villages.

4.3.1 Determination of Village candidates for the capital city.

Determination of the village candidates for West Bogor Regency capital city here was done by using the analysis function of new location-allocation. In this analysis of the new location-allocation, the centroid of the village acts as a service center (facility) or as the capital of regency, while the centroid of the district acts as a party that requires service (demand). In accordance with the purpose of the study, which is conducting a comparative review, the analysis of the new location-allocation determines the village capital candidates based on 3 scenarios, namely: (1) Cigudeg District as a candidate for the West Bogor Regency capital, and all villages in the Cigudeg District have the opportunity to become the location of prospective capital cities; (2) All villages within the West Bogor Regency administrative area basically have the opportunity to be chosen as the location of prospective capital cities; (3) The geographical centroid location of the West Bogor Regency has the opportunity to be chosen as the location of the prospective capital city.

The first scenario is basically to ensure that spatially the Cigudeg Village is indeed chosen among 15 villages in the Cigudeg District area. Scenarios 2 and 3 are intended to get selected villages that will be used as a comparison for the first scenario.

The data used were the Indonesian topographic digital map scale of 1:25.000 downloaded from http://tanahair.indonesia.go.id/portal-web/download/per region [13]. The layers used as analysis material were: (a) Road layer, (b) Administrative layer, and (c) DEM (digital elevation model). While data on population per district was downloaded from https://bogorkab.bps.go.id/statictable / 2017 / [7].

The following was the sequence of analysis stages for the determination of village candidates for the capital city of West Bogor Regency:

(a) Creating the village centroid layer, the district centroid layer and the West Bogor Regency centroid layer. For the district centroid layer, the attribute table was completed with data of the population per district. Data of the population per district was used as a weight in the new location-allocation analysis. Fig 2. below explains the flow of making a centroid layer.

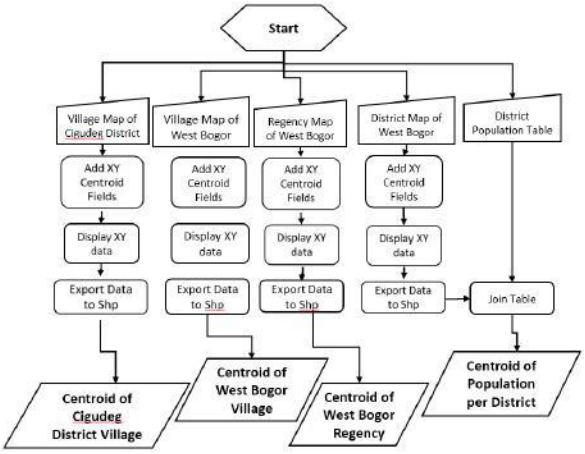

Fig 2. Flow map for making a centroid (midpoint)

(b) Setting up a network dataset. Since network analyst only works on network data, then compiling network data sets is very necessary. Setting up a network dataset conducted in a Personal Geodatabase of ArcGIS 10.2.1 using Road layer and Turn layer as input. Fig. 3. below is the flow of setting up a network dataset.

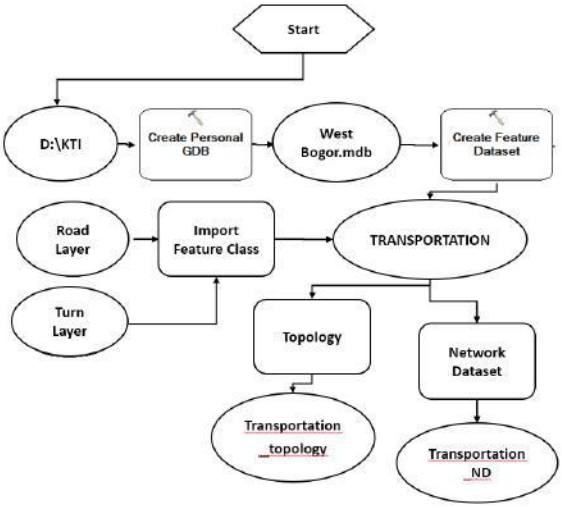

Fig. 3. Flow of setting up network datasets

(c) New location-allocation analysis to determine selected villages. This analysis used impedance settings: Distance (meters), Travel from: Demand to Facility; U-turn: allowed; Output type: straight line; Restriction: Turns; Problem Type: Maximum Attendance (maximum visit); Facility to Choose: 1; Impedance Cutoff: 40000 meters; and impediment transformation: Linear. Fig.4. below explains the analysis stage of New location-allocation.

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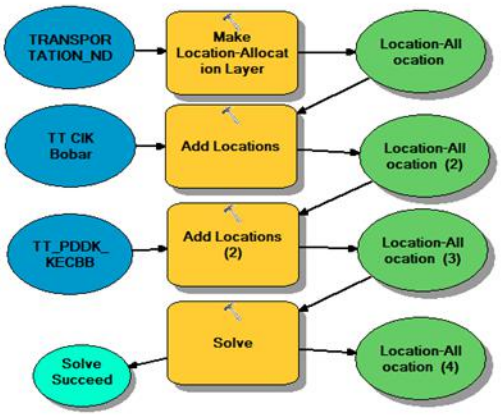

Fig.4. Model builder of analysis function new location-allocation

4.3.2 Calculation of the distance to the selected villages.

Calculation of the distance to the selected village was carried out using the analysis function of the New closest facilities. This function of analysis was aimed to obtain the shortest travel route between the districts to the selected village. This analysis will produce a map that describes the road segments that are passed and the mileage tables from 14 districts to 3 selected villages. Fig. 5. explains the flow of analysis of the New closest facility.

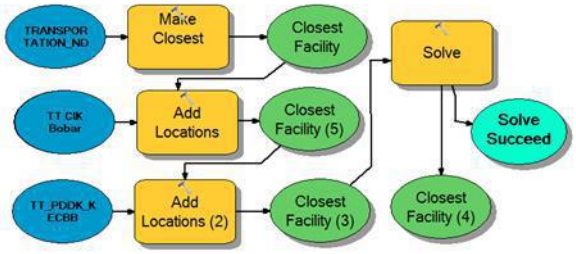

Fig.5. Model builder of analysis function for new closest facility.

4.3.3 Estimation of travel time to the selected villages.

The estimation of travel time from each district to the selected villages was carried out with the help of the Google Maps application. Google Maps was used to calculate the travel time of travel routes from the centroid of each district to the three selected villages resulted from of the New closest facility analysis.

The following were the stages of work to get estimated travel time from each district to the 3 selected villages:

(a) Inserting 17 centroid coordinates into the Google Maps application manually. 3 centroid points are the selected village centroids. The other 14 centroid points are the midpoint of the district.

(b) Recording of travel time from 14 districts to 3 selected villages through the Google Maps application. Recording of travel time carried out for 7 days during rush hour (06.00 – 09.00 AM and 04.00 – 07.00 PM) and normal hour. Fig.6. below describes the stage of entering the centroid coordinates of 14 districts and 3 selected villages into the Google Maps application and recording travel time data and public transportation costs.

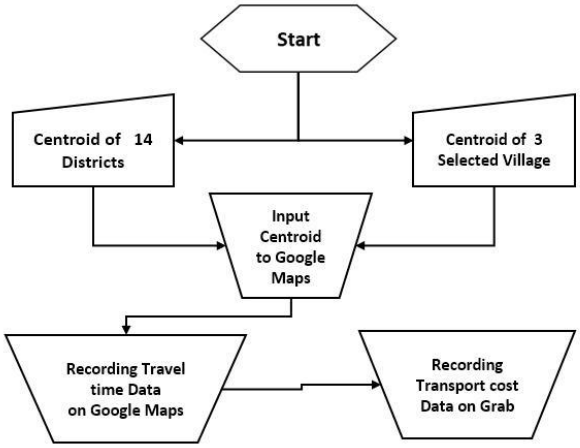

Fig. 6. Flow of recording travel time estimation and public transportation cost from 14 districts to 3 selected villages

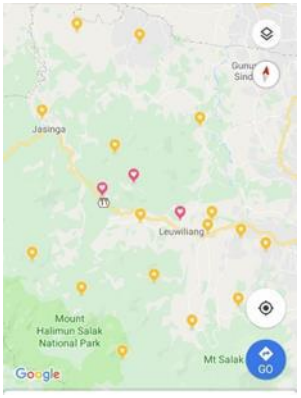

Fig 6. 17 Centroid coordinates on Google Maps application

4.3.4 Estimation of public transportation cost to the selected villages.

Calculation of public transportation cost was done with the help of the Grab application. Grab application in this case was used to calculate the cost of public transportation from each district centroid to the three selected villages vice versa. Recording of public transportation cost was carried out for 7 days during peak (rush) hour (06.00 – 09.00 AM and 04.00 – 07.00 PM) and normal hour. Recording of public transportation cost was carried out at the same time as recording travel time.

The analysis of travel time and the cost of public transportation is intended to get the average travel time

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estimation and transportation cost from each district to the three selected villages. Calculations were carried out using a simple statistical formula.

$$\bar{x} = \frac{x_1 + x_2 + x_3 + \ldots\ldots + x_n}{n}$$

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_1 \quad (1)$$

Where:
X = average value
Xn = recording data
n = number of samples

4.3.5 Accessibility analysis

Accessibility analysis is aimed to determine which village among these 3 selected villages that has the highest level of accessibility. The simple statistical comparative analysis was adopted to compare the accessibility levels to the 3 chosen villages. The parameters used as a comparison are (1) distance, (2) travel time and (3) public transportation costs. Determination of the accessibility level refers to the basic principal of accessibility, introduced by Liu and Zhu, (2004) who said that the closer the origin and destination to the transportation system the higher the level of accessibility. The less time and money spent on the trip, the greater the accessibility. Determination also refer to Ilhami (1990) in Mahathir (2017) who stated that the capital or selected location should has the smallest total physical distance so that it is easily accessible from all regions for smooth operation in government services

V. RESULTS

5.1 Scenario 1

In the first scenario, where 15 villages in the Cigudeg District are involved as candidates, the analysis of the new location-allocation defines Cigudeg Village as a chosen candidate (Fig. 7) With the choice of Cigudeg Village as the capital city, as it shown in Table 1. the local government must provide public services with a service distance of up to 29,03 km. The statistical calculation as shown on the right side of Table 1. shows that the range of local government services reached a total of 296, 79 km with an average service range of 21,2 km. The minimum service range is experienced by Leuwisadeng District with a distance of only 8,14 km.

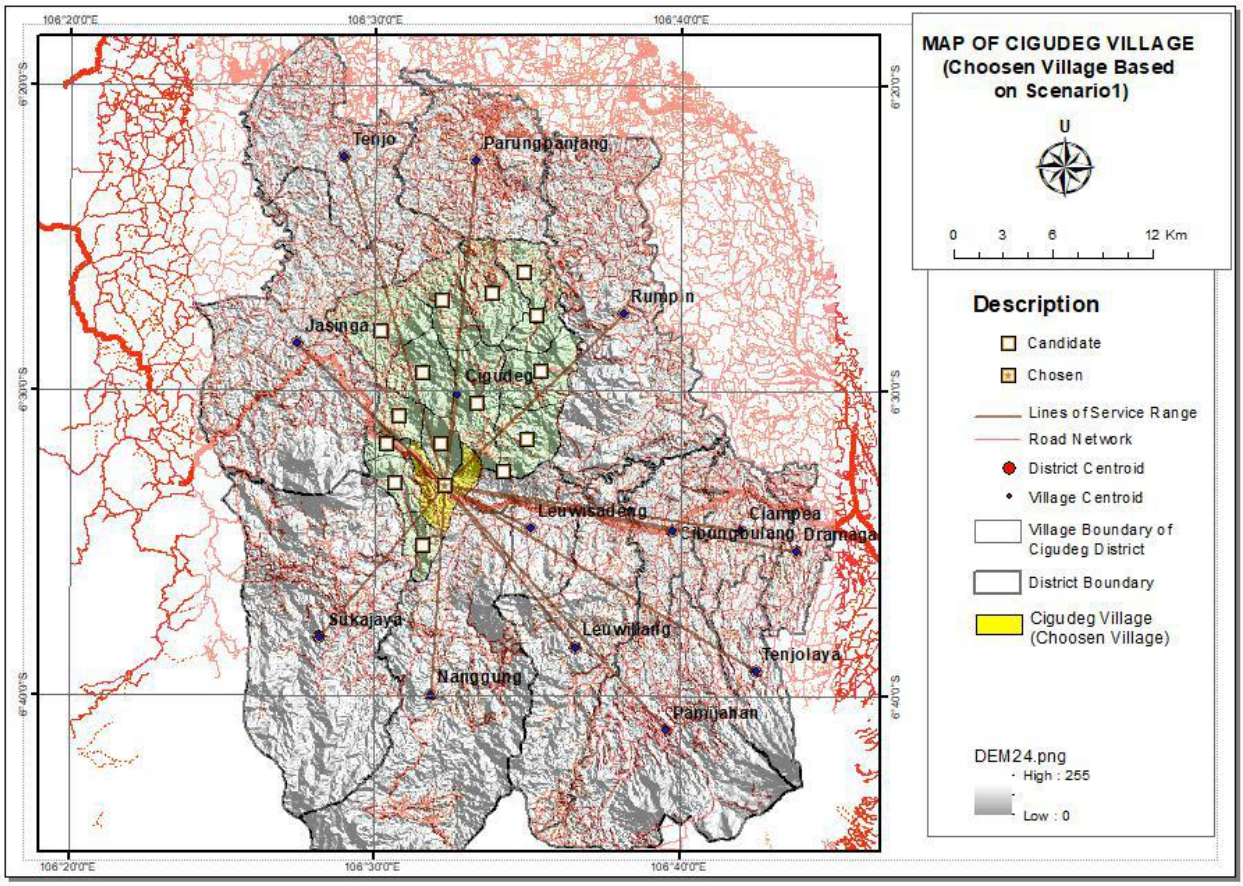

Fig. 7. Results of new location-allocation analysis based on scenario 1

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Table.1. Range of Cigudeg Village Services as Capital City Candidates

Location_Allocation_Desa_Cigudeg

FID	Weight	District - Village	Distance from Village
0	0,44	Ciampea - Cigudeg	22,5 km
1	0,59	Cibungbulang - Cigudeg	16,21 km
2	0,72	Cigudeg - Cigudeg	11,15 km
3	0,28	Dramaga - Cigudeg	28,83 km
4	0,62	Jasinga - Cigudeg	15,21 km
5	0,38	Leuwiliang - Cigudeg	24,68 km
6	0,8	Leuwisadeng - Cigudeg	8,14 km
7	0,59	Nanggung - Cigudeg	16,46 km
8	0,29	Pamijahan - Cigudeg	28,59 km
9	0,32	Parungpanjang - Cigudeg	27,14 km
10	0,37	Rumpin - Cigudeg	25,26 km
11	0,58	Sukajaya - Cigudeg	16,85 km
12	0,33	Tenjo - Cigudeg	26,75 km
13	0,27	Tenjolaya - Cigudeg	29,03 km

Statistics of Location_Allocatic
Field: Distance from Village
Statistics:
Count: 14
Minimum: 8,14
Maximum: 29,030001
Sum: 296,799999
Mean: 21,2
Standard Deviation: 6,783617
Nulls: 0

5.1.1 Mileage

From the new closet facility analysis by determining the village of Cigudeg as the chosen village, the 14 shortest travel routes were obtained, that are the route from 14 districts to Cigudeg Village. The detailed results of the new closest facility can be seen visually in Fig. 8, where there are 14 blue lines that describe in detail the road segments passed by each route. Based on the analysis of the new closest facility, the 14 travel routes from each district to Cigudeg Village produce route distance as shown in Table 3.

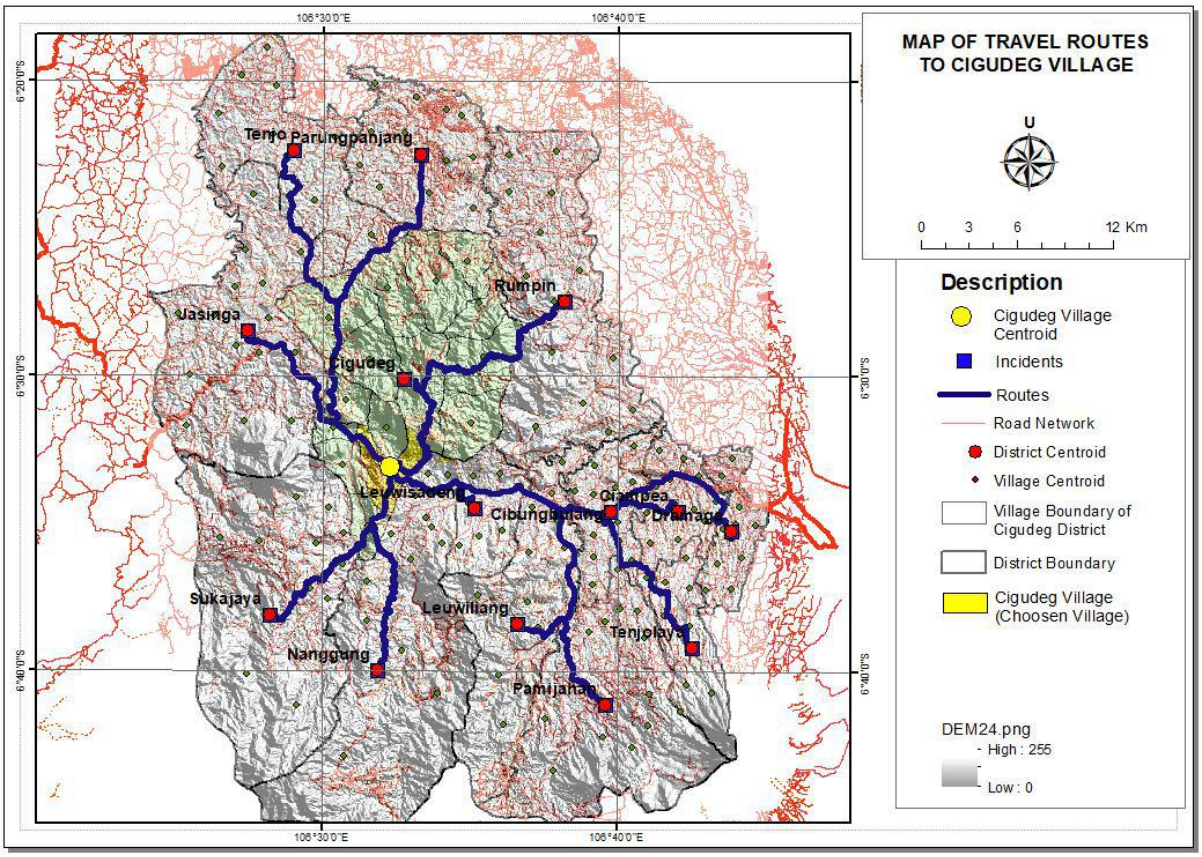

Fig. 8. Results of new closest facility analysis based on scenario 1

Table 2. Average Mileage of Each District Towards Cigudeg Village

Travel Routes to Cigudeg Village

FID	Travel Route	Travel Distance
6	Leuwisadeng - Cigudeg	8,14 km
2	Cigudeg - Cigudeg	11,15 km
4	Jasinga - Cigudeg	15,21 km
1	Cibungbulang - Cigudeg	16,21 km
7	Nanggung - Cigudeg	16,46 km
11	Sukajaya - Cigudeg	16,85 km
0	Ciampea - Cigudeg	22,5 km
5	Leuwiliang - Cigudeg	24,68 km
10	Rumpin - Cigudeg	25,26 km
12	Tenjo - Cigudeg	26,75 km
9	Parungpanjang - Cigudeg	27,14 km
8	Pamijahan - Cigudeg	28,59 km
3	Dramaga - Cigudeg	28,83 km
13	Tenjolaya - Cigudeg	29,03 km

Statistics of Rute_ke_Desa_Cigudeg
Field: Travel Distance
Statistics:
Count: 14
Minimum: 8,14
Maximum: 29,030001
Sum: 296,799999
Mean: 21,2
Standard Deviation: 6,783617
Nulls: 0

The statistical calculation on the right side of Table 2. shows that the longest route is 29,03 km, that is the Tenjolaya District – Cigudeg Village route. The shortest route to Cigudeg Village is experienced by Leuwisadeng District with a distance of 8,14 km. The total number of routes from 14 districts to Cigudeg Village reaches 296.79 km or an average of 21,2 km.

5.1.2 Travel time estimation

From the results of observations for 7 days on the travel time estimation from each district to Cigudeg Village, it shows that each route requires a daily average travel time as described in Table 3.

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The Table 3. describes that difference in travel time during rush hour with normal hours for each route ranges from 1 to - 4 minutes, with an average for all routes around – 1,52 minutes. This this actually indicates that the speed of vehicles during the normal hour tends to be slower.

Table 3. The Average Travel Time From 14 Districts to Cigudeg Village

No	District	Average Travel Time (Minutes)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	71,57	72,86	-1,29	72,21
2	Cibungbulang	39,00	41,43	-2,43	40,21
3	Ciampea	52,14	55,00	-2,86	53,57
4	Leuwiliang	50,43	53,00	-2,57	51,71
5	Lewisadeng	27,14	26,14	1,00	26,64
6	Pamijahan	67,43	69,29	-1,86	68,36
7	Rumpin	66,71	69,00	-2,29	67,86
8	Jasinga	28,00	29,14	-1,14	28,57
9	Parung Panjang	53,14	55,57	-2,43	54,36
10	Nanggung	58,14	58,14	0,00	58,14
11	Cigudeg	29,29	28,57	0,71	28,93
12	Tenjo	71,43	72,57	-1,14	72,00
13	Sukajaya	62,29	63,14	-0,86	62,71
14	Tenjolaya	71,86	76,00	-4,14	73,93
Total		748,57	769,86	-21,29	759,21
Average		53,47	54,99	-1,52	54,23

For this small difference, the rush hour and normal hour travel times can be considered to be no difference, and the average daily travel time can be used as a benchmark for travel time estimation. The total amount of daily travel time to Cigudeg village reaches 759,21 minutes or an average of 54,2 minutes.

5.1.3 Public transportation cost estimation.

Based on observations for 7 days on public transportation costs using 2-wheeled and 4-wheeled vehicles, passengers who travel from the district to Cigudeg Village vice versa, must issue transportation costs as shown in Tables 4 and 5.

Table 4. The Average 2 Wheeled Transport Costs from each District to Cigudeg Village Vice Versa

No	District	Average GrabBike Transportation Fee (IDR x1000)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	90,71	85,00	5,71	87,86
2	Cibungbulang	46,14	42,57	3,57	44,36
3	Ciampea	68,00	63,43	4,57	65,71
4	Leuwiliang	58,43	58,29	0,14	58,36
5	Lewisadeng	23,43	23,43	0,00	23,43
6	Pamijahan	92,29	86,43	5,86	89,36
7	Rumpin	93,86	87,00	6,86	90,43
8	Jasinga	36,14	35,86	0,29	36,00
9	Parung Panjang	86,29	80,29	6,00	83,29
10	Nanggung	51,43	51,14	0,29	51,29
11	Cigudeg	22,14	20,71	1,43	21,43
12	Tenjo	100,43	94,43	6,00	97,43
13	Sukajaya	55,14	53,57	1,57	54,36
14	Tenjolaya	100,00	93,86	6,14	96,93
Total		924,43	876,00	48,43	900,21
Average		66,03	62,57		64,30

Table 5. The Average 4 Wheeled Transport Costs from each District to Cigudeg Village Vice Versa.

No	District	Average GrabCar Transportation Fee (IDR x1000)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	129,43	124,86	4,57	127,14
2	Cibungbulang	82,43	75,71	6,71	79,07
3	Ciampea	105,43	101,86	3,57	103,64
4	Leuwiliang	89,00	85,57	3,43	87,29
5	Lewisadeng	43,14	42,00	1,14	42,57
6	Pamijahan	128,43	122,86	5,57	125,64
7	Rumpin	132,71	131,43	1,29	132,07
8	Jasinga	59,14	57,00	2,14	58,07
9	Parung Panjang	121,86	110,29	11,57	116,07
10	Nanggung	77,43	75,43	2,00	76,43
11	Cigudeg	43,86	43,14	0,71	43,50
12	Tenjo	124,00	123,00	1,00	123,50
13	Sukajaya	78,43	73,57	4,86	76,00
14	Tenjolaya	136,29	131,86	4,43	134,07
Total		1351,57	1298,57	53,00	1325,07
Average		96,54	92,76	3,79	94,65

From the two tables above, it can be seen that there are differences in transportation costs during rush hour and normal hours. This difference has become the provision of Grab as the manager of online transportation where there is a difference in tariffs of IDR 5000 between rush hour and normal hours which apply to both GrabBike and GrabCar. If the transportation cost of each of these sub-districts are summed and then the average value is taken, the transportation cost to and from Cigudeg Village is around of IDR 64.300 for 2 wheeled vehicle and IDR. 94.650 for 4 wheeled vehicle.

5.2 Scenario 2

The new location-allocation analysis in scenario 2, where 166 villages plus the centroid of The West Bogor Regency were included as candidates, determines that the village of Leuwiliang deserved to be selected as a village of capital city of The West Bogor Regency. The location of the Leuwiliang Village can be seen in Fig. 9.

With the determination of Leuwiliang Village as a candidate for the government center of The West Bogor Regency, the local government will has a range of services as far as 38,94 km (Table 6). below describes the range of services from Leuwiliang Village as a candidate for the center of government

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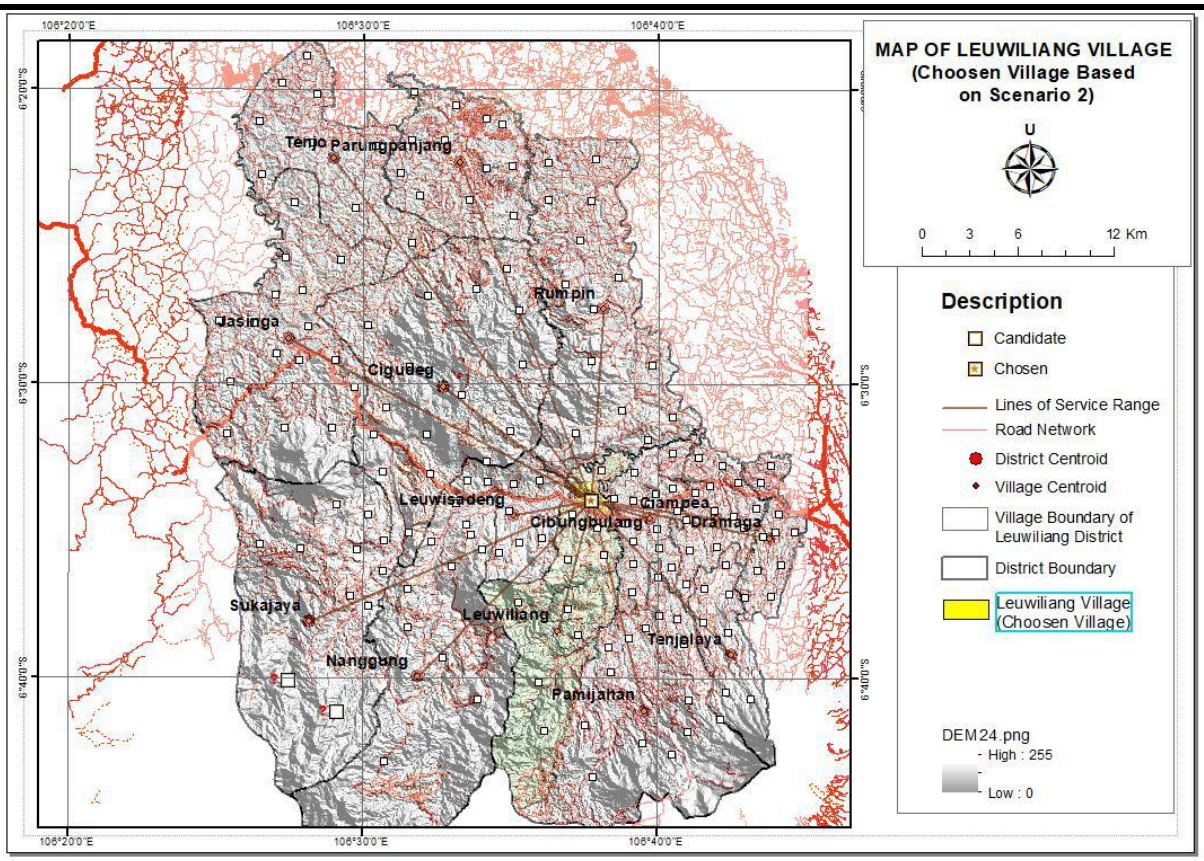

Fig. 9. Results of new location-allocation analysis based on scenario 2

Table 6. Range of Leuwiliang Village Services as a Capital City Candidate

Location_Allocation_Desa_Leuwiliang

FID	District - Village	Weight	Distance from Village
1	Cibungbulang - Leuwiliang	118193,38	4,68 km
6	Leuwisadeng - Leuwiliang	62076,21	6,75 km
0	Ciampea - Leuwiliang	116500,28	10,96 km
5	Leuwiliang - Leuwiliang	81197,26	13,45 km
2	Cigudeg - Leuwiliang	71862,92	17,18 km
3	Dramaga - Leuwiliang	63071,9	17,3 km
8	Pamijahan - Leuwiliang	80324,54	17,36 km
13	Tenjolaya - Leuwiliang	33234,3	17,49 km
10	Rumpin - Leuwiliang	78406,01	17,67 km
7	Nanggung - Leuwiliang	37057,37	23,01 km
11	Sukajaya - Leuwiliang	20323,05	25,94 km
4	Jasinga - Leuwiliang	30441,62	27,4 km
9	Parungpanjang - Leuwiliang	15782,79	35,19 km
12	Tenjo - Leuwiliang	1904,69	38,94 km

Statistics of Location_Allocatio
Field: Distance from Village
Statistics:
Count: 14
Minimum: 4,68
Maximum: 38,94
Sum: 273,32
Mean: 19,522857
Standard Deviation: 9,464996
Nulls: 0

Based on Table 6. Parungpanjang District dan Tenjo District located at the distance as far as more then 35 km. it means that the service range of Leuwiliang Village is further than Cigudeg Village which is less than 30 km away. However, when viewed from the total number of services, the total service range of Leuwiliang Village is 273.32 km. This total is lower than the total range of cigudeg village services of 296,79 km. The average service range of Leuwiliang Village is around 19,52 km which is lower than Cigudeg Village that has average of 21,2 km.

5.2.1 Mileage

Analysis of the New closest facility by setting Leuwiliang Village, as a service center produces 14 travel routes as illustrated in Fig. 10. The blue line illustrates the shortest route from 14 districts to Leuwiliang Village vice versa. Based on the analysis of the new closest facility, the 14 travel routes have the average mileage as described in Table 7. below.

Table 7. Average Mileage of Each District Towards Leuwiliang Village

Travel Routes to Leuwiliang Village

FID	Travel Routes	Travel Distance
1	Cibungbulang - Leuwiliang	4,68 km
6	Leuwisadeng - Leuwiliang	6,75 km
0	Ciampea - Leuwiliang	10,96 km
5	Leuwiliang - Leuwiliang	13,45 km
2	Cigudeg - Leuwiliang	17,18 km
3	Dramaga - Leuwiliang	17,3 km
8	Pamijahan - Leuwiliang	17,36 km
13	Tenjolaya - Leuwiliang	17,49 km
10	Rumpin - Leuwiliang	17,67 km
7	Nanggung - Leuwiliang	23,01 km
11	Sukajaya - Leuwiliang	25,94 km
4	Jasinga - Leuwiliang	27,4 km
9	Parungpanjang - Leuwiliang	35,19 km
12	Tenjo - Leuwiliang	38,94 km

Statistics of Rute_perjalanan_Ke
Field: Travel Distance
Statistics:
Count: 14
Minimum: 4,68
Maximum: 38,94
Sum: 273,32
Mean: 19,522857
Standard Deviation: 9,464996
Nulls: 0

Statistical calculation on the shortest route from 14 districts to Leuwiliang Village as shown on the side of Table 7. shows that the shortest route is the route of Cibungbulang District - Leuwiliang Village as far as 4,8 km. The longest route is the route of Tenjo District - Leuwiliang Village, that is 38,94 km away. The total number of travel routes is 273.32 km. The average route for all districts to Cigudeg Village is 19,52 km.

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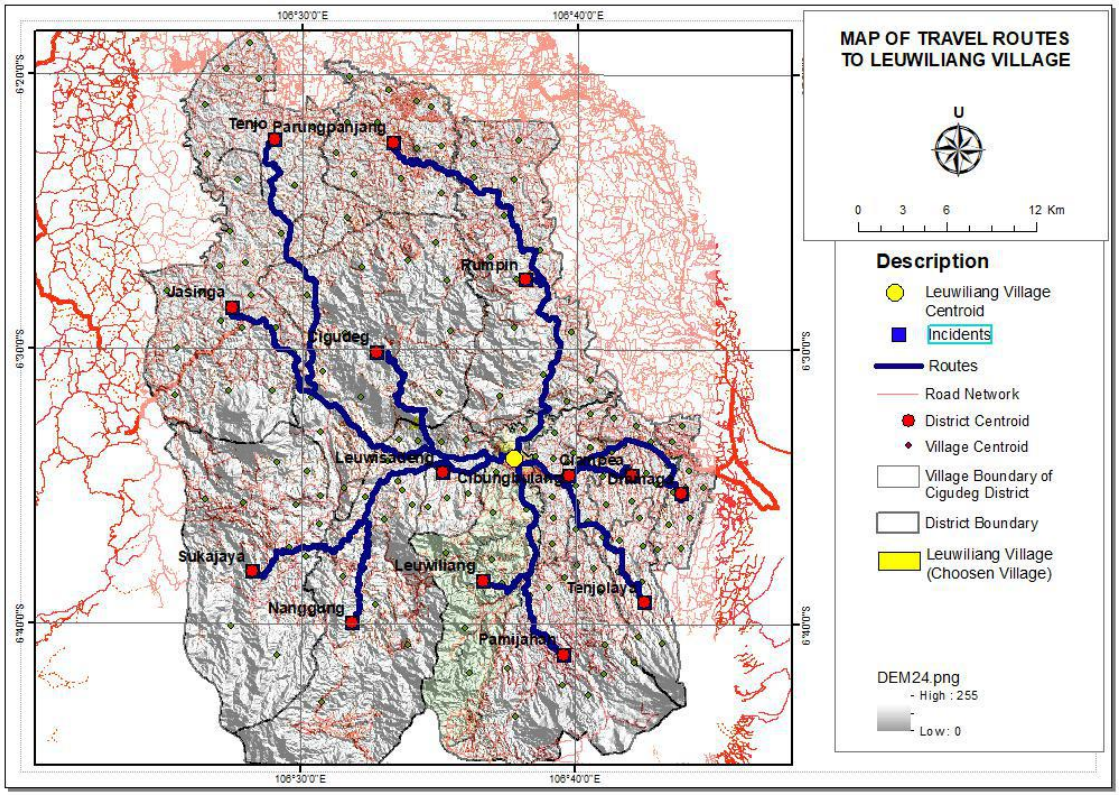

Fig. 10. Results of new closest facility analysis based on scenario 2

5.2.2 Travel time estimation

The following Table 8. contains the recapitulation of travel time observations on Google Maps from 14 districts to Leuwiliang Village vice versa. It shows on the Table 8. that the difference travel time between rush hour and nomal hour for each route is between 0,29 minute up to -3,57. The average travel time difference for all route is – 1,47 minutes. The minus symbol in travel time difference indicates that during normal hour, the average speed of vehicle towards the Leuwiliang Village of tends to be slower. The time difference of only 1,47 minutes in this study can be considered insignificant so that the average daily travel time per route can be used as a benchmark for time estimation.

Table 8. Average Travel Time From 14 Districts to Leuwiliang Village Based on Google Maps

No	District	Average Travel Time (Minutes)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	47,86	49,14	-1,29	48,50
2	Cibungbulang	17,57	19,57	-2,00	18,57
3	Ciampea	29,86	33,43	-3,57	31,64
4	Leuwiliang	31,86	32,86	-1,00	32,36
5	Lewisadeng	18,86	18,57	0,29	18,71
6	Pamijahan	45,86	46,86	-1,00	46,36
7	Rumpin	43,14	44,00	-0,86	43,57
8	Jasinga	49,71	50,86	-1,14	50,29
9	Parung Panjang	77,86	78,86	-1,00	78,36
10	Nanggung	68,86	70,00	-1,14	69,43
11	Cigudeg	47,00	48,00	-1,00	47,50
12	Tenjo	91,57	94,00	-2,43	92,79
13	Sukajaya	80,29	82,29	-2,00	81,29
14	Tenjolaya	51,71	54,14	-2,43	52,93
Total		702,00	722,57	-20,57	712,29
Average		50,14	51,61	-1,47	50,88

In refer to the Table 8. the fastest travel time occurs on the Cibungbulang District - Leuwiliang Village route with an average travel time of 18,57 minutes for the distance of 4,68 km. The second is the Leuwisadeng District - Leuwiliang Village route with a travel time of 18,57 minutes for the distance of 6,75 km. The longest travel time occurs on the route of Tenjo District - Leuwiliang Village with an average travel time of 92,79 minutes for the distance of 38,94 km. The second longest travel time occurred on the route of Sukajaya District - Leuwiliang Village with a travel time of 81,29 minutes for the distance of 25,94 km. The sum of the travel time of the above 14 routes produces the average travel time to Leuwiliang Village vice versa by 50,88 minutes.

5.2.3 Public transportation cost estimation

The Public transportation cost that must be borne by passengers who travel from 14 districts to Leuwiliang Village vice versa can be seen in Table 9 and Table 10.

Table 9. describes in detail the transportation cost from each district to Leuwiliang Village using 2-wheeled vehicle. The table shows that the route of Cibungbulang District - Leuwiliang Village and Leuwisadeng District - Leuwiliang Village route only requires a fee of IDR 12.000 and IDR 13.290 with a difference in tariff between rush hour and normal hour less than IDR 1000. On the other hand, the Tenjo District - Leuwiliang Village route need the transportation cost of IDR 134.290 followed by 2 other routes, namely Parung Panjang District -

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Leuwiliang Village with IDR. 90.360 and Sukajaya District - Leuwiliang Village with IDR 94.290.

Table 9. The Average 2 Wheeled Transportation Cost from each District to Leuwiliang Village Vice Versa

No	District	Average GrabBike Transportation Fee (IDR x1000)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	40,14	38,00	2,14	39,07
2	Cibungbulang	12,29	11,71	0,57	12,00
3	Ciampea	22,14	25,00	-2,86	23,57
4	Leuwiliang	23,29	22,29	1,00	22,79
5	Lewisadeng	13,43	13,14	0,29	13,29
6	Pamijahan	45,29	44,14	1,14	44,71
7	Rumpin	46,71	43,14	3,57	44,93
8	Jasinga	81,00	75,43	5,57	78,21
9	Parung Panjang	93,14	87,57	5,57	90,36
10	Nanggung	77,00	70,57	6,43	73,79
11	Cigudeg	54,57	51,57	3,00	53,07
12	Tenjo	136,57	132,00	4,57	134,29
13	Sukajaya	99,14	89,43	9,71	94,29
14	Tenjolaya	49,71	47,86	1,86	48,79
Total		794,43	751,86	42,57	773,14
Average		56,74	53,70	3,04	55,22

The sum of all these routes results in an average transportation cost headed to Leuwiliang Village vice versa of IDR 55.220 with the difference in tariffs between rush hour and normal hour of IDR 3.040. This tariff difference is below the standard set by Grab which is IDR. 5000.

The following Table 10. describes the transportation cost that must be borne by passengers who traveling from the districts to Leuwiliang Village vice versa using the 4 wheeled vehicle.

Table 10. The 4Wheeled Transportation Cost from each District to Leuwiliang Village Vice Versa.

No	District	Average GrabCar Transportation Fee (IDR x1000)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	78,57	72,14	6,43	75,36
2	Cibungbulang	25,43	23,29	2,14	24,36
3	Ciampea	48,86	47,86	1,00	48,36
4	Leuwiliang	53,14	48,29	4,86	50,71
5	Lewisadeng	30,29	30,57	-0,29	30,43
6	Pamijahan	88,29	84,57	3,71	86,43
7	Rumpin	70,71	67,43	3,29	69,07
8	Jasinga	115,29	107,57	7,71	111,43
9	Parung Panjang	148,43	143,71	4,71	146,07
10	Nanggung	104,71	99,57	5,14	102,14
11	Cigudeg	93,29	86,00	7,29	89,64
12	Tenjo	175,14	178,57	-3,43	176,86
13	Sukajaya	131,57	120,86	10,71	126,21
14	Tenjolaya	96,29	87,71	8,57	92,00
Total		1260,00	1198,14	61,86	1229,07
Average		90,00	85,58	4,42	87,79

Similar to the 2-wheeled transportation mode, the lowest transportation cost occurs on the Cibungbulang District - Leuwiliang Village route which is IDR 24.360 with a difference in tariff of IDR 2.140. The second lowest is the Leuwisadeng District - Leuwiliang Village route with a fare of IDR 30.430 and tariff difference of IDR 210. The highest transportation cost is also experienced by the Tenjo District – Leuwiliang Village route with the amount of IDR 176.860 with a difference in tariff of IDR 3.430.

The sum of the 14 transportation costs for the 4-wheeled transportation mode results in an average transportation cost of IDR 87.790 with an average tariff difference of IDR 4.420, which is still under the Grab standard.

5.3 Scenario 3

For the scenario 3, 15 villages in the Cigudeg District were involved together with the geographic centroid of West Bogor Regency and promoted to be candidates. The new location-allocation analysis in this scenario determined the geographic centroid of West Bogor Regency as the chosen location. Since the geographic centroid of West Bogor Regency located at the southern part of Bayuresmi Village, we call this centroid by the name Bayuresmi 2. The result of new location-allocation analysis of this third scenario can be visually seen on Fig. 11.

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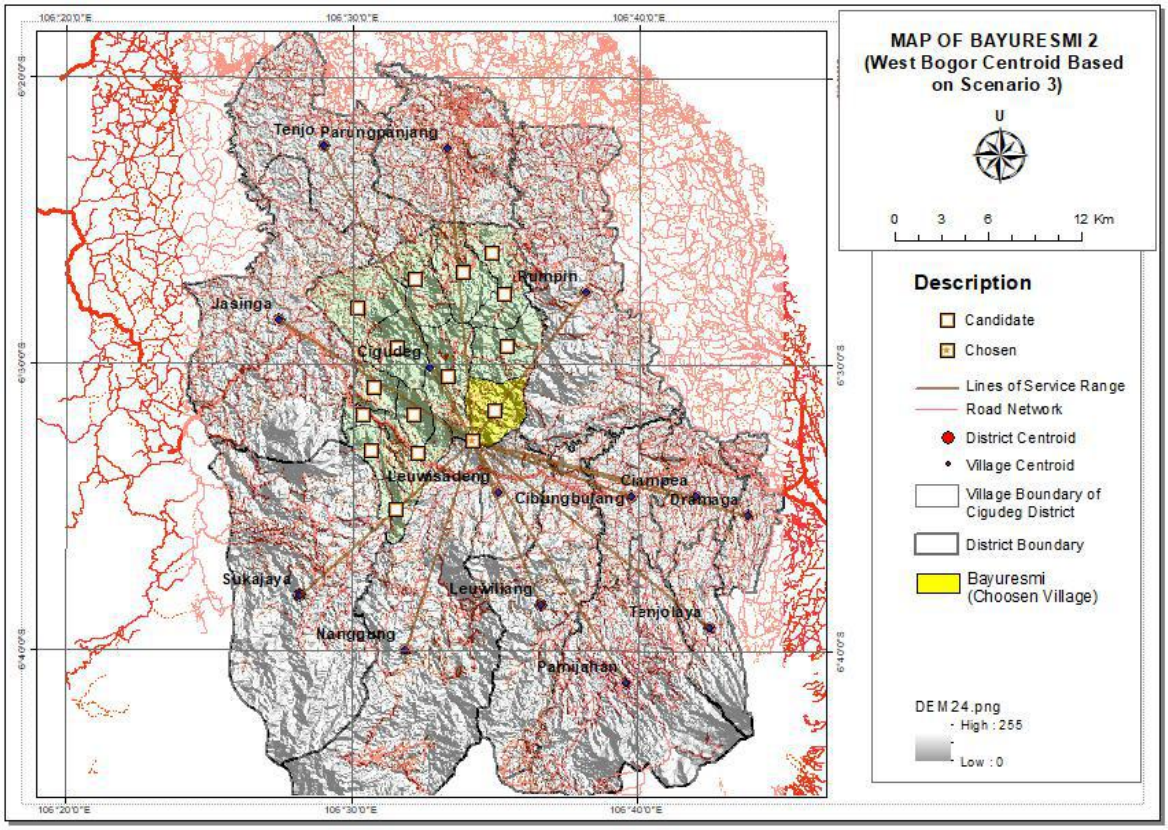

Fig. 11. Results of new location-allocation analysis based on scenario 3

With the election of Bayuresmi 2 as a candidate for the government center of West Bogor Regency, this government center will have a range of services as shown in Table 11.

Table 11. Range of Services of Bayuresmi 2 as a Capital City Candidate

Location_allocation_Bayuresmi_2

FID	District_Village	Weight	Distance from Village
6	Leuwisadeng - Bayuresmi 2	64968,46	5,2 km
2	Cigudeg - Bayuresmi 2	100411,8	8,12 km
1	Cibungbulang - Bayuresmi 2	90891,76	12,84 km
0	Ciampea - Bayuresmi 2	83764,25	19,12 km
7	Nanggung - Bayuresmi 2	44983,18	19,37 km
5	Leuwiliang - Bayuresmi 2	57176,52	21,31 km
10	Rumpin - Bayuresmi 2	62402,85	22,23 km
11	Sukajaya - Bayuresmi 2	25577,60	22,31 km
4	Jasinga - Bayuresmi 2	40442,66	23,26 km
8	Pamijahan - Bayuresmi 2	52461,53	25,21 km
3	Dramaga - Bayuresmi 2	40405,92	25,45 km
13	Tenjolaya - Bayuresmi 2	21186,05	25,65 km
12	Tenjo - Bayuresmi 2	9326,613	34,8 km
9	Parungpanjang - Bayuresmi 2	15799,19	35,19 km

Field: Distance from Village
Statistics:
Count: 14
Minimum: 5,2
Maximum: 35,19
Sum: 300,06
Mean: 21,432857
Standard Deviation: 8,216126
Nulls: 0

Table 11. shows that if Bayuresmi 2 elected as the government center, then the local government must serve the needs of the community for more than 35 km away from the regency capital. The statistical calculation shows that Bayuresmi 2 has the shortest range of service of 5,2 km. The longest range of service is 35,19 km away. The total range of service reach 300,6 km. The average service range is 21,05 km. For the furthest service range, Bayuresmi 2 has a range of services closer than Leuwiliang Village (38,94 km) but is much farther than Cigudeg Village (29,03 km). However, in terms of the total service range, Bayuresmi 2 has the longest service range (300,06 km) compared to Cigudeg Village (296,80 km) and Leuwiliang Village (273,32 km)

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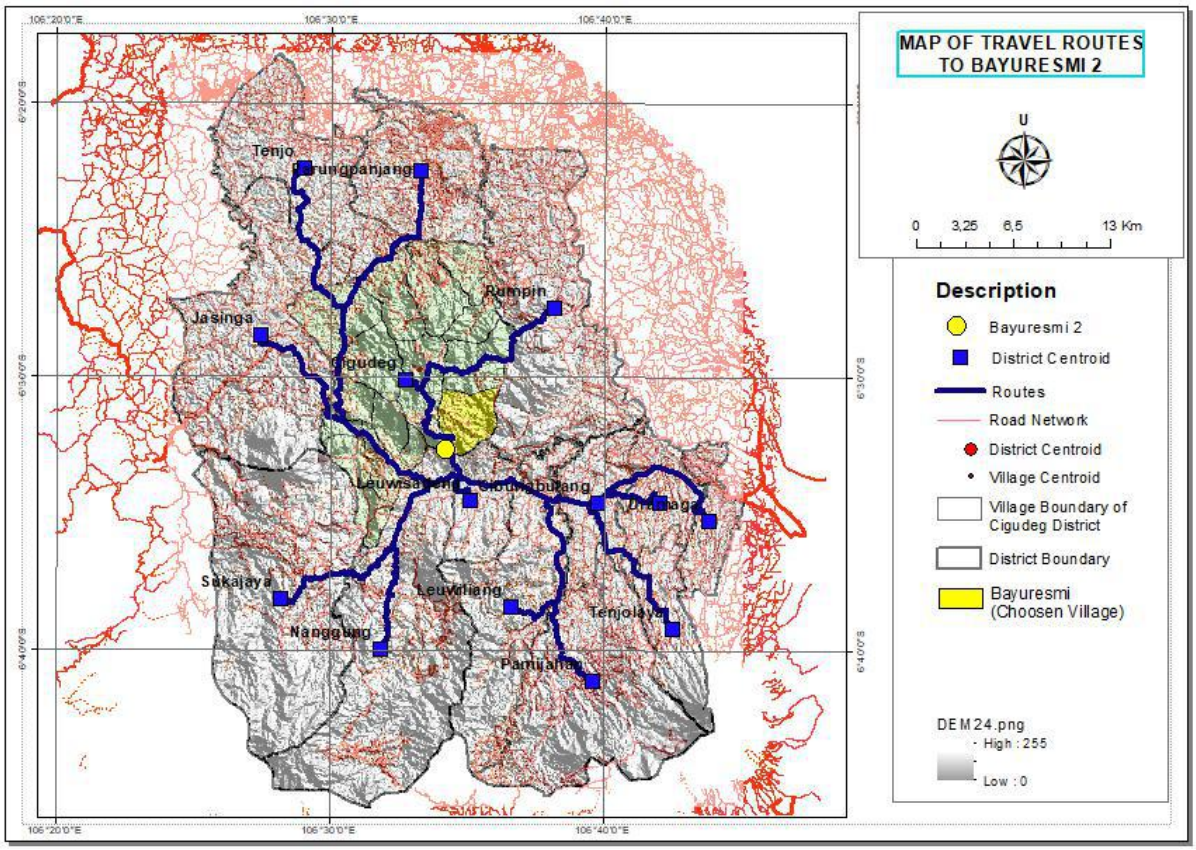

Fig. 12. Results of new closest facility analysis based on scenario 3

5.3.1 Mileage

Based on the result of new closest facility, the shortest route to reach Bayuresmi 2 from each district is as shown visually in the Fig. 12. The blue line is the shortest routes connecting Bayuresmi 2 with each district. These routes have mileage as shown in Table 12.

Table 12. Travel Routes 14 Districts to Bayuresmi 2

Travel Route to Bayuresmi2

FI	Travel Route	Travel Distance
6	Leuwisadeng - Bayuresmi 2	5,2 km
2	Cigudeg - Bayuresmi 2	8,12 km
1	Cibungbulang - Bayuresmi 2	12,84 km
0	Ciampea - Bayuresmi 2	19,12 km
7	Nanggung - Bayuresmi 2	19,37 km
5	Leuwiliang - Bayuresmi 2	21,31 km
10	Rumpin - Bayuresmi 2	22,23 km
11	Sukajaya - Bayuresmi 2	22,31 km
4	Jasinga - Bayuresmi 2	23,26 km
8	Pamijahan - Bayuresmi 2	25,21 km
3	Dramaga - Bayuresmi 2	25,45 km
13	Tenjolaya - Bayuresmi 2	25,65 km
12	Tenjo - Bayuresmi 2	34,8 km
9	Parungpanjang - Bayuresmi 2	35,19 km

Field: Travel Distance

Statistics:
Count: 14
Minimum: 5,2
Maximum: 35,19
Sum: 300,06
Mean: 21,432857
Standard Deviation: 8,216126
Nulls: 0

Statistical calculations on Table 12. shows that the shortest route is the route from Leuwisadeng District to Bayuresmi2, that is 5,2 km. The farthest route is the route from Parung Panjang District to Bayuresmi2 that is 35,19 km. The total number of travel routes from all districts to bayuresmi 2 is 300,06 km with an average value of 21,43 km.

5.3.2 Travel time estimation

The following Table 13. describes in detail the travel time needed to travel to Bayuresmi 2 on the above 14 routes.

Table 13. Travel Time Average Travel from 14 Districts to Bayuresmi2 Based on Google Maps

No	District	Average Travel Time (Minutes)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	81,00	80,00	1,00	80,50
2	Cibungbulang	52,00	52,71	-0,71	52,36
3	Ciampea	62,71	65,57	-2,86	64,14
4	Leuwiliang	60,71	62,43	-1,71	61,57
5	Lewisadeng	42,14	42,14	0,00	42,14
6	Pamijahan	77,43	80,14	-2,71	78,79
7	Rumpin	77,29	79,29	-2,00	78,29
8	Jasinga	54,14	54,86	-0,71	54,50
9	Parung Panjang	81,29	82,57	-1,29	81,93
10	Nanggung	77,29	77,29	0,00	77,29
11	Cigudeg	18,29	18,57	-0,29	18,43
12	Tenjo	96,86	97,86	-1,00	97,36
13	Sukajaya	87,71	88,71	-1,00	88,21
14	Tenjolaya	84,43	87,86	-3,43	86,14
Total		953,29	970,00	-16,71	961,64
Average		68,09	69,29	-1,19	68,69

It can be seen on the Table 13, that the fastest travel time occurred on the route of the Cigudeg District - Bayuresmi 2 with a travel time of 18,43 minutes for a distance of 8,12 km. The second fastest travel time occurs on the route of Leuwisadeng District - Bayuresmi 2 with a travel time of 42,14 minutes for a distance of only 5,2 km. The table also explains that the longest travel time occurs on

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the route of Tenjo District - Bayuresmi2 with a travel time of 97.36 minutes for a distance of 34,8 km. The second longest travel time occurs on the route of Sukajaya District - Bayuresmi 2 with a travel time of 88,21 minutes for a distance of 22,31 km. The difference in travel time between rush hour and normal hour seems to be no significant (-1,19 minutes). Therefore, the average daily travel time can be used as a benchmark for travel time estimation. The total daily travel time to go to Bayuresmi 2 is 961,41 minutes with an average daily travel time of 68.69 minutes

5.3.3 Public transportation costs

This Table 14. describes the cost of public transportation for 2-wheeled transportation modes that must be borne by passengers who traveling from district to Bayuresmi 2 and vice versa.

Table 14. The Average 2 Wheeled Transport Costs from each district to Bayuresmi 2 Vice Versa

No	District	Average GrabBike Transportation Fee (IDR x1000)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	91,57	86,86	4,71	89,21
2	Cibungbulang	50,43	44,71	5,71	47,57
3	Ciampea	62,86	65,00	-2,14	63,93
4	Leuwiliang	63,57	60,00	3,57	61,79
5	Lewisadeng	36,86	34,00	2,86	35,43
6	Pamijahan	95,71	80,43	15,29	88,07
7	Rumpin	88,43	81,86	6,57	85,14
8	Jasinga	69,86	66,43	3,43	68,14
9	Parung Panjan	119,29	110,86	8,43	115,07
10	Nanggung	70,43	67,43	3,00	68,93
11	Cigudeg	11,00	11,00	0,00	11,00
12	Tenjo	136,43	125,14	11,29	130,79
13	Sukajaya	92,14	87,43	4,71	89,79
14	Tenjolaya	99,43	92,86	6,57	96,14
Total		1088,00	1014,00	74,00	1051,00
Average		77,71	72,43	5,29	75,07

For the case of scenario 3 as it shown in the Table 14, there is a slightly significant difference in transportation costs during rush hour and normal hour with a range between IDR. 0 up to IDR 15.290 or an average of IDR 5.290.

Table 14. also shows that the route of the Tenjo District - Bayuresmi 2 requires transportation costs of IDR 130.000 for mileage of 34,8 km with travel time of 97,36. This is the highest transportation costs among 14 routes. The second highest transportation cost occurs on the route of Parung Panjang District - Bayuresmi 2 at a cost of IDR 115.070. The total daily public transportation cost to Bayuresmi2 using 2 wheeled vehicle reach IDR 1.051.000 or an average of IDR 75.070.

The following Table 15. describes the cost of 4-wheeled public transportation modes that must be borne by passengers when traveling from the district to Bayuresmi 2 and vice versa. Referring to data shown in Table 15. the difference in tariffs between rush hour and normal hour varies greatly from IDR. - 570 - up to IDR 18.430. or an average of IDR 6.170. Furthermore, the lowest transportation cost occurs on the route of Cigudeg District - Bayuresmi 2 at a cost of IDR 21.710, with the difference in tariff of IDR – 570.

Table 15. The Average 4 Wheeled Transport Costs from each District to Bayuresmi 2 Vice Versa.

No	District	Average GrabCar Transportation Fee (IDR x1000)			
		Rush Hour (S)	Normal hours (TS)	Difference	Daily
1	Dramaga	124,43	114,71	9,71	119,57
2	Cibungbulang	80,14	70,71	9,43	75,43
3	Ciampea	93,57	93,00	0,57	93,29
4	Leuwiliang	90,57	87,86	2,71	89,21
5	Lewisadeng	57,43	56,71	0,71	57,07
6	Pamijahan	129,57	111,14	18,43	120,36
7	Rumpin	118,00	112,71	5,29	115,36
8	Jasinga	93,43	90,71	2,71	92,07
9	Parung Panjang	148,14	137,43	10,71	142,79
10	Nanggung	104,00	101,57	2,43	102,79
11	Cigudeg	21,43	22,00	-0,57	21,71
12	Tenjo	164,57	154,57	10,00	159,57
13	Sukajaya	127,00	122,29	4,71	124,64
14	Tenjolaya	135,00	125,43	9,57	130,21
Total		1487,29	1400,86	86,43	1444,07
Average		106,23	100,06	6,17	103,15

Eight travel routes from 14 existing routes spend transportation cost above IDR 100.000 with the highest fare of IDR 159.570 that is for the Tenjo District - Bayuresmi 2 route. Five other routes require transportation costs between IDR 50.000 up to IDR 95.000. The total amount of daily public travel cost using 4-wheeled vehicles from 14 districts to Bayuresmi 2 is 1.444.070 or an average of 103.150

5.4 Accessibility Analysis

A simple statistical comparison method used in this accessibility analysis was arranged in a table form as shown in Table 16. The comparable parameters include; (1) travel distance; (2) travel time; (2) 2-wheeled public transportation cost; and (4) 4-wheeled public transportation cost. The value of each parameter that was compared is the average value.

Table 16. Travel Distance, Travel Time and Public Transportation Cost of the Three Scenarios

Chosen Village	Accessibility Parameter			
	Distance (Km)	Travel Time (Minute)	2-Wheeled Cost (IDR x1000)	4-Wheeled Cost (IDR x1000)
Cigudeg	21,20	54,23	64,30	94,65
Leuwiliang	19,52	50,88	55,22	87,79
Bayuresmi 2	21,43	68,69	75,07	103,15

Based on the Table 16. Leuwiliang Village has the lowest value for all parameters of accessibility. The second order is held by Cigudeg Village, while the third order is held by Bayuresmi 2. Figure 13. is a graph describes visually the comparison of accessibility parameters from three selected villages.

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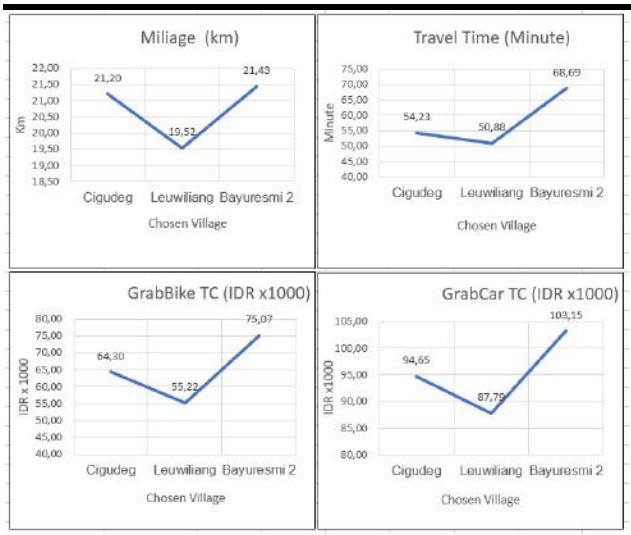

Fig. 13. Graph of accessibility parameters of Cigudeg Village, Leuwiliang Village and Bayuresmi 2

VI. DISCUSSION

The results of comparative analysis presented in the form of tabulation and graphs show that Leuwiliang Village has accessibility parameters with the lowest value for all parameters (travel distance, travel time and public transportation cost). Bayuresmi 2 as it shown on Table 16. has accessibility parameters with the highest value for all parameters. While Cigudeg Village has accessibility parameters with values between Leuwiliang Village and Bayuresmi 2 for all parameters.

In Refer to accessibility criteria which mention that the closer the origin and destination to the transportation system, the higher the level of accessibility, and the less time and money spent on the trip, the greater the accessibility [18], Leuwiliang Village seems to be a village that meets these criteria. In other word, Leuwiliang Village has the highest level of accessibility compared to Cigudeg Village and Bayuresmi 2.

The status of Leuwiliang Village as a village with the highest level of accessibility has actually been detected by only looking at the total range of services, when referring to the argument that states that the capital or location chosen must have the smallest total physical distance so that it is easily accessible from all regions for smooth service in government services [19].

If we look at the Service Range Table in the three scenarios, Cigudeg Village has a total range of services of 296.80 km (Table 3). Leuwiliang Village has the total range of services of 273.32 km (Table 7.), and Bayuresmi 2 with a total range of services of 300.06 km (Table 11).

It is clear that of the three total service ranges, the village of Leuwiliang has the shortest total service range. This can be interpreted that among the three selected locations, Leuwiliang Village is in the most appropriate location to become the center of government of West Bogor Regency.

The choice of Leuwiliang Village as the most suitable village to become the candidate for the capital of West Bogor Regency in this study, raises one question. What was the basis of the expert team in 2008 in determining the village of Cigudeg as the candidate for the capital of West Bogor Regency?

In an interview with a member of the expert team involved in the Cigudeg Village study in 2008, said that the determination of Cigudeg Village of was based on the index of centrality. The centrality index is one of the methods used in regional planning, which is usually used in conjunction with scalogram methods, analysis of cities, gravity and other methods [21]. When was questioned is not the centrality index based on the completeness of the infrastructure facilities, while the facts show that the complete infrastructure facilities of the Cigudeg Village are not as complete as Leuwiliang Village, he replied that the main reason was to find areas that are still empty.

This main reason indirectly leads to a conclusion that the appointment of Cigudeg Village as a candidate for the capital in 2008 was based more on political considerations. The choice of territory on the basis of political considerations is permissible in regional planning (Regional Layout Plans) as long as it does not conflict with the allocation of space in the spatial pattern. In the interview, he was also asked if he was basically looking for vacant land, why was not choose Bayuresmi Village which is the centroid of West Bogor Regency. The reason given was that Bayuresmi Village is located in a hilly area.

Even though there has been an answer that Bayuresmi Village was not chosen for topographic reasons, this study still proposes Bayuresmi 2 as a candidate for the capital (scenario 3). There are 3 reasons that support the need for centroid points in West Bogor Regency to be included in the selection of capital candidates. First, existing technology makes it possible to modify landscapes through civil engineering. Second, many countries move their capital to the center of the region on the basis of balance and equal distribution of services [18]. Third, many cities in Indonesia are in hilly areas which with the correct city arrangement produce beautiful urban areas.

This research actually expects Bayuresmi 2 to be chosen as the location of the prospective capital city with the highest level of accessibility. Unfortunately, the results of the analysis on each accessibility parameter put Bayuresmi 2 in third place. The failure of Bayuresmi 2 to be in the first position is certainly related to the availability of the existing road network around Bayuresmi 2.

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The failure of Bayuresmi 2 to be in the first position is certainly related to the availability of the existing road network around Bayuresmi 2. We can see clearly in Fig. 14 that of the 14 routes to Bayuresmi 2, only one route that has a direct connection to Bayuresmi 2, that is Rumpin District. 13 other routes must pass through Leuwiliang Village or Cigudeg Village. These 13 routes also utilize the Bogor Rangkasbitung collector road which is also used by the route to Cigudeg Village and towards Leuwiliang Village. Bayuresmi 2 is also in a position of approximately 3 km from the Bogor Rangkasbitung collector road. Such conditions certainly impact on the addition of the length of the 13 routes, and enlarge the total route distance to Bayuresmi2.

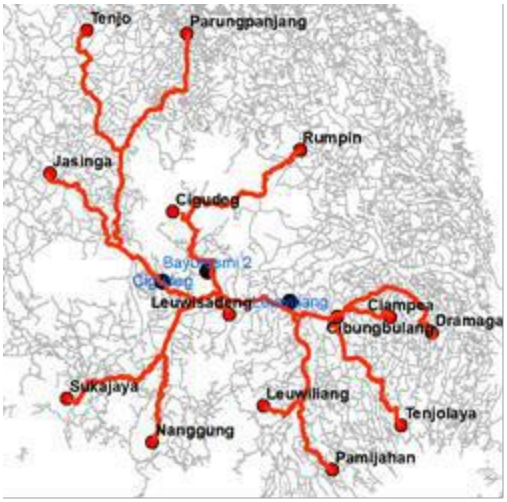

Fig. 14. Availability of road network around Bayuresmi 2

The level of accuracy of the research results using network analyst is highly dependent on the availability and accuracy of road network data and traffic engineering data. Accuracy of attributes in the classification of road functions will clearly separate the road that can be passed by motorized vehicles and roads that can only be passed on foot. Accuracy in this case will affect the determination of the shortest route by motorized vehicles. Accuracy of traffic engineering data, such as one-way or two-way roads, the presence of red lights, prohibition to turn, prohibition to make U-turn will also affect the selection of the shortest route.

In this study, errors caused by traffic engineering could be considered very small, because there is no one-way road implementation, no restricted turn, no U-turn ban and only a few red lights are found in the West Bogor Regency. In contrast to the road network, there are differences in data presented by network analyst with data presented by Google Maps.

The Fig. 18 is an example of the difference in distance that occurs on the Bayuresmi 2 - Rumpin District route. According to Google Maps the shortest distance route Bayuresmi 2 - District Rumpin is 27,1 km, while according to the new closest facility 22,23 km (Table 12). The route taken by the two is also different. In this case, because Google data is always updated [34], and each road segment is corrected through field checks, it is suspected that error occur in the road function classification system of the road layer of topographical map.

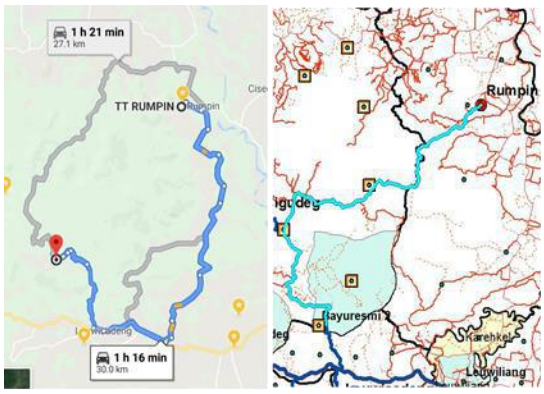

Fig. 15. Route selection presented by Google Maps and by network analyst

It is suspected that the road segment that should actually function as a trail is grouped into motorized road functions. This error clearly influences the accuracy of the Bayuresmi 2 accessibility parameters.

Network analysis, actually also equipped with the ability to estimate travel time. However, to be able to estimate the travel time, network analyst requires the daily road profile data (street daily profile). Compiling such network datasets requires extensive data collection and processing, which can be very expensive or not feasible for some applications [34]. The difficulty in compiling the daily road profile table also seems to be the cause of the lack of network analyst used in spatial analysis. Therefore, in this study, the estimation of travel time was done using the Google Maps application.

6.1 Google Maps as a tool for estimation of travel time

On the research of Estimating O–D Travel Time Matrix by Google Maps API [34] they found several benefits of using Google Maps in estimating travel time compared to ArcGIS Network Analyst. These advantages include: (1) no need to prepare a road network; (2) using a newer road network; and (3) calculating congestion in high traffic and rush hour areas. But they also alluded the weaknesses of using Google Maps, for instance all data used in calculations is managed by Google Maps. Users do not have control over any quality or editing rights, while it is believed that there is no data entirely error-free [34].

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The advantages of Google Maps were indeed proven in this research. Without having to prepare time-consuming data, and simply open the Google Maps application on a smartphone, then determine the place of origin and destination, Google Maps instantly provides data on the route, and travel time according to the mode of transportation used. The data presented is definitely a real time data.

Google is also able to offer alternative routes that can be chosen according to the behavior of the traveler. Fig. 18 is an example of how Google offers 3 alternative routes that can be chosen to travel from Bayuresmi2 to Rumpin District with 4 wheeled private transportation modes. The first route is 27.1 km with a travel time of 1 hour 21 minutes, the second and third routes are 30,0 km with a travel time of 1 hour 16 minutes. The three routes offered by Google are even different from the shortest route set by the network analyst with a distance of 22.23 km (Table 12). For the public transportation mode Google only offers a route with the fastest travel time. In the above case, Google offers a route with a distance of 30.0 km with a travel time of 1 hour 16 minutes.

In relation to the level of accessibility of a place or location, Google Maps is clearly able to offer a number of route options, complete with the estimated travel time and distance, including the modes of transportation used. This Google Maps offer, indirectly provides many options for travelers to choose whether based on the fastest time or the shortest distance, and the mode of transportation used. These Google Maps capabilites contribute greatly in determining the level of accessibility of a place. Logically, the more routes offered, and the more choices of transportation modes that can be used, the greater the opportunity or access to reach the place, which means the higher the level of accessibility of the place. Therefore, regardless of the level of data accuracy as questioned [34]. Estimating travel time and distance with the Google Maps application seems to be more effective and efficient with a more accountable degree of accuracy.

Regrettably, Google Maps is not equipped with an analysis function as found on network analyst. The new location-allocation analysis function in this study clearly contains errors due to the quality of road data that still has to be questioned. Difference in distance as in the case of the shortest distance Bayuresmi 2 - Rumpin District is evidence of an error in road data derived from the Indonesian topographic map.

6.2 Grab application as a tool for estimation of public transport cost.

Network analyst, basically also equipped with the ability to estimate the cost of travel. But as well as travel time, procurement of travel cost data requires the cost of survey and processing data that is very large and complex. Therefore, the presence of online transportation services is considered as a way out in data collection of public transportation cost estimation.

The main reason for using online transportation services in estimating public transportation cost is because these modern service models are in great demand by all levels of society, and are expected to become models of future transportation services. The tariff system issued by online transportation services that must be paid by passengers indirectly represents a system of public transport fares that can be accepted by the public. Another reason is that the door-to-door service system implemented by online transportation services is able to eliminate un-measured travel costs such as those that occur in travel systems based on stops and routes. Therefore, the estimation of public transportation cost using online transportation services is more measurable and realistic and simultaneously reflects the total cost of travel from the place of origin to the destination.

The estimation of public transportation cost based on the Grab application used in this study is known to not always be in-line with the recommended route offered by Google Maps for public transportation. Fig. 16 below is an example of a case that occurred on the journey from Bayuresmi 2 to Pamijahan District. It appears on the Fig. 16 that Grab took a different and farther route than the one recommended by Google Maps. This difference in distance clearly affects the total transportation costs that must be borne by passengers. The difference between the recommended route of Google Maps and the route chosen by Grab does not only occur on the route of Bayuresmi 2 – Pamijahan District, but also on other routes that are the object of research.

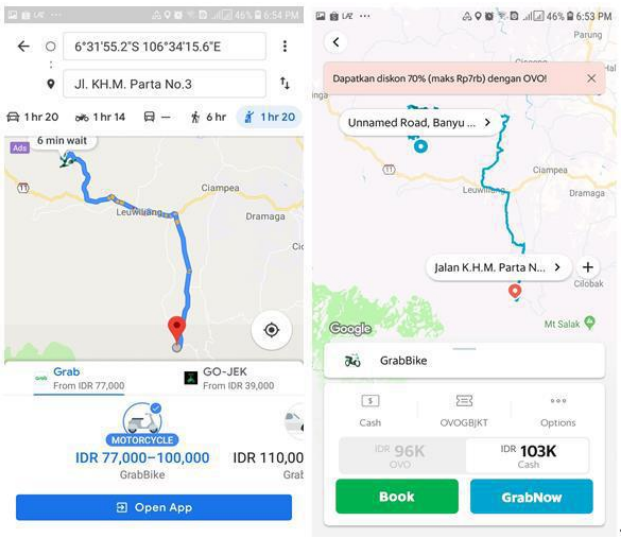

Fig. 16. Differences in travel routes between route recommended by Google Maps and route chosen by Grab

However, the case of the different route that affects on the total cost of public transportation, as well as the case of the determination of the tariff per km which is also said to be affected by weather conditions, is not the focus of this study. Estimating public transportation costs in this study is based solely on the IDR numbers listed in the Grab application. In this study, the IDR number stated in the Grab application is considered as the official and legal transportation fee issued by the company, as a result of an internal decision, and accepted by the passenger as service satisfaction.

VII. CONCLUSIONS

From a series of analysis carried out in an effort to answer the research objectives, it is concluded that Cigudeg Village is not included in the village with the highest level of accessibility. The highest accessibility value is precisely held by Leuwiliang Village, the chosen village as a result of scenario 2. Bayuresmi 2, which from the beginning was expected to be the location with the highest level of accessibility apparently is only in third place.

VIII. RECOMMENDATIONS

Since the results of the research have appointed Leuwiliang Village as the village with the highest level of accessibility, it is suggested to the West Java provincial government to take more consideration in the determination of Cigudeg Village as the candidate for the capital of West Bogor Regency.

It is recommended to repeat this study by using travel data and travel time completely from Google Maps to see if there are differences in results compared to the results of the current study. This is important for network analyst development as a science that is able to adapt to technological developments.

It is recommended for coaches who involved in the network analyst training to continue to carry out various network analyst applications in solving problems related to services, and make them as materials to develop a research-based learning model that is believed to provide better understanding to participants in the learning process.

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Responses of date Palm Seedling to co-Inoculation with Phosphate Solubilizing Bacteria and Mycorrhizal Arbuscular Fungi

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Abstract**—** *With the broad aim of biologically improving P uptake by date palm, in this work we use the beneficial phosphorus solubilizing microorganisms like arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) characterized by a remarkable ability to solubilize phosphorus and tolerance to hard pedoclimatic conditions. For this study, a pot experiment was conducted under greenhouse condition to assess the effect of associations between mycorhizal fungus (Glomus mosseae) (M) with a range of ten bacterial strains (B).*

An essay of different treatments (control seedlings, seedlings inoculated by arbuscular mycorhizal fungi (AMF) and seedlings inoculated by a combination of PSB and AMF) showed the progressive evolution of microbial activity.

The results indicated that the rhizosphere interactions between Glomus mosseae and PSB strains (Pseudomonas striata, Bacillus subtilis) significantly improved growth parameters including root dry weight (23,66%), root length (24%), leaf dry weight (67,7%), leaf length (26,9%), promote P mineralization in soil P (142,3%) uptake by date palm seedling as compared to control, and percent root colonization in date palm seedling was also recorded by the combined inoculation of Glomus mosseae and PSB strains (Pseudomonas striata, Bacillus subtilis).

Keywords**— Bacteria, date palm seedling, fungi, growth, inoculation, interaction.**

I. INTRODUCTION

Phosphorus is an important key element in the nutrition of plants. Although phosphorus is present both in organic and inorganic forms in soils but its availability is limited to plants and mostly restricted due to complex formations with other nutrients (Sharma et al. 2013).

The soils in Djerid region are calcareous in nature and due to the high pH much of phosphorus is not available for plant uptake and growth promotion (Mtimet 2016; Zougari et al. 2016). However, to respond to the growing demands, in this region, to improve the absorption of phosphorus and compensate this poor availability of this element, it is necessary to manipulate phosphorus solubilizing microorganisms (arbuscular mycorhizal fungi and phosphorus solubilizing bacteria), which convert the insoluble forms of P to an accessible form by plants (ortho-phosphate) (Costa et al. 2015). Currently inoculation of these microorganisms have gained popularity (Parkash et al. 2011) to be used as biofertilizers instead of high input chemical fertilizers in crop production system (Kennedy et al. 2004).

A synergistic relation between arbuscular mycorhizal fungi (AMF) and phosphorus solubilizing bacteria (PSB) had been observed (Fazli et al. 2015). These observations also showed that a combined application of *Glomus fasciculatum* and *Azotobacter* increase the concentration of P, K and N uptake by the mulberry (Morus nigra) leaf of 10, 16 and 5.8 %, respectively.

Therefore, it seems more appropriate to consider this technique is more interesting. Indeed, it is becoming increasingly clear that influencing the microbial diversity of soil, it would be possible to improve fertility (Beauregard 2010).

Several research results on soil microorganisms were published during the last decade, the factors influencing the growth of these microorganisms and the changes in their community are likely to act together and their combined effects are difficult to predict (Oehl et al. 2003; Castillo et al. 2006). Nevertheless, the work of Schrey et al. (2007) is in keeping with the small number of previous studies that have addressed the practical use of co-inoculation with phosphate solubilizing bacteria (PSB) and mycorrhizal arbuscular fungi (AMF) in agriculture.

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In Djerid region, no attempts have been made so far to investigate the effect of PSB and AMF inoculation on date palm crop. Therefore, the aim of the present study was to investigate the effect of AMF and PSB strains inoculation on growth, mycorrhization and P uptake of date palm seedling in the arid conditions.

II. MATERIALS AND METHODS

The material used in this work is the AM fungal spore and phosphorus solubilizing bacteria (PSB) of the host plant: *Phoenix dactilifera L.* (Date palm).

The collection of a representative soil sample was taken from Jerid palm groves. AMF spores were isolated using the wet-sieving (125 and 45 µm) and decanting method described by Gerdemann and Nicolson (1963). Spores and spore clusters were transferred into Petri dishes and counted in three replications under binocular microscope with magnification of 40X and divided in groups in relation to morphological characteristics as shape, size, color, presence of structures like sporiferous saccule, subtending hypha. Although, the same morphotypes of mycorrhizal fungal spores (*Glomus mosseae)* were isolated in collaboration with Eastern cereal and Oilseed Research Centre (ECORC) Ottawa). Approximately 50 spores were isolated and counted for each pot and stored at 4°C for a maximum of two days before pots application.

The AMF colonization rate of date palm seedlings roots was determined using the magnified line-intersect method of McGonigle et al. (1990).

Phosphate solubilizing bacteria (PSB) were collected from the rhizosphere of date palm. Samples of soil were suspended in phosphate buffer saline and serial dilutions were spread on Pikovskaya's (PVK) agar containing $Ca_3H_2PO_4$ as the phosphate source. The bacterial isolates were characterized by conventional tests on API 20 E gallery, API 50CH gallery and on specific reactive media in collaboration with Environmental Health Laboratory (EHL) Tozeur.

The ability of the bacteria to solubilize insoluble phosphate was qualitatively determined from the clear light zone surrounding colonies (Figure 1) according to Edi-Premono et al. (1996) by the solubilization index = [ratio of the total diameter (colony + halo zone) to the colony diameter].

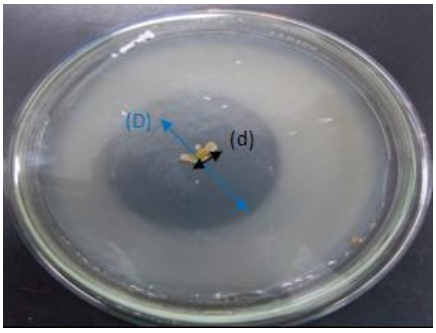

Fig.1: Halo zone around the colony on Pikovskaya (PVK) agar confirm phosphate solubilizing bacteria. (D: total diameter (colony + halo zone) and d: colony diameter).

For the inoculums preparation the selected PSB strains were inoculated separately in 250 ml flasks containing 100 ml of Pikovskaya's broth. The flasks were kept in a growth chamber at 28°C on an orbital shaker at 120 rpm. After 7 days 1 ml of the broth culture was spread on Pikovskaya (PVK) agar plates to determine the microbial concentration. Finally, the bacterial cultures were adjusted to a concentration of approximately 10^8 cfu / ml (Murray et al. 2003).

The soils of Djerid region were selected for cultivation of date palm seedlings grown in plastic pots under greenhouse conditions at the regional research center of oasis agriculture. Pots were filled with 3kg sterilized soils of Djerid region. Three replications were prepared for each treatment.

the considered soils studied at all depths are generally characterized by coarse textures, slightly alkaline to alkaline pH values, very low cation exchange capacity (CEC); the percentage of active limestone is moderately high (Table 1).

Table.1: Chemical and textural characteristics of the soils from Djerid region

Depth (cm)	pH	EC (mmhos/cm)	CEC (mmol (+)/kg)	CaCO3 (%)		Texture class
				Total	Active	
0-20	8.8 ±0.02	7.4 ±0.12	5.3	12.7 ± 1.01	7.3 ±0.09	SCL
20-40	8.7 ±0.05	5.3 ±0.01	4.5	12.5 ±1.17	8.1 ±0.38	SL
40-60	7.4 ±0.01	5.2 ±0.16	4.5	10.1 ±1.20	8.0 ±1.07	SL

SCL: Sandy Cay Loam, SL: Sandy Loam, EC: electrical conductivity, CaCO3: calcium carbonate

The date seeds were disinfected with 3.5% NaOCl and then rinsed with distilled water 3 times after 10 min. The disinfected date seeds were transferred to incubator at 28°C to accelerate pre-germination seed activities.

The experiment includes the following treatments:

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1- Control treatment (T): untreated soil

2- M: Spores of the AMF (*Glomus mosseae*) (50 spores per pot)

3- Bi + M; (Bi: (B1, B2, …, B10)) ; M: Spores of the AMF (*Glomus mosseae*)

All date palm seedlings were harvested after 135 days and the growth parameters, such as_root length, root dry weight, leaf length and leaf dry weight were recorded. The roots were washed out with tap water to remove the soil particles. After drying for 48 hours at 60°C, the samples were weighted, grinded and further analyzed for phosphorus concentration and accumulation by date palm seedlings by the method as described by Pauwels et al. (1992).

To determine root percentage colonization, the roots were cleared in 10% KOH, rinsed once with water, soaked in 2% HCl and stained with Trypan blue solution. After rinsing three times with water, roots were stored in lactoglycerol. Percent colonization of various AMF structures and the overall association was determined under the microscope by the magnified intersections method (McGonigle et al. 1990)

Soil samples were collected, from each treatment, 135 days after inoculation. These soil samples were air dried ground and sieved through a 2 mm sieve. Soil samples were analyzed for assimilable P by the following procedures described by Pauwels et al. (1992).

The statistical treatment of results is achieved through the STATISTICA Version 5 software, (Beaux et al. 1991). Analysis of variance of two factors by Fisher's F test is performed to verify the equality of the means of hypothesis risk threshold of 5%. It is supplemented by multiple comparisons of means by the Newman Keuls test when the equality of averages hypothesis is rejected, according to Dagnelie (1986).

III. RESULTS

3.1. Phosphorus solubilizing bacteria associated with date palm

We isolated and purified a total of 10 bacterial strains from the rhizosphere of date palm seedling by repeated streak culture on PVK medium. Initially, all isolates were tested for their phosphate solubilizing activity using PVK medium.

The most efficient and competitive phosphorus solubilizing bacteria are selected: B1: *Pseudomonas horyzihabitans*, B2: *Pseudomonas aeruginosa*, B3: *Serratia liquefacien*, B4: *Pseudomonas striata*, B5: *Bacillus subtilis*, B6: *Citrobacter freundii*, B7: *Moraxella spp*, B8: *Serratia odorifera*, B9: *Non-fermenter spp* and B10: *Bacillus cereus)*

3.2. The determination of the bacterial effectiveness

The phosphate solubilization index of the date palm seedling isolates varied from 2.4 to 4.3 (Figure 2).

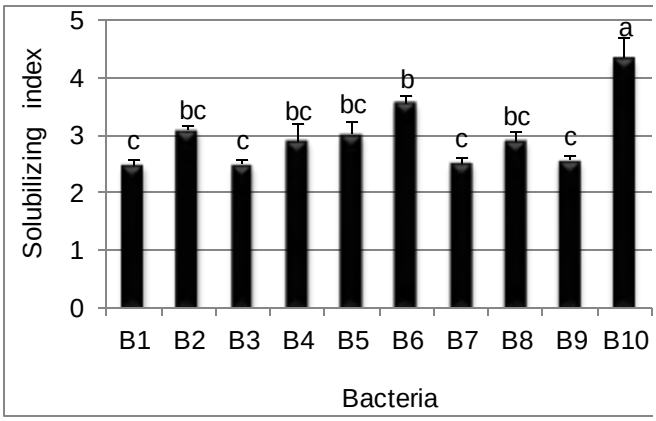

Fig.2: The phosphate solubilization index of the date palm seedling isolates (Bars with different letters represent statistical significant differences according to Newman Keuls test (p≤0.05).

Bacillus cereus (B10) exhibited the highest phosphate solubilization index (4.3).

Several early studies revealed that many phosphorus solubilizing bacteria are normally present in the soil. They can remove and release phosphorus from soil.

3.3. Phenotypic analysis

The influence of combinations (*Glomus mosseae* (M) and PSB) on growth and phosphorus nutrition was studied nine months after inoculation

3.3.1. Root length

Test results showed that the inoculation of *Glomus mosseae* (M) and PSB strains influenced the root length of date palm seedlings as compared to the treatments having single or no inoculation. The maximum (31 cm) root length with 24% increase over control (T) and single inoculation (M) was observed. The treatments (B4+M; B5+M and B10+M) have somewhat higher values than the other treatments, but statistical analysis shows some stability of the root length for all treatments (Table 2).

Table.2: Root length, root dry weight, leaf length and leaf dry weight as influenced by the inoculation of AMF (Glomus mosseae (M)) and PSB.

	Root length	Root dry weight	Leaf length	Leaf dry weight
B1+M	26,6 a	1,136 ab	29,1 ab	1,763 cd
B2+M	26,6 a	1,312 a	28,2 b	2,121 bc

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B3+M	28,2 a	1,174 ab	31,3 ab	2,009 c
B4+M	30,9 a	1,372 a	32,8 a	2,520 a
B5+M	31,2 a	1,388 a	32,9 a	2,677 a
B6+M	29,0 a	1,339 a	31,3 ab	2,335 b
B7+M	29,4 a	1,177 ab	30,4 ab	2,405 ab
B8+M	29,6 a	1,209 ab	31,1 a	2,386 ab
B9+M	27,6 a	1,341 a	25,0 c	1,961 c
B10+M	31,2 a	1,434 a	31,5 a	1,994 c
M	25,1 a	1,356 a	30,1 ab	1,779 cd
T (Control)	25,4 a	1,031 b	26,1 bc	1,550 d

(Values followed by different letters represent statistical significant differences according to Newman Keuls test ($p \leq 0.05$)).

3.3.2. Root dry weight

The dual inoculation (AMF and PSB) increased root dry weight of date palm seedlings significantly ($p \leq 0.05$) over control (T). The maximum (1.42 g) root dry weight with 37.9% increase over control was observed.

The influence of combination (PSB (B_i and *Glomus mosseae* (M)) on root dry weight was studied and the results showed that treatments (B10+M) gives the highest value, but there are no significant differences for the majority of treatments (Table 2).

3.3.3. Leaf length

Based on comparisons of treatments it's fair to say that treatments ((B4+M; B5+M; B8+M and B10+M) give the highest values (Table 2), but the minimum leaf length of date palm seedling (25 cm) was noted in the combined inoculation (B9+M). The experiment showed that there are bacteria that are auxiliaries of mycorhization, so-called "mycorrhiza helper bacteria". While others have inhibitory activity on the development of mycorrhizae.

3.3.4. Leaf dry weight

The highest total dry weight was obtained when date palm seedlings were inoculated with AM fungi (*Glomus mosseae)* incorporated with bacteria B4 and B5 as compared to the control and other treatments. Plant inoculated with *Glomus mosseae* (M) plus bacteria B5 was found to be two times greater of leaf dry weight than that of the control (T)

The results showed that co-inoculation of fungus and phosphorus solubilizing bacteria (PSB), in comparison with single inoculation (M) and the control (T), of any of them caused a significant increase in leaf dry weight (Table 2).

3.4. Mycorrhization of date palm seedling

The majority of treatments generate a sizeable rate of mycorrhizal hyphae that exceeds 50% (Figure 3).

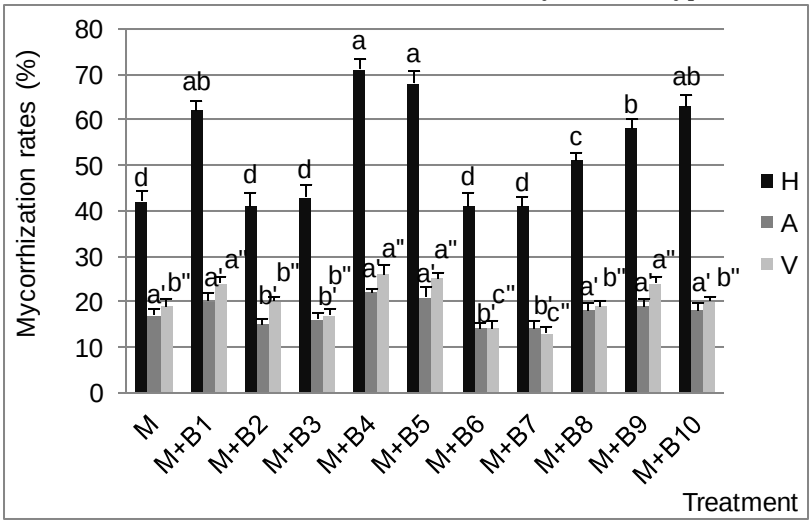

Fig.3: Effects of treatments on mycorrhization rate; hyphal ( ), arbuscular, ( ) and vesicular ( ) in the roots of date palm seedling. (Bars with different letters represent statistical signicant differences mycorrhization rate hyphal (a,b,c); mycorrhization rate arbuscular (a',b',c') and mycorrhizationrate vesicular (a",b",c") according to Newman K euls test (P<0.05)).

It is evident that the treatment (M+B7) is characterized by a limiting effect on the appearance and the development of fungal structures (hyphae, arbuscule, and vesicle). The minimum values of hyphal growth rate, arbuscular growth rate and vesicular growth rate were 41%, 14% and 13% respectively.

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The present study demonstrated the benefits of combinations between M+Bi (*Glomus mosseae* (M)) and phosphorus solubilizing bacteria strains (Bi)) for growth of fungal structures in comparison with single inoculation (M). The bacterial community can change the result of mycorrhizal establishme.

In addition, it was found that *Glomus mosseae* spores development was positively (R^2= 0.69) correlated with PSB population (Figure 4)

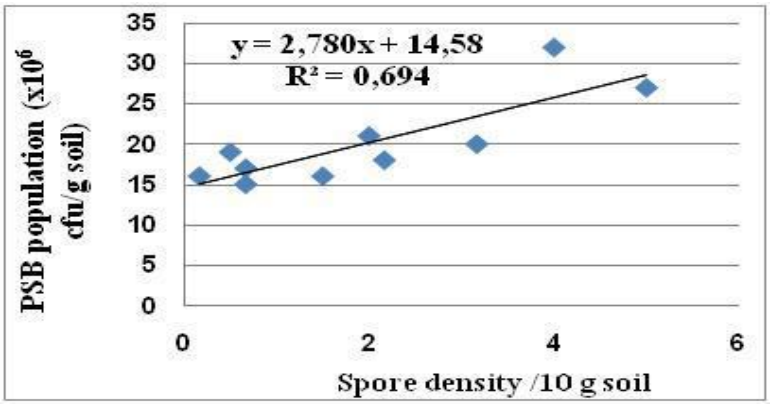

Fig.4: Correlation between spores density of the mycorrhizal fungus (Glomus mosseae) and PSB population in date palm seedlings

3.5. Phosphorus content

Based on comparisons of treatments it's fair to say that the highest P uptake was recorded in the treatment of AMF inoculated with *Pseudomonas striata* (B4) *and Bacillus subtilis* (B5).The minimum P uptake was found in "B6+M" treatment.

Based on Figure 5, some of the treatments have low phosphorus levels in comparison with treatment (M), which implies the ineffectiveness of these associations for biological uptake of this mineral element.

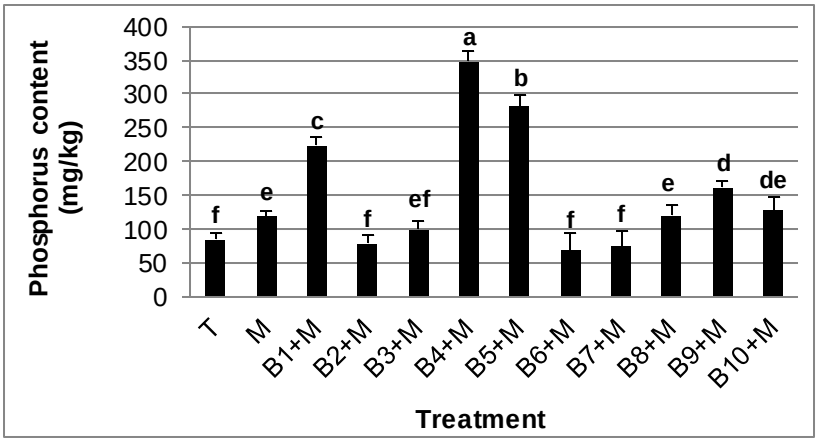

Fig.5: Effect of different treatments on phosphorus content of date palm seedlings. (Bars with different letters represent statistical significant differences according to Newman Keuls test ($p \leq 0.05$).

Interference and correlation of all these results showed several types of biological relationships between different microorganisms in their habitat (symbiosis, mutualism, antagonism).

Arbuscular mycorrhizal (AM) associations have been shown to reduce damage caused by soil-borne plant pathogens.

IV. DISCUSSION

The dual inoculation with AMF and PSB is an important approach to decrease the use of chemical fertilizers and improve P supply for sustainable crop production. In our study, we inoculate ten strains of PSB with AMF and investigated its effect on different plant growth parameters. The synergistic effect of AMF and some PSB influenced P solubility and stimulated date palm roots to absorb nutrients from soil and thus enhanced the overall plant growth as compared to the treatments having single inoculation (M) and the control (T). The microbial activities stimulated nutrients uptake and plant growth may be due to hormones such as auxin or gibberellic acid production as stated by Minaxi *et al*. 2013; Kang *et al*. 2012.

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In this study, we observed a significant growth benefit of the synergistic association of date palm seedlings with AMF and some PSB. Our results showed that combined inoculation of plants with AMF and PSB resulted in increased AMF colonization, which is an important indicator of plant nutrition. The synergistic interaction of some PSB and AMF significantly increasing plant biomass, plant growth and amelioration of phosphorus uptake compared with untreated plants (Osorio and Habte 2001 and Kohler et al. 2007). While, we found that some PSB strains reduced AMF colonization in date palm seedling, consistent with observations made by Alqarawi et al. (2014) in Ephedra aphylla and Hashem et al. (2015) in Vigna unguiculata.

The experiment showed that there are bacteria that are auxiliaries of mycorhization, so-called "mycorrhiza helper bacteria". While others have inhibitory activity on the development of mycorrhizae. However, plants inoculated with (M+B3, M+B6 and M+B7) showed significantly lower mycorrhizal colonization than plants inoculated with (M+B1, M+B4, M+B5 and M+B10). This result matches those found by Garbaye and Bowen (1989) who demonstrated that the rhizosphere microflora could have a positive or negative impact on the mycorrhizal symbiosis, depending on the bacterial isolates.

Several early studies revealed that many phosphorus solubilizing bacteria are normally present in the soil (Buchan 1983; Sidat et al. 1999). They are efficient in storing polyphosphate and can remove and release phosphorus from soil (Peix et al. 2003).

The bacterial community can change the result of mycorrhizal establishment (Marschner et al. 2001). Arbuscular mycorrhizal (AM) associations have been shown to reduce damage caused by soil-borne plant pathogens. These results confirm those from other authors (Ben Kaled et al. 2003; Chabot et al. 1993 and Baquall and Das 2006).

V. CONCLUSION

To improve phosphorus uptake by date palm, this work was realized to study the biological alternatives (phosphorus Solubilizing Bacteri (PSB) and mycorrhizal fungus (*Glomus mosseae*)) in order to solve phosphate's blocking assimilation characterizing soils in southern Tunisia.

This work has also highlighted the presence of bacterial strains that have phenotypical and genetical diversity. These bacteria seem to be adapted to the extreme conditions of the oasis.

Phenotypic analysis showed that treatments (B4+M; B5+M and B10+M) have somewhat higher values than the other treatments. These results suggest a synergistic relationship between some phosphorus solubilizing bacteria and arbuscular mycorrhizal fungi that can enhance date palm productivity.

According to the experimental results, it seems that co-inoculation (B4+M and B5+M) of date palm seedlings, can have a very strong effect on the uptake of phosphorus from soil. The other treatments showed the ineffectiveness of these associations for biological uptake of this mineral element.

The growth parameters, root colonization and phosphorus uptake responses of date palm seedlings to co-inoculation of AMF and PSB showed that they depend on a combination of fungal and bacterial strains. According to the results of this study, co-inoculation (B4+M and B5+M) significantly increased all parameters studied.

Generally, interference and correlation of all these results showed several types of biological relationships between different microorganisms in their habitat (symbiosis, mutualism and antagonism).

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