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FOREWORD

I am pleased to put into the hands of readers Volume-4; Issue-1: Jan-Feb 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: March, 2019

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Physicochemical Status of Sitalakkhya River, an Ecologically Critical Area (ECA) of Bangladesh

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***Abstract*—** *The study was conducted to assess the water quality of the Sitalakkhya River, Narayangonj, Bangladesh and observe its temporal changes forthe physicochemical parameters of water from March to May, 2015. The present study showed that the color of water was deep black or black and emittedobnoxious smell. It was found in the study that the temperature, transparency, TDS, EC, pH, DO, alkalinity, hardness, NO_3^-, Cl^- and BOD were ranged from 31.5-34.6^{0}C, 15.25-27.30 cm, 291-781 ppm, 558-2664 ppm, 7.57-8.60, 1.4-3.6 ppm, 123-435 ppm, 203-332 ppm, 0-89.13 ppm, 51.68-237.36 ppm and 3.21-17.3 ppm, respectively. Most of the observed parameters have exceeded permissible limits except pH and Cl^- indicating higher concentrations of organic and inorganic pollutants present in the river.According to the study,significant strong positive correlations were found in temp. vs pH (r= 0.774, p< 0.05), temp. vsCl^- (r= 0.954, p< 0.05), temp. vs BOD (r= 0.747, p< 0.05), TDS vs EC (r= 0.955, p< 0.05), pH vsCl^- (r= 0.808, p< 0.05), pH vs BOD (r= 0.864, p< 0.05) and DO vsNO_3^- (r= 0.758, p< 0.05) in March; temp. vsNO_3^- (r=0.915, p< 0.05), TDS vs EC (r= 0.949, p< 0.05), TDS vs DO (r= 0.765, p< 0.05) and DO vshardness (r= 0.745, p< 0.05) in April andtransparency vsNO_3^- (r= 0.906, p< 0.05), TDS vs EC (r= 0.922, p< 0.05), TDS vs pH (r= 0.836, p< 0.05), EC vspH (r= 0.982, p< 0.05) and pH vs hardness (r= 0.764, p< 0.05) in May. Significant strong negative correlations were found in EC vs alkalinity (r= -0.745, p< 0.05), DO vsBOD (r= -0.876, p< 0.05) and NO_3^- vs BOD (r= -0.765, p< 0.05) in March and TDS vsCl^- (r= -0.774, p< 0.05), EC vsCl^- (r= -0.758, p< 0.05), hardness vs BOD (r= -0.775, p< 0.05) and NO_3^-vsCl^- (r= -0.763, p< 0.05) in April.The hydrochemistry of the water body has revealed that the water is unsafe for human health, aquatic organisms and also for household, irrigational and industrial uses without proper treatment.*

Keywords*— *Sitalakkhya River, water quality, physicochemical parameter.

I. INTRODUCTION

Bangladesh is one of those polluted countries, which currently holds 1176 industries that discharge about 0.4 million m^3 of untreated waste to the rivers in a day [17]. In terms of quality, the surface water of the country is vulnerable to pollution from untreated industrial effluents and municipal wastewater, runoff from chemical fertilizers and pesticides, and oil and lube spillage in the coastal area from the operation of sea and river ports. Water quality also depends on effluent types and discharge quantity from different types of industries, types of agrochemicals used in agriculture, and seasonal water flow and dilution capability by the river system [7].

Dhaka city is surrounded by a number of rivers and canals of which Turag, Sitalakkhya, Buriganga, Dhaleshwari and Balu are the important ones. The surface water along these peripheral rivers of Dhaka city is known to be highly polluted due to municipal and industrial untreated waste waters that are discharged into these rivers [15]. Pollution is so severe in the Buriganga, Sitalakkhya and Balu rivers that it is almost impossible to treat the water for making it suitable for human use. For this critical situation, on September 2009, Department of Environment (DoE) declared Buriganga,Sitalakhya and Balu rivers as Ecologically Critical Area (ECA) [8].

The river Sitalakkhya is one of the most prominent rivers in the flood plain region of Bangladesh. It is located in Narayangonj City, the second most vital industrial zone of the country. In spite of being an Ecologically Critical Area huge quantities of untreated domestic and industrial wastes are being released every day in the Sitalakkhya River. The water quality of this river is deteriorating rapidly, especially during dry season at certain reaches of the river [1].

The wastes, effluents and agrochemicals contain heavy metals, toxic substances, germs and nitrogen containing toxic substances which pollute the natural system of Sitalakkhya River and it actually acts as a sink. Thus a serious environmental hazards is created which endangers human health and cause problems to aquatic lives. So, the Sitalakkhyariver needs attention to save it from destruction. The objective of the present study is to assess the water quality of Sitalakkhyariver by analyzing physicochemical parameters and make a temporal correlational analysis.

II. MATERIALS AND METHODS

2.1 Study area

The study was conducted from March to May, 2015 at the Sitalakkhya River in Narayangonj (Fig. 1). Sitalakshya River is a distributary of the Brahmaputra. In its initial stages it flows in a southwest direction and then east of the city of Narayangonj in central Bangladesh until it merges with the Dhaleshwari near Kalagachhiya. The river is about 110 kilometers (68 miles) long and at its widest, near Narayangonj, it is 300 meters (980 ft) across.

2.2 Sample collection

Water samples were collected from 6 sites of the river namely S-1 (Taraboghat), S-2 (Taitkkakhal), S-3 (Shenpara), S-4 (Kachpur Bazar) and S-5 (Monjilkhola) and S-6 (Shiddhirgonj) from March to May, 2015. Samples were collected in 250 ml black coated plastic bottles with three replications from each point. Prior to sample collection, all bottles were cleaned with distilled water.

2.3 Sample analysis

The water quality parameters such as temperature and pH were determined by the thermometer and digital pH meter (model-pH Scan WP 1, 2, Malaysia), respectively. Transparency was measued by secchi disc by following proper method [21]. Electrical Conductivity (EC) and Total Dissolved Solids (TDS) were determined by digital EC meter and digital TDS meter (model-HM digital, Germany), respectively. Dissolved oxygen (DO) was determined by digital DO meter (model-D. 46974, Taiwan). Alkalinity was measured by titration method and the EDTA method was used to determine the hardness of water. The biological oxygen demand (BOD) was measured by two steps incubation method [21 and 12].

2.4 Statistical analysis

The physicochemical parameters for all the study sites were analyzed by calculating Pearson's correlation coefficient (r) value.

III. RESULTS AND DISCUSSIONS

3.1 Physicochemical Properties of the Study Area

The observed water color was black in all sampling stations. But phytoplankton enriched dark greenish blue, red or brown color is good for fishes [6] and the standard is colorless [23].

During the study period (at all sampling stations) the maximum water temperature 34.6^{0}C was recorded in May (S6) and minimum 31.5^{0}C was recorded in March (S1) (Table 1). In case of river water temperature, the DoE standard for sustaining aquatic life is within 20 to 30^{0}C both in dry and wet season [4] and all the observed values exceeded the standard limit indicating higher level of pollutants in water. In the study, temperature showed significant strong positive correlation with pH ($r= 0.774$, $p< 0.05$), Cl^- ($r= 0.954$, $p< 0.05$) and BOD ($r= 0.747$, $p< 0.05$) in March and NO_3^- ($r=0.915$, $p< 0.05$) in April. A study found the temperature of the Sitalakkhya river varied from 20.5 to 31.3^{0}C [13]. Many mills and factories are constructed on the bank of this river who use the river's water for cooling purpose and also dispose the wastes into the river thus increase the temperature.

The maximum water transparency value 27.30 cm was recorded in May (S5) and minimum 15.25 cmwas recorded in March (S1) (Table 1). According to WHO, the standard value of transparency is 40 cm and transparency levels are low at all sampling points. In the study, transparency showed significant strong positive correlation with NO_3^- ($r= 0.906$, $p< 0.05$) in May.

The observed maximum TDS value 781 ppm was recorded in April (S1) and minimum 291 ppmwasrecorded in May (S1) (Table 1).High TDS value indicatesthe presence of an appreciable quantities of bicarbonates, sulfates and chlorides of Ca, Mg and Na [14].In the study, TDS showed significant strong positive correlation with ECin March ($r= 0.955$, $p< 0.05$), April ($r= 0.949$, $p< 0.05$) and May ($r= 0.922$, $p< 0.05$); DO ($r= 0.765$, $p< 0.05$) in April and pH ($r= 0.836$) in May and significant strong negative correlation with Cl^- ($r= -0.774$, $p< 0.05$) in April.A study found the TDS of the Sitalakkhyariver varied from 80 to 754 ppm [13]. Dumping of industrial wastes is mainly responsible for increasing TDS level in this river.

The observed maximum EC value 2664 µS/cm was recorded in March (S2) and minimum 558 µS/cm was recorded in May (S1) (Table 1).The standard value of EC is 250 µS/cm [23] and all the values were greater than the permissible limit indicating presence of ionic compounds as well as higher concentration of inorganic pollutants in

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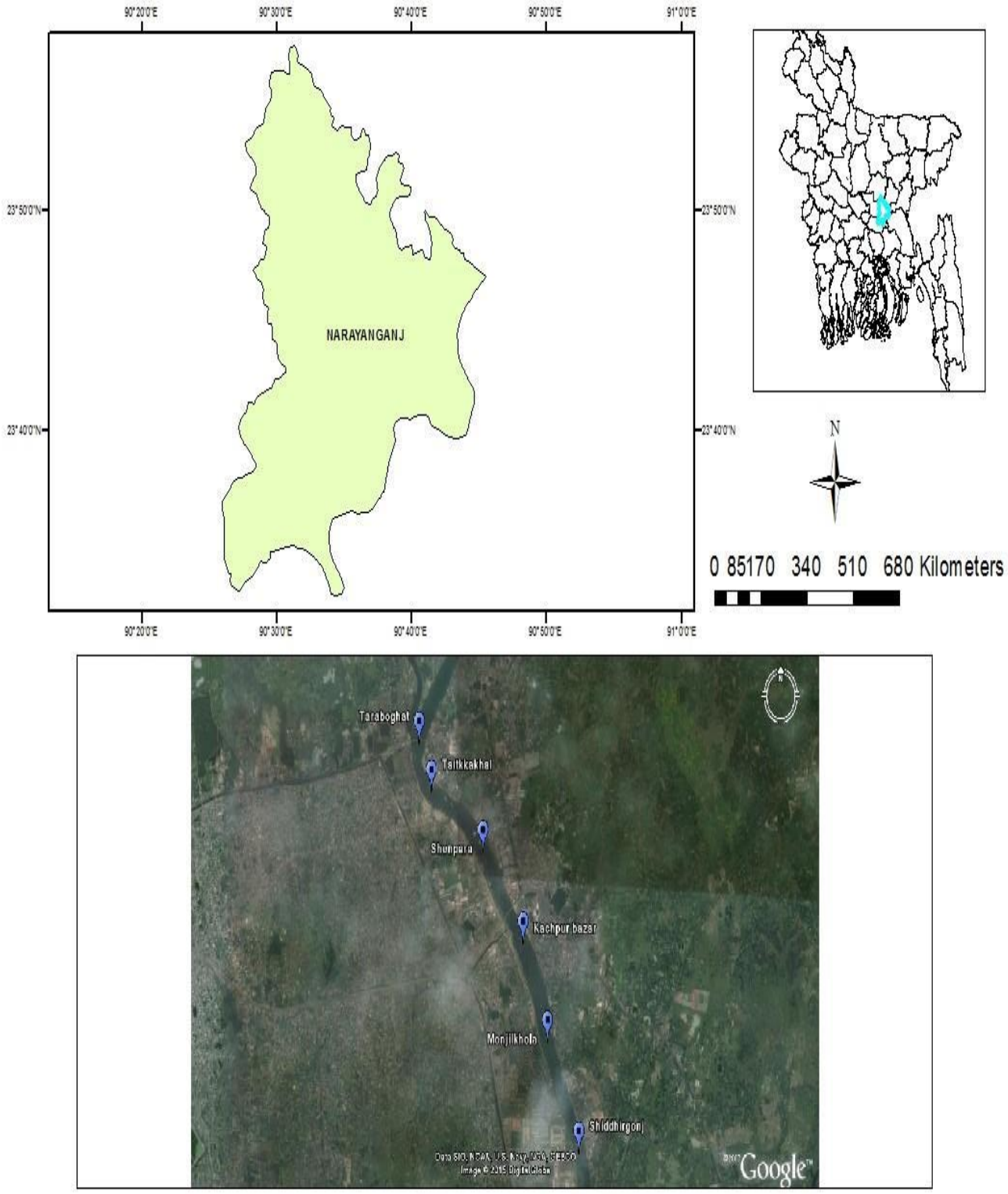

Fig. 1: Map showing the study area with sample collection points.

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water.In the study, EC showed significant strong positive correlation with pH (r= 0.982, p< 0.05) in May; significant strong negative correlation with alkalinity (r= -0.745, p< 0.05) in March and Cl^- (r= -0.758, p< 0.05) in April.A study found the EC of the Sitalakkhyariver varied from 121 to 1167 µS/cm [13]. Releasing chemical wastes containing ionic compounds causes increase in EC level in this river.

In this study, the maximum pH value 8.60 was recorded in April at Station- 2 (S2) and minimum 7.57was recorded in May at Station- 1 (S1) (Table 1) and all the observed values were within the standard range (6.5-9.0). In the study, pH showed significant strong positive correlation with Cl^- (r= 0.808, p< 0.05) and BOD (r= 0.864, p< 0.05) in March and hardness (r= 0.764, p< 0.05) in May. A study found the pH of the Sitalakkhya river varied from 6.9 to 8.0 [13]. Most of the industries use lime to neutralize the acidic condition of the effluents before releasing into the atmosphere thus the pH level of this river water is almost neutral.

The maximum DO value 3.6 ppm was recorded in May (S6) and minimum 1.4was recorded in March (S3) (Table 1). The standard range of DO for fish culture is from 5 ppm to saturation [16] and all the observed values were below the standard level. DO levels of 3 ppm or lower should be regarded as hazardous to lethal under stream and lake conditions [9]. Such low value of DO indicates presence of high organic compounds in water and doesn't support the survival of aquatic life. In the study, DO showed significant strong positive correlation with NO_3^- (r= 0.758, p< 0.05) in March and hardness (r= 0.745, p< 0.05) in April and significant strong negative correlation with BOD (r= -0.876, p< 0.05) in March. A study found the DO of the Sitalakkhyariver varied from 0.5 to 3.5 ppm [13]. Huge industrial and household loads are responsible for decreasing DO level in this river and also indicates huge microbial growth in water.

The maximum alkalinity 435 ppm was recorded in March (S4) and minimum 123 ppmwas recorded in May (S3) (Table 1). The standard level of alkalinity is <100 ppm [18] and all the observed values were higher than this standard value indicating high alkaline condition of water.

The maximum hardness 332 ppm was recorded in April (S2) and minimum 203 ppmwas recorded in March (S4) (Table 1). The counter-ions associated with the bicarbonate and carbonate fraction of alkalinity are the principal ions responsible for hardness [3]. The standard level of hardness is 123 ppm [12] and all the observed values were higher than this standard value. In the study, hardness showed significant strong negative correlation with BOD (r= -0.775, p< 0.05) in April. Increasing level of chemical wastes disposal causes high hardness level of water.

In the present study, the maximum Nitrate(NO_3^-) value 89.13 ppm was recorded in April (S3) and minimum 0 ppmwas recorded in March (S3 and S4) (Table 1). Nitrate reactions (NO_3^-) in fresh water can cause oxygen depletion. Thus aquatic organisms depending on the supply of oxygen in the stream can perish. Non-point pollution sources such as agriculture and livestock may have contributed to the increased NO_2^- and NO_3^- in the rivers of developing countries especially in Bangladesh and Indonesia [20]. Here, most of the observed values were higher than the standard NO_3^- value which is 10 ppm [22]. In the study, NO_3^-showed significant strong negative correlation with BOD (r= -0.765, p< 0.05) in March and Cl^- (r= -0.763, p< 0.05) in April. Disposal of wastes containing high ionic compounds causes high NO_3^- level.

The maximum Chloride(Cl^-) value 237.36 ppm was recorded in April (S2) and minimum 51.68 ppmwas recorded in March (S1) (Table 1). Here, all the observed values were within the standard level of Cl^- which is 600 ppm [23].

The maximum BOD value 17.3 ppm was recorded in April (S3) and minimum 3.21 ppmwas recorded in May (S2) (Table 1).The BOD values showed high fluctuations primarily due to the addition of effluent from industries and also because of dilution by river water [2].Unpolluted waters typically have BOD values of 2 ppm or less [5]. Here, most of the observed valueswere higher than the standard BODvalue which is 5-7 ppm [10] because of the discharge of huge untreated sewage in the study area.It also indicated the presence of comparatively more organic waste in the river water [19].High BOD level indicates high amount of biodegradable wastes in the river water.

3.2 Pearson Correlation

Pearson correlations were done among the analyzed physicochemical parameters of Sitalakkhyariver (Table 2, 3 and 4).

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Table 1. Variations in physicochemical properties of Sitalakkhya River (March-May, 2015).

Parameters	**Sampling Stations**	**March**	**April**	**May**	**Standards**
Temperature (^{0}C)	S1	31.5	32.3	33.5	20-30^{o}C [11]
	S2	32.6	31.8	34.3	
	S3	33.7	32.2	33.8	
	S4	33.4	32.2	34.3	
	S5	33.1	32.2	34.4	
	S6	33.4	32.4	34.6	
	Mean ±SD	32.95±0.80187	32.18±0.20	34.15±0.41352	
Transparency (cm)	S1	15.25	19.57	21.50	40cm [23]
	S2	16.25	22.15	25.25	
	S3	16.8	16.6	23.70	
	S4	18.35	19.1	19.50	
	S5	17.07	26.57	27.30	
	S6	15.75	19.50	22.75	
	Mean ±SD	16.58±1.09	20.58±3.42	23.33±2.75	
TDS (ppm)	S1	663	781	291	500pp [22]
	S2	729	394	300	
	S3	670	412	316	
	S4	675	618	319	
	S5	674	424	309	
	S6	656	420	326	
	Mean ±SD	677.83±26.07	508.16±157.25	310.16±12.92	
EC (µS/cm)	S1	1288	1184	558	250 µS/cm [23]
	S2	2664	738	581	
	S3	1267	772	615	
	S4	1298	1175	623	
	S5	1398	792	608	
	S6	1364	778	613	
	Mean ±SD	1546.5±549.70	906.5±212.23	599.66±24.95	
pH	S1	7.88	8.59	7.57	6.5-8.5 [22]
	S2	7.98	8.60	7.77	
	S3	8.30	8.58	8.00	
	S4	7.99	8.26	8.09	
	S5	8.04	8.58	7.97	
	S6	8.07	8.07	7.92	
	Mean ±SD	8.04±0.14	8.45±0.23	7.88±0.18	
DO (ppm)	S1	2.2	3.4	3.55	4-6 ppm [22]
	S2	2.1	3	2.55	
	S3	1.4	2.8	2.4	
	S4	2.0	2.9	2.9	
	S5	2.6	2.6	2.8	
	S6	2.3	2.9	3.6	
	Mean ±SD	2.11±0.13	2.93±0.27	3.13±0.21	

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Alkalinity (ppm)	S1	415	180	135	>100 ppm [18]
	S2	365	232	130	
	S3	385	236	123	
	S4	435	240	147	
	S5	425	212	162	
	S6	420	260	150	
	Mean ±SD	407.5±26.78	266.66±27.56	141.16±14.42	
Hardness (ppm)	S1	216	320	230	123 ppm [12]
	S2	208	332	243	
	S3	218	224	256	
	S4	203	294	251	
	S5	226	222	263	
	S6	215	232	238	
	Mean ±SD	214.33±8.02	270.66±50.56	246.83±12.16	
NO_3^-(ppm)	S1	17.05	86.16	46.43	10 ppm [22]
	S2	2.90	2.99	49.16	
	S3	0	89.13	47.33	
	S4	0	88.43	44.49	
	S5	17.22	85.55	48.29	
	S6	18.71	87.15	46.57	
	Mean ±SD	9.32±9.22	73.24±34.44	47.045±1.63	
Cl^-(ppm)	S1	51.68	197.51	136.39	600 ppm [23]
	S2	55.75	237.36	138.44	
	S3	66.63	209.75	137.95	
	S4	62.17	207.15	136.11	
	S5	63.4	215.27	139.01	
	S6	65.25	225.14	142.05	
	Mean ±SD	60.81±5.85	215.36±14.12	138.33±2.1	
BOD (ppm)	S1	8.1	9	6	5-7 ppm [10]
	S2	11	7.25	3.21	
	S3	17.3	14	6.87	
	S4	13.2	6.1	8.29	
	S5	9.5	10	7.38	
	S6	11	13.27	5.91	
	Mean ±SD	11.68±3.24	9.94±3.18	6.28±1.75	

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Table.2: Pearson Correlations among the different parameters of surface water in Sitalakkhyariver in March, 2015.

	Temp.	Transpar.	TDS	EC	pH	DO	Alkalinity	Hardness	NO_3^-	Cl^-	BOD
Temp.	1										
Transpar.	0.617	1									
TDS	-0.133	0.087	1								
EC	-0.2	-0.149	0.955*	1							
pH	0.773*	0.228	-0.176	-0.235	1						
DO	-0.343	-0.176	-0.038	0.076	-0.660	1					
Alkalinity	0.072	0.314	-0.724	-0.745*	-0.290	0.467	1				
Hardness	-0.016	-0.324	-0.387	-0.344	0.291	0.293	0.093	1			
NO_3^-	-0.413	-0.614	-0.483	-0.284	-0.395	0.758*	0.436	0.601	1		
Cl^-	0.954*	0.471	-0.367	-0.401	0.808*	-0.251	0.196	0.239	-0.173	1	
BOD	0.747*	0.475	-0.016	-0.150	0.864*	-0.876*	-0.298	-0.186	-0.765*	0.657	1

* Correlation is significant at 0.01 level (2 tailed).

Table.3: Pearson Correlations among the different parameters of surface water in Sitalakkhyariver in April, 2015.

	Temp.	Transpar.	TDS	EC	pH	DO	Alkalinity	Hardness	NO_3^-	Cl^-	BOD
Temp.	1										
Transpar.	-0.270	1									
TDS	0.345	-0.239	1								
EC	0.321	-0.263	0.949*	1							
pH	-0.529	0.270	0.058	-0.087	1						
DO	0.049	-0.384	0.765*	0.602	0.112	1					
Alkalinity	-0.005	-0.266	-0.668	-0.483	-0.693	-0.528	1				
Hardness	-0.530	-0.060	0.529	0.493	0.244	0.745*	-0.384	1			
NO_3^-	0.915*	-0.255	0.348	0.388	-0.339	-0.128	-0.072	-0.597	1		
Cl^-	-0.599	0.314	-0.774*	-0.758*	-0.131	-0.327	0.573	0.046	-0.763*	1	
BOD	0.508	-0.313	-0.425	-0.539	-0.149	-0.275	0.289	-0.775*	0.421	-0.005	1

* Correlation is significant at 0.01 level (2 tailed).

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Table.4: Pearson Correlations among the different parameters of surface water in Sitalakkhyariver in May, 2015.

	Temp.	Transpar.	TDS	EC	pH	DO	Alkalinity	Hardness	NO_3^-	Cl^-	BOD
Temp.	1										
Transpar.	0.264	1									
TDS	0.638	-0.168	1								
EC	0.591	-0.052	0.922*	1							
pH	0.554	-0.027	0.836*	0.982*	1						
DO	-0.010	-0.411	-0.019	-0.314	-0.437	1					
Alkalinity	0.629	0.193	0.314	0.342	0.326	0.360	1				
Hardness	0.312	0.479	0.373	0.678	0.764*	-0.718	0.300	1			
NO_3^-	0.099	0.906*	-0.377	-0.326	-0.296	-0.425	-0.155	0.187	1		
Cl^-	0.657	0.427	0.520	0.285	0.142	0.264	0.333	-0.018	0.347	1	
BOD	-0.021	-0.344	0.449	0.593	0.611	0.052	0.450	0.484	-0.686	-0.272	1

* Correlation is significant at 0.01 level (2 tailed).

IV. CONCLUSION

The present status of waste water management in Bangladesh is not satisfactory and most of the waste water is discharged indiscriminately without maintaining any sort of proper steps which is degrading the water quality critically. The present study was conducted to evaluate the physicochemical parameters of water collected from 6 sites of Sitalakkhyariver from March- May, 2015 and observe temporal changes of the parameters. According to the study, water temperature, transparency, TDS, EC, pH, DO, alkalinity, hardness, NO_3^-, Cl^-, BOD were ranged from 31.5-34.6^{0}C, 15.25-27.30 cm, 291-781 ppm, 558-2664 ppm, 7.57-8.60, 1.4-3.6 ppm, 123-435 ppm, 203-332 ppm, 0-89.13 ppm, 51.68-237.36 ppm and 3.21-17.3 ppm, respectively indicating presence of high organic and inorganic compounds or pollutants in water.

In the study, pH showed significant strong positive correlation with Cl^- (r= 0.808, p< 0.05) and BOD (r= 0.864, p< 0.05) in March and hardness (r= 0.764, p< 0.05) in May; DO showed significant strong positive correlation with NO_3^- (r= 0.758, p< 0.05) in March and hardness (r= 0.745, p< 0.05) in April; DO showed significant strong negative correlation with BOD (r= -0.876, p< 0.05) in March; hardness showed significant strong negative correlation with BOD (r= -0.775, p< 0.05) in April and NO_3^-showed significant strong negative correlation with BOD (r= -0.765, p< 0.05) in March and Cl^- (r= -0.763, p< 0.05) in April.

The present study found that the physicochemical condition of Sitalakkhya River was very poor in the month of March and April. But with the increase in the water flow the condition improved slightly in the month of May. From the compression with the previous year's data the study found that the situation is getting worse with time.

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Chemical Composition and Functional properties of *Caesalpina bonduc* and *Monodara myristica* seed flours

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***Abstract*—** *The chemical composition, nature minerals, anti-nutrients, functional and physicochemical properties of Caesalpina bonduc and Monodara myristica seed flours were determined. The results for proximate compositions in the samples were for Caesalpinia bonduc flour were moisture (7.89%), Ash (2.96%), Crude protein (18.26%), Crude fibre (12.54%), fat content (23.59%) and Carbohydrate (34.76%) while for Monodora myristica flour were: moisture (5.05%), Ash content (2.81%), crude fibre (4.69%), crude protein (24.62%), fat content (7.01%) and carbohydrate (55.82%). The mineral contents in (mg/l) for Caesalpinia bonduc and Monodara myristica seed flours were: K(36.68), (101.87), Ca(40.98), (59.43), Na (475.65), (463.57), Mg (13.20), (14.80), Zn (61.80), (66.20), P (18.5), (23.86), Ni (14.70), (13.40), Fe (12.80), (28.3), Cu (0.06) and (0.08) respectively. These results showed that the seeds samples contained some valuable minerals that are useful in bone formation as well as physiological and metabolic activities. The results of the functional properties for Caesalpina bonduc and Monodora myrictca seeds flours were: gelation capacities (51.40%), (53.80%), water absorption capacities (113.00), (121.50), emulsion capacities(56.25%), (56.00%), swelling capacities(7.10), (6.80) and oil Absorption capacities (137.50%), (115.50) and foaming capacities were (5.05) and (5.00) respectively. The anti-nutritents contents for Caesapina bonduc and Monodora myristica reported in this study were: Phytate (0.406%), (0.37%) Saponin (0.184%), (0.172%), Oxalates (0.068%), (0.056%), Tannins (0.083%) and (0.074%) respectively.This result suggested that the seed flours have low anti-nutritional content and may be useful in food formulations like doughes and baked products. It also confirmed that the seed samples may be good substitutes for wheat flour and Soy flour as extender in binder formulation.*

***Keywords*— chemical composition, functional properties, Caesalpina bonduc, Monodara myristica and seed flours.**

I. INTRODUCTION

Plants are primary sources of medicines, fibre, food, shelter and other items of everyday use by humans. The root, stems, leaves, flowers, fruits and seeds provide food for animals and human (Hemingway, 2004). Plants serve as indispensable constituents of diet supplying the body with mineral salts, vitamins and certain hormone precursors in addition to protein and energy (Oyenuga and Fetuga, 1975). Seeds possess nutritive and calorific values which make them necessary in diets (Odoemelarin, 2003). Among these plants are: *Monodora myristica* and *Caesalpinia bonduc* popularly known as African nutmeg and fever nut respectively.

Morphologically, African family is a berry that grows mild in the evergreen forest of West Africa (Burn *et al*, 2009). The seeds are economically and medicinally important (Okafor, 1987; Okigbo, 1977)

The kernel obtained from the seed is a popular condiment used as a spicing agent in both African and continential food in Nigeria

They are romantic and mostly used as condiment in food as flavouring agent. When in powdered form, may be taken as stimulant or stomadic to relieve constipation.

Caesalpinia bonduc is widely grown in India, in the plain waste lands and coastal areas. It is popularly referred to as "Ayo" in the southwest parts of Nigeria. The plant has a very large straggling thorny shrub, branches armed with hooks and straight hard yellow prickles. Fruits inflated pods cover with wiry prickles with seed grew with a smooth shinny surface. The seed is bitter but has no toxic effect on human body with consumption. The root of the plant is used in curing fevers, cough, and asthma while the leaves are

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useful in curing elephantiasis, intestinal worms and fever. However, there is limited information of the nutritional evaluation of these seeds. Thus, the main objective of this study is to investigate the nutritional evaluations for domestic and industrial applications.

II. MATERIALS AND METHODS

The samples used for the work were purchased in neighborhood market, Akure, Ondo State, Nigeria. The seeds were separated from the shells, dried and ground into flour, then packaged and stored in freezer at -4° prior to analysis. The oils from the samples flours were extracted by soxlet apparatus using n-hexane (40-60°C).

Proximate analysis

The moisture and Ash contents were determined using an oven and dry ashing method. (AOAC, 2005).

Crude protein and fat were determined according to the method described by (AOAC, 2005).

Nitrogen was converted to crude protein by multiplying by a factor of 6.25. The crude fiber was determined by adding 2g (w_1) of the sample into 500ml conical flask; 200ml of boiling 1.25% H_2SO_4 was added and boiled for 30 minutes. The mixture was filtered through muslin cloth and rinsed with hot distilled water. The sample was scrapped back into the flask and 200ml of boiling 1.25% NaOH was added and allowed to boil again for another 30 minutes.

Filtered and then rinsed with 10% HCl twice with industrial methylated spirit and allowed to drain and dry. The residue was scrapped into a crucible, dried in the oven at 105°C, allowed to cool in a desiccators and weighed (W_2); then placed in a muffle furnace at 300°C for 30 minutes and finally allowed to cool at room temperature and weighed (w_3) (AOAC, 2005).

$$\% \text{ crude fiber} = \frac{w_2 - w_3}{W_1} \times 100$$

The carbohydrate content was calculated by difference.

% carbohydrate = (100 – (% moisture + %ash + % crude fiber + % crude fat + % crude protein).

Minerals Analysis

The minerals were analysed by dry ashing the samples at 550°C to constant weight and dissolving the ash in 100ml standard flask using dionized water with 3ml of 3MHCl. Sodium and potassium were determined using Flame photometer (model 405, Corning, UK). All other minerals were determined by Atomic Absorption Spectrophotometer (Perkin and Elmer model 403, USA), (AOAC, 2005; Pearson, 1976).

Protein functional properties determination

Water absorption capacities and oil absorption capacities of *Caesalpinia bonduc* and *Monodora myristica* seed flour were determined by using the combination of the procedure of Shathe *et al* (1982) and Sisulki (1962)

The bulk densities of seed flours were determined using the standard analytical recommended by Narayana and Marasinga Rao (1984) with little modification. The least gelation and foaming capacities of seed flour were determined by modifying the method of (Offman and Garcia, 1977). The emulsion capacities for *Caesalpinia bonduc* and *Monodora myristical* seed flours were also determined using the procedure of AOAC (1990).

Anti nutrients determination

The anti nutritional contents of the seed sample flours were determined using the standard analytical method recommended by AOAC (2005). These include; phytate, oxalate, tannin, saponins contents of the flour samples.

III. RESULTS AND DISCUSSION

Table 1 showed the proximate compositions of *Caesalpinia bonduc* and *Monodora myristica* seeds flours. The protein content (18.26%) and 24.62%) for *C. bonduc* and *M.myristica* were a significantly lower than (44.5%0 reported for *Afzehia africana* (Ogungbele, 2014), but were in close range with water melon (24.30%) limon bean flour (22.7%). and pigeon pea (22.4%) reported by Oshodi *et al*, (1989). However these values were significantly higher than those of cereal crops (quinoa, 6.3% (Ogungbenle), pear millet (7.6%) (Oshodi *et al.*, 1999). Protein serves as the building block of the body. This showed that only M.myristica can contribute significantly to human daily requirement of 23-56g of protein (Witto, 1973). Fat is essential in the diet as it increase the palability of food. The fat content (34.4%) for *M.myristica* seed flour was significantly higher than that of *C.bonduc* seed flour (7.01%). This value was lower than that of periwinkle (74.74%), (Ogungbenle, 2012), gourd seed (50.5%) and yellow melon (51.9%) reported by Ogungbenle, 2003) but significantly higher than (18.9%) *Afzelia africana* seed. (Ogungbenle, 2004). This suggested that *Monodora myristica* seed flour can be grouped as oil-rich crop.The ash content of *C. bonduc* and *M. myristica* seed flours were in close rangeHowever, these values (2.96%) and (2.81%) in this study were lower than (4.93%) reported for *Afzelia africana* (Ogunbenle, 2014). Ash is an indicator of the quality of inorganic compounds (minerals) in the sample. The recommended value for Ash in nuts seed and tubers should fall in the range 1.5- 2.5% (Pomeranz and Clifton, 1981). These values fall within this range, this suggested

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that, they can be recommended for animal feeds. The carbohydrates contents for the samples, *C.bonduc* (34.76%), *M. myristica* (55.82%) and crude fibres contents (12.54%) and (4.69%) were significantly higher than the values (10.06%) and (2.6%) for Huran crepitan seed flour reported by Adeleke *et al*, (2009). The carbohydrate helps to regulate protein and fat metabolism. The high values suggested that both *Caesalpina bonduc* and *Monodara myristica* seed flour will be a source of energy for daily requirement.

The mineral contents (*mg/kg*) of *Caesalpina bonduc* and *Monodora myristica* seed flours were shown in Table 2.The least abundant minerals are Cu, Ni, Cr and Fe. While sodium was found to be the most abundant mineral (475.65), and (1264.19) for *Caesalpina bonduc* and *Monodara myristica* seed flours respectively. Sodium plays crucial role in maintaining osmotic pressure of the body fluid which protects the body from excessive fluid loss. It deficiency leads to muscular weakness and mental convulsion.

Calcium, an important mineral required for bone formation and neurological function was found to be present at significant level especially in Monodora myristica seed flour (121.45%) that of *Caesalpinia bonduc* seed flour with value (40.98).However, this value was lower than melon (130.7 mg/kg) and higher than pumpkin (*72.3 mg/kg*) and gourd seeds (54.9 mg/100g) respectively reported by Olaofe *et al*, 1994). The mean values of potassium in this study were for C. bonduc (36.68) and M.myristica (*353.93mg/mg*). It has been reported that magnesium as an activator of many enzyme systems and maintains the electrical potential in nerves. Phosphorous is always found with calcium in the body both contributing to the food Ca/K ratio is *Monodora* seed and greater than 1. Thus suggest that they would serve as good sources of mineral for bone formation.

Table.1: Proximate compositions of C.bonduc and M.myristica seed flours

Parameters	*Caesalpinia bonduc*	*Monodora myristica*
Moisture	7.89 ± 0.00	4.88 ± 0.13
Crude fat	23.59 ± 0.00	34.44 ± 0.01
Crude Protein	18.26 ± 0.10	10.79 ± 0.10
Ash	2.96 ± 0.04	5.99 ± 0.13
Crude fibre	12.54± 0.01	35.85 ± 0.10
Carbohydrates	34.76 ± 0.00	8.05± 0.01

Table.2: Mineral compositions of Caesalpinia bonduc and Monodora myristica seed flours.

Parameters minerals (mg/g)	Caesalpiniabondue seed flour(mg/g)	Monodoramyristica seed flour (mg/g)
Potassium	36. 68 ±0.02	353.95 ±0.10
Calcium	40 98 ±0.05	121.45 ± 0.021
Sodium	475.65±0.39	1264.19±0.03
Magnesium	13.20 ± 0.01	13.90 ±0.02
Zinc	61.80 ±0.02	21.50 ± 0.03
Phosphorous	18.5 ±0.10	17.2 ±0.20
Nickel	14.70 ± 0.00	16.60±0.61
Ferrous	12.8 ±0.30	13.70 ±0.15
Chromium	12.8 ±0.01	13.7 ± 0.15

Table 3 presents the results for protein functional properties of *Caesalpinia bonduc* and *Monodora myristica* seed flours. The result for water absorption capacities were *Caesalpinia bonduc* flour (113.00) and *Monodora myristica* seed flour (121.50). However, these values were lower compared to the value reported for defatted gourd seed (199.00), white melon (221.0) Ogungbenle,(2004).Water absorption capacity is a critical function of protein in various foods like doughes and baked products (bread and cakes). Hence, these seed flours may be useful for these food formulations (Oshodi *et al*., 1999). The foaming capacities for the samples were Caesalpina bonduc (5.05%) and Monodora myristica (8.16%) respectively. These value were significantly lower than those of soya bean flour(70.0%),sun flower (23.0%),reported by Linn *et al*, 1974 and pigeon flour (68.0%)reported by Oshodi and Ekpengin, (1989). These low values reported in this study suggested that the seed flour samples may not be rich in

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flexible protein in molecules which rapidly reduce the surface tension to give a foaming ability (Gration and Plutips, 1980).

The emulsion capacities values were; *Caesalpina bonduc* (56.25%), *Monodora myristica* flour (55.77%).these value were lower than Africa yam bean flours (90%) and (95.10%)reported for sun flower flour by Linn *et al* ,1974). However ,the values were higher than soy flour (18.00%),wheat flour (7.0-110%)as reported by Linn *et al*, 1974) and (49.40%)reported by Fagbemi and Oshodi (1991) which suggested that they may be good substitutes for wheat flour ,Soy flour and full fat flulted pumpkin as meat additives/extender and binder formulation.

The oil absorption capacities were; *C.bonduc* (137.5%) and *M. myristica* (182.5%) respectively. These values in the present study showed that the seed flours have high oil absorption capacities and would be very useful in food formulation.

Table.3: Protein functional properties (composition) of C.bonduc and M.myristica seed flours.

Parameters	*Caesalpinia bonduc* seed flow	*Monodora myristica* seed flow
Loosed Bulk density	0.4878± 0.01	0.4348 ± 0.02
Packed bulk density (s/me)	0.625± 0.01	0.5714± 0.00
Gelation capacity (%)	51.40± 0.33	56.20± 0.01
Water absorption capacity (%)	113.00±0.05	121.50± 0.01
Oil Absorption capacity (%)	137.50± 0.5	182.50± 0.20
Emulsion capacity (%)	56.25± 0.10	55.77± 0.02
Foaming capacity (%)	5.05±0.04	8.16±0.02
Selling capacity	7.10±0.10	6.60±0.01

Table.4: Antinutrients composition of C. bonduc and M.myristica seed flours.

Parameters (%)	Caesalpinia bonduc seed flour	Monodora myristica seed flour
Phytate	0.406 ± 0.02	0.371± 0.02
Saponin	0.184 ± 0.01	0.172± 0.01
Oxalate	0.068 ± 0.00	0.056 ± 0.00
Tannins	0.083 ± 0.00	0.074 ± 0.00

The results in Table 4 showed that the seed flours have a very low level of tannins (%) *C. bonduc* (0.083%) and *M.myristica* (0.074%) and Oxalates (0.68%) and 0.056%) respectively. Tannic acid (tannins) inhibit minerals especially iron (Fe) in human body. It also forms complex with proteins, starch, cellulose and minerals.

Also, saponins values for seed flours were *C.bonduc* (0.184%), *M.myristica* (0.172%) respectively. These values were however lower when compared with values reported for *S.nignum* seeds (0.66%) by (Akutugwo *et al*, 2007). Saponin has been reported to possess but beneficial and deleterious properties and exhibit structure on biological activities (savage 1993). The phytate levels were; (0.406%) and (0.371%) for *C.bonduc* and *M.myristica* seed flours. A high concentration of phytate could cause adverse effect in the digestability (Akintayo and Bayer, 2002). It also forms complexes with Zn^{2+}, Fe^{2+} and CO^{2+}. However, these values were significantly lower than 1.0% recommended value.

IV. CONCLUSION

This present study suggested that *Ceasalpinia bonduc* and *Monodora myristica* seed flours were rich on important foods nutrients compared to some oil seeds and nuts. The protein functional properties result showed that the seed flour might be a good substance in food formations such as doughes (breads and cakes).

Also, the anti-nutrients analysis showed that seed flours contained low concentrations of anti-nutrients, thus allowing the proper intake of valuable minerals when consumed.

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Succession of Plant Spesies Following a Forest Fire on Mount Talang, West Sumatera

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Abstract**—** *This research was conducted from May to July 2018. Observation of vegetation was carried out in the ex-fire of Talang Mount area, while identification of vegetation was carried out in the Herbarium Biology Departmen, Faculty of Science and Mathematics Andalas University. The purpose of this study is to identify vegetation that grows after forest fire, to determine the effect of forest fire on the succession processes. Vegetation analysis in natural forest and post-combustion forest was carried out by making observation plots of vegetation with map sizes for seedling level of 2mx2m, sapling level of 5mx5m, pole level of 10mx10m and tree level with a size of 20mx20m. Observation data is carried out with important value index analysis, There are 22 identified vegetations that grow after forest fire. The influence of forest fire on the existence of the succession process indicated by the presence of plants that grow in the post-burning forest area, namely Similac sp and A6 plants.*

Keywords**— Analysis of vegetation, natural forest, post-burnt forest.**

I. INTRODUCTION

Indonesia is one of the countries included in megadiversitas, which is a country that has a high diversity of flora. Forest is a natural resource which is very important because it constitutes a biological diversity as a source of germplasm, the source of the seeds of natural vegetation, the source of forest products such as timber and non-timber, regulating water management, protection against floods and erosion as well as soil fertility, protection of biodiversity for interests of science, culture, recreation, tourism, and so on (Istigono, 2004).

Mount Talang is one of the active volcanoes located in West Sumatra and is administratively located in the district of Koto Anau, Solok District. This mountain has the altitude (elevation) where 2,597 m asl (PVMBA, 2007). On February 1, 2018 Mount Talang forest area affected by fire, the fire started from the top of the mountain and then spread to the southern and eastern slopes of Mount Talang. The negative impact caused by fire in the mountain area is certainly large enough cover damage to parts of the surrounding forests, declining biodiversity, the loss of the seeds of natural vegetation, declining economic value of forests and soil productivity.The impact of wildfires covering all aspects of the ecosystem such as fauna, soil, water, climate, air, and humans. Moreover, the response to the forest fires will impact on the reduction of plant species composition, decrease in the quality and quantity of tree stands caused by heat. Due to the warming fire, the plant's metabolic processes will be disrupted and damaged even death. On the other hand the impact of forest fires will encourage various forms of adaptation of plants to fire, including promoting germination, seed dispersal, and breaking dormancy of certain seeds.

Utomo (2013) adds that the next generation that will appear is determined by the compatibility of the seeds in the ground to be able to grow and flourish. Information about seed reserves in the soil is important in the study of the ecology of an ecosystem because it can describe the existing vegetation on it and also to determine the potential of other types of plants that will grow in these habitats. Ore reserves in secondary forests play an important role as a source of seed to plant colonization process in the succession process.

An examination of the structure and composition of species, forest fires will affect the succession, which would allow the emergence of new types of vegetation and the loss of vegetation types existing in the region. Rahmasari (2011) adds that the succession is happening is an attempt to restore the ecosystem of the environmental conditions both biotic and abiotic components components. The succession of changes can be seen from the composition and structure of the forest vegetation.

Based on these descriptions, the authors conducted a research on "Succession of Plants Spesies Following A Forest Fire on Mount Talang, West Sumatera".

II. MATERIALS AND METHODS

This research was conducted from May to July 2018. Observation of vegetation was carried out in the ex-fire of Talang Mount area, while identification of

vegetation was carried out in the Herbarium Biology Departmen, Faculty of Science and Mathematics Andalas University. While the tool needed is a map of the location of the study, the Global Positioning System (GPS), thermo-hygrometer, rope, scissors, tape measure, compass, machete, commando knives, drill belgi, camera, identification books and stationery vegetation. Vegetation analysis in natural forest and post-combustion forest was carried out by making observation plots of vegetation with map sizes for seedling level of 2mx2m, sapling level of 5mx5m, pole level of 10mx10m and tree level with a size of 20mx20m. Observation data is carried out with important value index analysis (Soerianegara and Indrawan, 1988).

With the formula:

Important Value Index = Relative Density + Relative Frequency (For seedlings and saplings)

Important Value Index = Relative Density + Relative Frequency + Dominance Relative (For pole and tree level)

III. RESULT AND DISCUSSION

3.1 Identification of the Vegetation

Based on the observations of vegetation were carried out in the field, found some vegetation after a forest fire, all the vegetation is identified in the Herbarium Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences (MIPA) Andalas University and there is one type of vegetation that can not be identified due to organ reproductive yet complete and include the type of vegetation that is rarely found in tropical rain forests. Vegetation identified species that grow after a forest fire can be seen in Table 1.

Based on observations in natural forests and forest after the fire, suspected early on natural forest vegetation is similar to the post-burn forest vegetation because of the condition of forest belonging to the secondary forest with the ambient temperature and humidity averaging nearly the same, namely 15.1ºC - 19.1ºC and an average humidity of 68%. Presumably because of the overlay in a forest ecosystem that dominates the vegetation growing on post-burn forest areas is similar to natural forests.

The existence of the new vegetation to grow after the fire indicates that fires can influence the seed in the soil deposits to encourage various forms of adaptation of plants to fire, including breaking dormancy of certain seeds. The presence of certain types of seeds are small and lightweight due to the condition of post-burn forest area that opens make the seed easily blown by the wind and can grow back. Utomo (2013) states that the generation plants that appear after fires compatibility is determined by seeds grains to grow and thrive. Ore reserves in secondary forests play an important role as a source of seed to plant colonization process in the succession process.

3.2 Analysis Of Vegetation

Based on observations of vegetation analysis performed on the natural forest area and forest area after the fire, the vegetation found seedlings, saplings, poles and trees. Importance Value Index (IVI) seedlings in natural forests and post-burn forest can be calculated from the sum of the relative density with relative frequency. In plants, the seedlings, the index value is important in natural forests and post-burn forest can be seen in Table 2.

In Table 2 it can be seen that the Family Poaceae can be found in natural forests and forest after the fire. Percentage of important value index *Melastoma trachyphyllum* plant on post-burn forest areas is also higher than the natural forest natural forest I and II. The high importance value index may show a mastery or domination is also high, this shows that the plant is a plant *Melastoma trachyphyllum* pioneer or a beginner who first occupy an open environment after the fire.

Similac sp and A6 plant are growing vegetation after forest fires that are not found in natural forests natural forests I and II. The existence of the new vegetation to grow after the fire indicates that fires can influence the seed in the soil deposits encourage the various forms of plant adaptations to fire. Pictures of *Similac* sp and A6 plants can be seen in Figure 1

Aciana *et al*., (2017) stated that the succession is a process that affects the turn of plants within a certain period. Then Saharjo and Cornelio (2011) adds the cause of the species composition of vegetation changed after the fires allegedly spread along the wind, birds and other animals that eat grains or fruits that help in the speed of growth of new vegetation in the area.

In plants saplings, the index value is important in natural forests and forest post-burn seen in Table 3.

In Table 3 it can be seen that no plant saplings growing in the forest on fire after post-burn forest I and II. This happens because the plant saplings belonging to natural regeneration saplings taller than 1.5 m with a trunk diameter of less than 10 cm, and this is also due to the short time period at the time of observation after the fire broke out three months makes no vegetation included in the category of saplings. In Table 3 types *Gahria javanica* explain important value index is the highest, followed by the type of *Melastoma trachyphyllum*, it indicates that the type of *Gahria javanica* and *Melastoma trachyphyllum* grow to dominate on both natural forest.

Importance Value Index poles on natural forests and post-burn forest seen in Table 4. In Table 4 it can be seen that there is no plant small trees growing on post-

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burn forest I and II post-burn forest. This happens because the poles are a category of young trees that have a trunk diameter of 10-20 cm. It is also due to the short time period at the time of observation after the fire broke out three months makes no vegetation types included in the category of small trees. This also shows that due to a fire will cause a great impact. This impact will cause the extinction of species of flora that existed before.

The highest index of significant value to both natural forest dominated by family Rhododendrum, then the type of *Vaccinicum vangiaefolium*, *Pandanus tectorius* and *Ficus microcarpa*. The dominant species in a plant community will have an important value index is high, so that the most dominant species of course have an index value of the greatest importance.

Important value index tree level in natural forests and post-burn forest can be seen in Table 5.

In Table 5 it can be seen that the percentage index of the highest importance which both natural forests are a type of plant *Vaccinicum vangiaefolium*. Index values of high importance can show a mastery or domination is also high. In Table 5 it can be seen that there is no vegetation level forest trees that grow on the first post-burn and post-burn forest II. This happens because the level of the tree is a category of adult trees have a diameter of more than 20 cm. With this condition requires a long time to be able to grow into a new plant within a time span of less than one year after the occurrence of a fire. Another possibility is happening is for a complex process of secondary succession requires a fairly long period of time or decades to reach balance or homeostasis.

Resosoedarmo et al., (1986) adds the speed of the succession process is also influenced by several factors including, the early community area damaged by disturbances. The more narrow area of land damage, the faster the process of community dynamics occur. Second, the plant species around the increasingly diverse causes more rapid succession process of vegetation. Thirdly, their presence would be life (seeds, fruits, spores, etc.) which is the main object in the succession of vegetation as well as the diffusion rate. Fourth, the newly formed substrate type also plays a role because it serves as a place to grow for the new plant, the better the quality of the substrate then the better the speed of succession. Fifth, the climatic conditions, especially the wind speed direction with seeds (seeds, fruits, spores, etc).

Table 1. Type of vegetation after wildfires

No.	Family / species	No.	Family / species
1.	*Poaceae*	13.	*Cyperus iria*
2.	*adiantaceae*	14.	*Cyperus kyllingia*
3.	*Gleiceniaceae*	15.	*Cyperus rotundus*
4.	*Ficus microcarpa*	16.	*Asystasia gangetica*
5.	*Melastoma trachyphyllum*	17.	*Gahria javanica*
6.	*Similac sp*	18.	*Croton hirtus*
7.	*Graptopyllum pictum*	19.	*Althernanthera sessilis*
8.	*Podocarpus neriifolius*	20.	*Fimbristylis mileacea*
9.	*Swietenia macrophylla*	21.	*Cleome rutidosperma*
10.	*blechnum vulcanicum*	22.	*Cleome gynandra*
11.	*Peperomia pellucida L*	23.	A6
12.	*Borreria latifolia*		

Description: A6 = species not yet identified its kind

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Table 2. Importance Value Index for seedlings in natural forests and forest post-burn

Family / Species	Importance Value Index (IVI)%			
	Natural forest I	Natural forest II	Post Burned forest I	Post Burned forest II
Poaceae	55.86	60.38	44.41	65.00
Gleiceniaceae	31.98	46.56	13.68	-
adiantaceae	10:13	-	13.68	-
lycopodium cernum	26.58	33.40	-	-
Melastoma trachyphyllum	-	19.66	102.06	98.33
Allaeophania rugosa	21.84	-	-	-
Similac sp	-	-	-	36.67
Graptopyllum pictum	10:13		13:09	-
blechnum vulcanicum	27.48	39.98	-	-
Gaultheria leucocarpa	15.99	-	-	-
A6	-	-	13:09	-
Total	199.99	199.98	200.01	200.00

Description: A6 = Species of unknown species

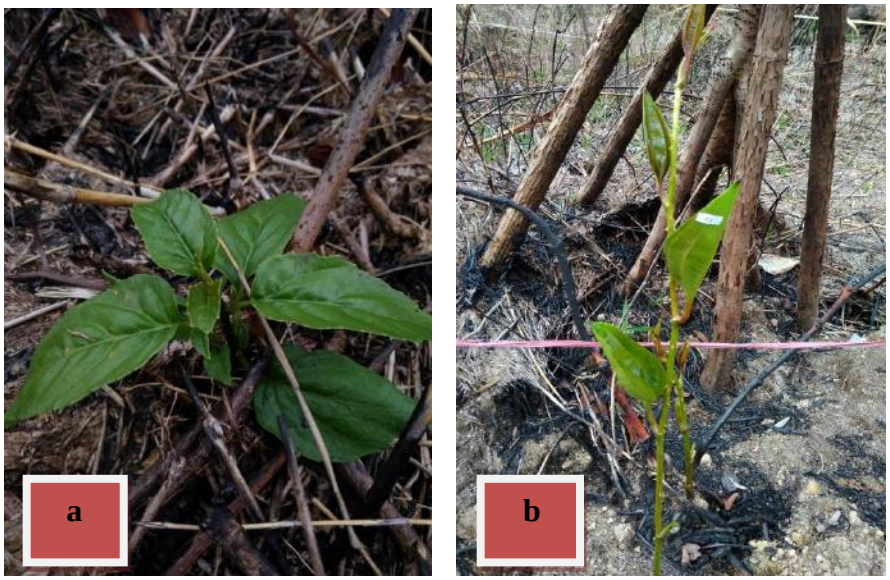

Fig.1: Vegetation growing seedlings after forest fires that are not found in natural forests. a) A6, b) Similac sp

Table 3. Importance Value Index saplings on natural forests and post-burn forest

Family / species	Importance Value Index (IVI)%			
	Natural forest I	Natural forest II	Post Burned forest I	Post Burned forest II
Melastoma trachyphyllum	38.99	48.19	-	-
Gahria javanica	86.90	69.80	-	-
Rhododendrum	37.21	-	-	-
Ficus microcarpa	36.90	-	-	-
Anaphalis javanica	-	42.35	-	-
A7	-	39.64	-	-
Total	200.00	199.98	-	-

Description: A7 = Species of unknown species

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Table 4. Importance Value Index poles on natural forests and post-burn forest

Family / Species	Importance Value Index (IVI)%			
	Natural forest I	Natural forest II	Post Burned forest I	Post Burned forest II
Rhododendrum	94.88	82.55	-	-
Vaccinicum vangiaefolium	76.99	57.98	-	-
Pandanus tectorius	68.38	62.00	-	-
Ficus microcarpa	59.75	52.12	-	-
Litsea Sp	-	45.26	-	-
Total	300.00	299.91	-	-

Table 5. important value index tree level on natural forests and post-burn forest

Family / species	Importance Value Index (IVI)%			
	Natural forest I	Natural forest II	Post Burned forest I	Post Burned forest II
Vaccinicum vangiaefolium	68.40	55.87	-	-
Podocarpus elangatus	55.37	52.61	-	-
Eucalyptus alba	55.87	45.28	-	-
Ficus microcarpa	60.53	47.25	-	-
Litsea sp	-	44.01	-	-
Macaranga mauritania	59.82	54.98	-	-
Total	299.99	300.00	-	-

IV. CONCLUSION

There are 22 identified species of vegetation that grow after a forest fire. The influence of forest fires on the succession process as indicated by the type of plant that grows in the post-burn forest areas namely *Similac sp* and A6 plants at the seedling stage that does not exist in natural forests and the increase in the percentage of importance value index (IVI) plant seedlings on post-burn forest like plants *Poacea* and *Melastoma trachyphyllum*.

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Adaptation of Variety Diversity on Growth and Production in the Area of Onion Plant Marginals (*Allium ascalanicum* L.)

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***Abstract*—** *The study aims to obtain variety with broad adaptations that are able to grow and produce optimal production on marginal fields of onion planting, which can improve farmers' welfare, reduce dependence on red onion imports, become exporters of onion to food sovereignty. The method that will be used is a Randomized Block Design (RBD) method consisting of five red onion variety as treatment, namely BimaBrebes, Tajuk, Bauji, Super Philip, and Local Enrekang variety. Each treatment was repeated three times. Data processing with SPSS Software, Version 22, and if there is a real influence, then a different test is performed with the Duncan Test at the level of ap= 0.05. The research was carried out on the experimental land of STIP PuangrimaggalatungSengkang. The parameters observed consist of the growth and production of onion plants. The results of the analysis showed that the onion variety that was quite adaptive was BimaBrebes variety onion and was able to produce an average production of 12.08 t ha^{-1}.*

***Keywords*—Adaptation, Onion, Marginal, Growth, Production.**

I. INTRODUCTION

Wajo is one of the districts that are considered marginal to the onion crop, because farmers do not know and are not touched by the cultivation of onion. There needs to be an effort to adjust the environment of some onion varieties to the location to be cultivated by conducting adaptation tests in the form of research. According to the Census in 2011, the consumption rate of red onion in Indonesia per capita per year reached 4.56 kg or 0.38 kg per capita per month (BPS, 2015). In order to meet the needs of the growing onions hence the need for a breakthrough technology that can improve the cultivation of onion production (Rahayu et al., 2016).

Adaptation of variety in the area considered marginal to the onion crop is early idea to support the technology development and land productivity as well as a vehicle of information science and knowledge. According to Kelvin (2014), onion cultivation technology development requires dissemination to farmers, including high yielding variety with productivity. According toIndah (2016), high-yielding variety of onion expected is adaptive variety that have high productivity, harvesting early maturing, resistant to pests and diseases, able to grow in the growth environment or agroekologi and quality of tubers in accordance with the wishes of consumers. Sembrani, Tuk-Tuk, Kuning and Medan variety have been able to adapt in the lowlands. Medan variety with a production range of 6.40-7.45 tons / ha (Sinaga et al., 2013).

Asrijal et al. (2018), onion that has the ability to grow quite well is onion of Bima variety, when compared with onion of Tajukand Super Philip variety, with the average yield at 10:43 t ha^{-1}. Better production in Moujung and Sumenep variety, namely production of 6.34-8.02 t ha^{-1}. Variety that are tolerant of Fusarium wilt attacks areMoujung (attack rate is 1.84%). while tolerant to Alternaria is Moujung and Sumenep (attack rate 9.60% - 15.86%) (Purbiati et al., 2010) ..successful growth until onion production, Besides is affected by the potential and quality of seeds or seedlings are sourced of various types of variety, also very dependent on the potential of the environment as a place of planting, including the altitude of the place, the condition of the climate and the level of soil fertility.

According to Suryana (2008), plants grow and develop fertile if the nutrients provided can be absorbed by a plant and in the form needed for its growth. Napitupulu and Winarto (2009), nutrients that are sufficient for onions can increase the weight of tuber crops. Damanik et al. (2010), potassium is needed for the process of photosynthesis and can increase the weight of onion bulbs. Furthermore, Fatmawati et al. (2015) NPK compound fertilizer can affect the growth and yield of onion, and can provide increased yields. According

Asrijal et al. (2018) red onions need fertile soil fertile and high in organic matter with the support of sandy clay or clay dust.

II. MATERIALS AND METHODS

This research was carried out on the experimental field of STIP PuangrimaggalatungSengkang. Using Randomized Block Design (RBD) consisting of five treatments of shallot varieties, namely the BimaBrebes, Tajuk, Bauji, Super Philip, and Enrekangvarieties, the treatment was repeated three times. The parameters were observed consisting of growth and production of red onion. Processing data with SPSS Software, Version 22, and if there is a real effect, a different test is done with Duncan Test at the level of ap= 0.05 (Gaspersz, 1991).

III. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Plant height (cm)

Results of analysis of variance showed that the onion variety treatment of showed highly significant effect on the height meter onion plants aged 35 and 42 days after planting. Duncan test showed that the treatment of onion variety of significantly different from the onion treatment of Enrekang Local, Super Philip and Bauji variety. However, it is not significantly different from Tajuk varieties (Table 1).

Table.1: Average Plant Height (cm) Onion Age 35 and 42 Planting After Days (PAD) in the Treatment Various of Onion Variety.

Plant Height	Various of Onion Variety (V)					PAD
	v_1	v_2	v_3	v_4	v_5	
Average	24.43a	23.40 a	21.00 b	20.27a	21.42 b	35
NP Duncan α = 0.05	*1.65*	*1.72*	*1.76*	*1.78*		
Average	32.41a	29.70 b	27.30 c	26.31c	27.72 c	42
NP Duncan α = 0.05	*1.52*	*1.58*	*1.62*	*1.64*		

Description: The same letters in the same row no significant effect on Duncan test level a p=0.05

3.1.2. Number of tubers (tubers)

Results of analysis of variance showed treatment of various varieties of onion plants showed a significant influence on the number of tubers age 70 HST. Duncan's test showed that the treatment of Bima varieties was significantly different from Tajukvarieties, Enrekang Local varieties, Super Philip varieties, and Bauji varieties. While the Tajuk varieties are significantly different from the Super Philip and Bauji varieties. However, it was not significantly different from the Enrekang Local varieties (Table 2).

Table.2: Average Number of Onion Tuber (cm) Onion Age 70 PAD in the Treatment Various of Onion Variety.

Number of Tuber	Various of Onion Variety (V)					PAD
	v_1	v_2	v_3	v_4	v_5	
Average	8.11a	7.04 b	5.73 c	4.47d	6.50 b	70
NP Duncan α = 0.05	*0.534*	*0.555*	*0.568*	*0.577*		

Description: The same letters in the same row no significant effect on Duncan test level a p=0.05

3.1.3. Production per hectare

Results of analysis of variance showed that the treatment of onion variety various of plants showed a significant influence on the production per hectare at the age of 77 days HST. Duncan's test showed that Bima variety were significantly different from Enrekang Local, Super Philip, and Bauji variety. However, it is not significantly different from the treatment of Tajuk variety. While the variety of Tajuk is significantly different from the treatment of Super Philip and Bauji variety. However, it was not significantly different from the Enrekang Local variety (Table 3).

Table.3: Average Production per Hectare of Onion Age 77 PAD on Different Varietyof Onion

Production per hectare	Various of onionvariety (V)					PAD
	v_1	v_2	v_3	v_4	v_5	
Average	12.08a	10.07ab	7.30 c	6.14c	8.24 bc	77
NP Duncan α = 0.05	*2.077*	*2.160*	*2.211*	*2.243*		

Description: The same letters in the same row no significant effect on Duncan test level a p=0.05

3.2. Discussion

3.2.1. Plant height

The observation of high onion plants aged 35 and 42 dap real effect on the variety of plant onion. BimaBrebes variety of onion plants showed higher high of 32.41 cm, while the lowest is 26.31 cm Bauji variety. This is due to the growing environmental condition factor matching onion variety of BimaBrebes compared with the treatment of other onion variety. According to Indah (2016), that improved variety of onion are expected variety adaptive high productivity, harvesting early maturing, resistant to pests and diseases, able to grow in the growth environment or agroekologi, and quality of tubers in accordance with the wishes of consumers.

3.2.2. Number of Bulbs

The observation of the number of bulbs of onion crop at the age of 70 real hstberpengaruh on different variety of onion crop. The BimaBrebes variety shows the highest number of onion tubers, namely 8.11 tubers, while the lowest is Bauji variety which is 4.47 tubers. This is due to factors of soil fertility conditions suitable with onion variety of BimaBrebes compared with the treatment of other onion variety. According to Asrijal et al. (2018), onions need fertile soil fertile and high in organic matter with the support of sandy clay or clay dust. The type of soil is good for growing onions is no Latosol soil, Regosol, grumosol, and Alluvial with a degree of acidity (pH) 5.5-6.5 soil and drainage and aeration in the soil goes well, the soil should not be inundated by water because can cause decay in tubers and trigger the emergence of various diseases (Sudirja, 2007).

According to Asrijal et al.(2018), a plant will grow and develop fertile if the nutrients provided can be absorbed by a plant and in the form needed. Napitupulu and Winarto (2009) stated that nutrients that are sufficient for onions can increase the weight of tuber crops. Onion plants can thrive and produce well, must be planted in a place that meets the growing environmental requirements including climate and soil fertility (Nasrullah, 2010).

3.2.3. Production per hectare

The observation of production per hectare of onions at the age of 77 days has a significant effect on various onion variety. The BimaBrebes variety shows that the highest yield per hectare of onions is 12.08 t.ha-1, while the lowest is Bauji variety which is 6.14 t.ha-1. This is due to genetic factors in the BimaBrebes variety of onions suitable for the environmental conditions of plant growth compared to other onion variety. According to Asrijal, et al., (2018), the growth and production of onions is influenced by the potential and quality of seeds or seeds sourced from various types of variety. Better production in Moujung and Sumenep variety, namely production of 6,34-8,0 2t.ha^{-1}. Variety that are tolerant of Fusarium wilt attacks are Moujung (attack rate is 1.84%), while those tolerant to Alternaria are Moujung and Sumenep (attack rate of 9.60% -15.86%) (Purbiati et al., 2010).

IV. CONCLUSIONS

Onion adaptability is quite high in Wajo, onion variety that have high adaptability are the variety of BimaBrebes. Further research is needed to get maximum production by adding manure and the best growth regulator. The government is expected to support the achievement of onion production centers in Wajo.

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Effect of Chitosan and Chlorocholine Chloride on the Minituberization of Cocoyam (*Xanthosoma sagittifolium* L. Schott)

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Abstract*—This study was carried out in order to evaluate the effect of chitosan (1, 2 and 3gL^{-1}) and chlorocholine chloride (5, 10 and 15mgL^{-1}) on the minituberization of cocoyam (Xanthosoma sagittifolium). Results showed that both phytohormones reduced the growth cycle of cocoyam from 6 to 5months with the best results obtained at the concentrations 2gL^{-1} and 15mgL^{-1} for chitosan (CTH) and chlorocholine chloride (CCC) respectively. The average number of leaves was greater in CTH at 2gL^{-1} (3.80±1.40) and 10mgL^{-1}(3.60±0.70) for CCC. The optimal height of the plant for CTH was obtained at 1gL^{-1} (17.87±5.47 cm) and 15mgL^{-1} (21.15±1.99 cm) for CCC. The average leaves surface was greater at 1gL^{-1} (36.39±17.02 cm^2) for CTH and 15mgL^{-1} (25.87±5.62 cm^2) for CCC. The number of minitubers harvested as well as the percentage of tuberization was maximum with 29minitubers at 2gL^{-1} and 23 for 15mgL^{-1}.The size of the minitubers was best at 3gL^{-1}(2.60±0.26 cm) for CTH and 10mgL^{-1} (0.56±0.27 cm) for CCC. The mass of the minitubers increased with best results obtained at 3gL^{-1}(2.80±0.84g) and at 10mgL^{-1} (0.20±0.36g) for CTH and CCC respectively.*

Keywords*— *chitosan, chlorocholine chloride, minitubers, minituberization,Xanthosoma sagittifolium.

I. INTRODUCTION

Cocoyam (*Xanthosoma sagittifolium)* is among the world's six most important root and tuber crops [1].It is pantropical and has been domesticated in most communities in Oceania, Africa, and Asia [2]providing sustenance for over 400 million people [3,4].Africa is the major producer with West and Central Africa, notably, Nigeria, Ghana, and Cameroon contributing to over 60% of the total African production [5].Thus, the importance of cocoyam to regional food security cannot be overstated. In spite of this growing importance, the production of cocoyam has been stagnant for many years. This is mainly due to (1): the low productivity of planting material [6],(2): the low availability of traditional planting material (corm cuttings) and (3): viral and fungal infections [7].In Cameroon, the main pathogen of cocoyam is*Pythium myriotylum*, which causes root rot and is responsible for up to 90% loss in yield in some plantations[8].Meristem culture technique is used to produce plants free of viruses and fungi especially in vegetative propagated plants [9].Plant tissue culture techniques have becomeapowerful tool for propagation of cocoyam to overcomemany problems facingtraditional methods of propagation. Different explants were used to produce disease free planting materials [10].

Many authors have shown that it is possible to produce tubers in *in vitro* conditions and that the tubers can be considered as seeds through the technique of microtuberization (*in vitro*) or minituberization (*in vivo*) [11].It has been showed that the addition of silver nitrate in the medium of microtuberization permits to inhibit the activity of ethylene what favorizes the good unrolling of tuberization in Irish potatoes [12].In agriculture, chitosan is used primarily as a natural seed treatment and plant growth enhancer, and as an ecologically friendly biopesticide substance that boosts the innate ability of plants to defend themselves against fungal infections [13].Agricultural applications of chitosan can reduce environmental stress due to drought and soil deficiencies, strengthen seed vitality, improve stand quality, increase yields, and reduce fruit decay of vegetables [14].Soluble chitosan helps acclimatization *in vivo*, and increase yield and seed quality of minitubers of treatments of different concentrations with

best minituber number and yield in controlled conditions also increased as shown in the work done on potato by [15].Chlorocholine chloride (CCC) is a biosynthesized inhibitor widely used in tissue culture media to promote microtuber formation [16].Although CCC stimulates tuber initiation by recalcitrant genotypes, it can inhibit microtuber growth in *Solanum tuberosum* cultivars that form tubers readily in its absence [17].

This research was aimed at studying the effect of chitosan and chlocholine chloride on the minituberization of cocoyam (*Xanthosoma sagittifolium* L. Schott). More specifically; to produce vitro plants of white cultivars of *Xanthosoma sagittifolium*, introduce minituberization in *Xanthosoma sagittifolium* through the supply of chitosan and chlorocholine chloride.

II. MATERIAL AND METHOD

2.1. Cocoyam plantlets culture

The plant material constitute of cocoyam *vitro* plants obtained through *in vitro* culture of the apex of white cultivar of *Xanthosoma sagittifolium* harvested from the farm. The explants issued from plants were disinfected according to the method of [18]modified. After one month of in vitro culture, young cocoyam plants with a root system and well developed leaves, agar was washed away with tap water, and then transferred in plastic pots containing a mixture of black soil, sawdust and sand in the ratio 2:1:1 readably sterilized in an oven (REPLEX mark) at 170°C for 48 hours. The union (pots + plantlets) were placed in the culture room. The union was then watered with tap water in the morning before sunrise and in the evening after sunset. The plants were head dressed with a transparent lid to keep a high humidity. After 14 days, the lids were taken off. The union was again left for 14 days before being transferred out of the culture room away from precipitations and sun rays. After others 14 days of acclimatization in ambient temperature, the plantlets were thus ready for the different treatments. The numbers of leaves were counted, height of plants measured with a ruler and the leaf surface was gotten by measuring the length and width and their averages were later calculated.

2.2. Effect of chitosan and Chlorocholine chloride on the growth of cocoyam

2.2.1. Morphological analysis

2.2.1.1. Treatment of plantlets and induction of minitubers

The induction of minitubers was realized on the action of chitosan (CH) and chlorocholine chloride (CCC) Thus, different treatments were realized. For each treatment, 45 plantlets were used divided in to 3 plots of 15 plantlets each. 10 mL of the mineral solution (constituted of macro and micro elements presents in the Murashige and Skoog solution) [19]were supplied to the plantlets every 10 days. The treatments of plantlets placed in plots with 5ml of different concentrations Chitosan (1 g/L, 2 g/L and 3 g/L) and chlorocholine chloride (5 mg/L, 10 mg/L and 15 mg/L) was apply after every 20 days. On day 100, the harvest took place due to total yellowing of the leaves of the treated plantlets.

The harvested minitubers were weighed and their height measured according to the different treatments and compared to the control.

2.2.1.2. Statistical analysis

During induction of minituberization, the height of plants was measured until harvest. The minitubers harvested was weight and their number was also determined. The percentage of tuberization was also determined for each treatment. All the statistical analysis were done using excel for the treatment and realization of curves and histograms. Student-Newman Keul's and Duncan's test with the least significant difference of 5 % were used for the comparative analyses of the results with the help of SPSS 16.0.

III. RESULTS AND DISCUSSIONS

3.1. Morphological analysis of cocoyam plantlet during minituberization

3.1.1. Average number of leaves of plants per treatment with time

The average number of leaves in the presence of CH, increased in all treatments from D_0 and then decrease till D_{100} with the highest obtain with 2g on D_{40} of 3.80±1.40b and the lowest at 3g on D_{100} of 1.90±0.57c. There exist no significant difference between the control 1g but it exists between the control, 2g and 3g. (Table.1). Also with CCC there exist a significant between the control, 5mg and 15mg on D_0, D_{40} and D_{80} as well as 10mg from D_{20} to D_{100}.The highest was obtained with 10mg on D_{20} of 3.60±0.70b and the lowest with 5mg on D_{100} of 2.10±1.29a (Table.1).

3.1.2. Average height of *X. sagittifolium* plants per treatment with time

There exist a significant difference between the control, 2g (D_{40}-D_{100}) and 3g (D_{80}-D_{100}) but no significant difference between the control and 1g (Table.1). The maximum average height for plants treated with CTH was obtained with 1g on D_{20} of 17.87±5.20ab cm and the minimum with the control on D_{80} of 8.69±3.76ab cm. In the presence of

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CCC, the average height of the plants decreased from D_0 to D_{100} in all treatments. There is a significant difference between the control and all treatments on D_0, D_{40} and D_{100} with a maximum height obtained with 15mg on D_{20} of 21.15±1.99a cm and a minimum with 5mg on D_{60} of 8.43±2.89a cm (Table.1).

3.1.3. Average leaf surface of *X. sagittifolium* plants with time

The average leaf surface of the plants in the presence CH, decreased from D_0 to D_{20} in all treatments including the control with the lowest value of 12.07±12.31a cm^2 obtained on D_{40} and the maximum average leaves surface with 1g on D_0 and D_0 and D_{40} of 36.39±17.02d cm^2 and 31.26±16.37c cm^2 respectively (Table.1). Also with CCC, the average number of leaf surface increased from D0 to D40 with a maximum of 25.87±5.62 cm^2 at 15 mg/L on D_{40}. After D_{40}, the average leaf surface decreased and the lowest value of 10.65±5.57a cm^2 was obtained at 5 mg/L on D_{80}.There exist a significant difference between the control and 5mg from D_{60}-D_{100} and with 10mg and 15mg from D_0-D_{80} (Table.1).

Table.1: Effect of chitosan and chlorocholine chloride on the growth of cocoyam plants

Parameters	Times (days)	Treatments						
		control	CTH (g/L)			CCC (mg/L)		
			1	2	3	5	10	15
Average Height of plants (HP) (cm)	D0	15.08±2.96a	17.87±5.47abc	15.59±4.81a	16.41±5.73a	15.33±3.29ab	20.44±4.38b	21.06±2.07b
	D20	14.69±3.21a	17.87±5,20ab	15.94±4.70a	16.82±6.97a	15.38±3.31a	20.52±4.35a	21.15±1.99a
	D40	12.70±3.71a	17.83±5.06b	16.75±4.65ab	16.22±6.96a	12.90±6.00ab	20.34±4.22a	20.85±1.74ab
	D60	9.29±4.69a	16.45±4.56b	15.64±2.68b	16.48±6.47a	8.43±2.89a	15.12±4.88a	14.49±4.44a
	D80	8.69±3.76ab	16.89±5.01c	14.65±3.88c	14.98±7.07a	7.71±3.44ab	14.53±2.82ab	12.47±3.97ab
	D100	9.76±3.21ab	14.92±3.67c	15.13±3.59c	15.67±5.54a	8.75±3.22a	12.06±6.15a	13.21±2.67a
Average Number of leaves (NL)	D0	3.20±1.14b	3.00±0.82b	2.90±1.10ab	2.20±0.63ab	2.60±0.52a	3.10±0.57b	3.00±0.47c
	D20	3.10±0.88a	3.20±1.55a	3.60±1.35a	2.70±1.25ab	3.30±0.48a	3.60±0.70b	3.00±0.67b
	D40	2.90±0.88ab	3.00±1.33ab	3.80±1.40b	2.60±0.97ab	3.00±0.82a	2.50±1.27b	3.00±0.94b
	D60	2.80±1.14a	2.05±0.85a	2.80±1.32a	2.20±0.92b	2.40±0.70a	2.40±1.08b	2.40±1.06b
	D80	2.80±0.94b	2.30±0.68ab	2.20±0.79ab	2.00±0.47c	2.50±1.354a	2.40±1.08c	2.80±1.03ab
	D100	2.70±0.68a	2.20±0.79a	2.30±0.48a	1.90±0.57c	2.10±1.29a	2.30±1.06abc	2.60±1.17bc
Average Leaves surface (SF) (cm^2)	D0	15.87±6.63a	36.39±17.02d	29.79±7.41cd	26.11±8.57bc	16.04±5.55a	20.98±5.79ab	24.31±5.05abc
	D20	15.20±6.92a	29.42±11.78b	25.91±8.29b	24.56±11.50b	14.98±5.30a	22.04±7.12ab	23.96±5.11b
	D40	12.07±12.31a	31.26±16.37c	28.40±10.71bc	26.90±10.62bc	12.72±5.42a	20.91±6.47ab	25.87±5.62bc
	D60	14.71±11.89ab	26.81±15.46c	24.19±8.59bc	26.39±10.64c	11.55±3.28a	21.79±6.59bc	18.67±7.81abc
	D80	12.57±6.37ab	26.82±15.60c	21.53±10.03bc	22.01±11.97bc	10.65±5.57a	22.23±10.43bc	17.6±7.72abc
	D100	15.54±6.19ab	20.72±5.16c	21.79±8.69c	21.92±8.77c	12.37±5.28a	19.17±9.85ab	18.74±4.41ab

3.2. Minitubers harvested according to the treatment of chitosan and chlorocholine chloride

From results, an increase in the number and weight of the minitubers was obtained with best results from CTH treatments compared to CCC treatments. The maximum results of 29 minitubers were obtained at 2 g/L with CTH and 23minitubers with CCC was obtained at 15 mg/L. The lowest values obtained were 9 minitubers at 3 g/L and 4 mg/L at 5 mg/L (Fig.1.).

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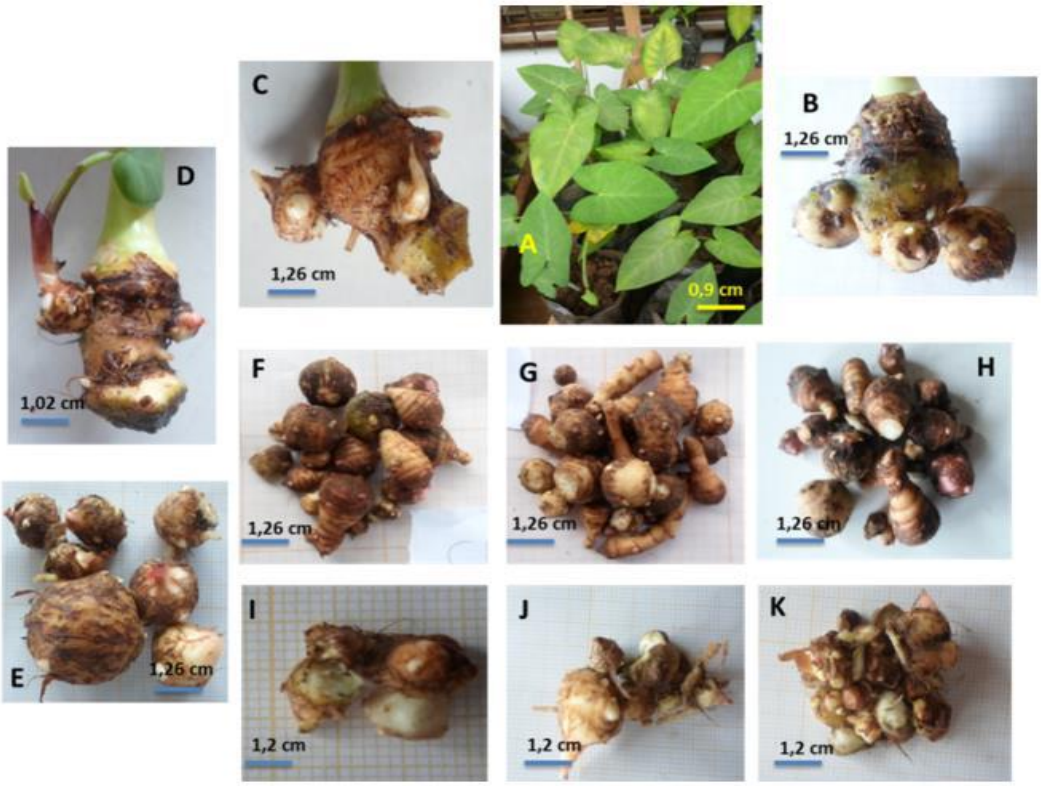

Fig.1: Minitubers harvested: Young plants ready for harvest (A): Plant treated with CTH(B):Plant treated with CCC(C): Control(D): Minitubers obtained from the; control(E), 1g CTH(F), 2g CTH(G), 3g CTH(H), 5mg CCC(I), 10mg CCC(J) and 15g CCC(K).

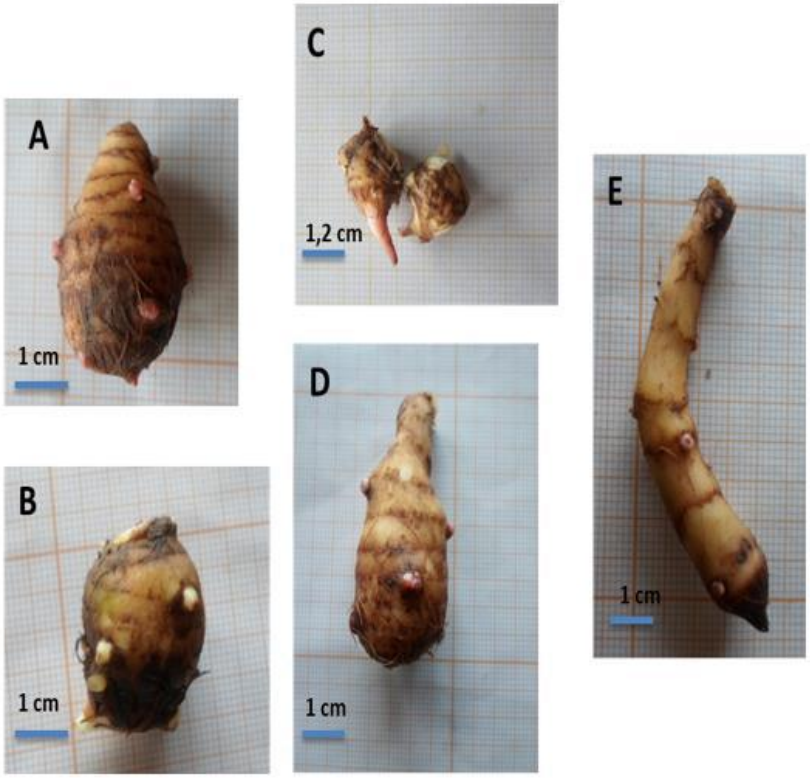

Fig.2: Shape of minitubers harvested: Oval (A), Round (B), Button (C), Pear (D) and crescent (E)

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3.2.1. Number of minitubers

The number of minitubers in the presence of CTH, increased with a maximum of 29 minitubers at 2 g/L and the lowest number of minitubers of 9 obtained with 3 g/L compared to the control of 18 minitubers with a great significance of 5% existing between all the treatments compared to the control (Fig.3). In the presence of CCC, the number of minitubers obtained increased with the concentration and a maximum of 23 minitubers at 15 mg/L and the lowest number of minitubers of 4 obtained with 5 mg/L with a great significant difference 5% between all the treatments and the control (Fig.4).

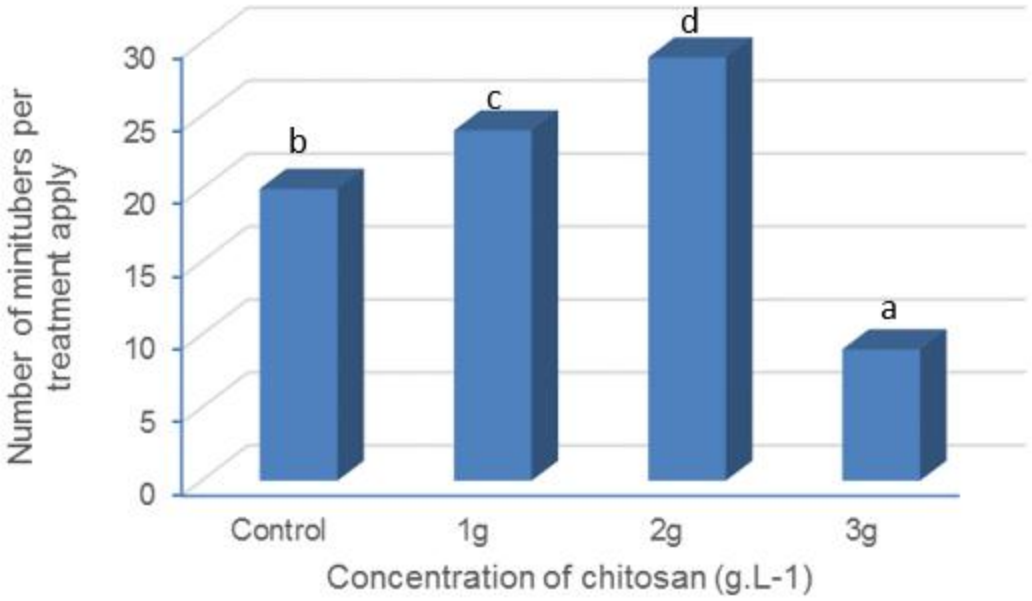

Fig.3: Number of minitubers per treatment of chitosan applied.

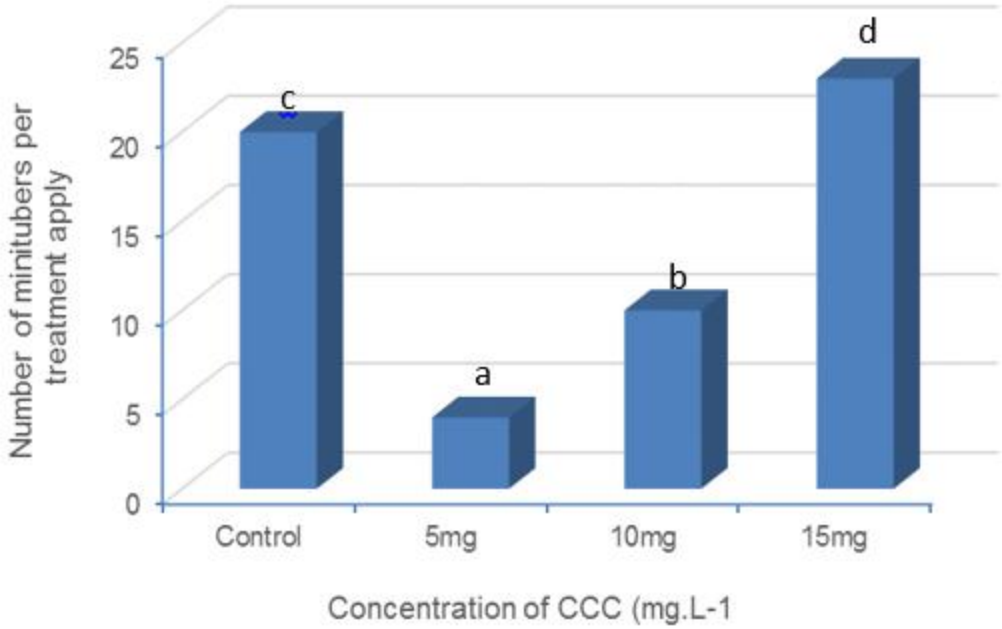

Fig.4: Number of minitubers per treatment of chlorocholine chloride applied.

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3.2.2. Percentage of tuberization

The percentage of tuberization in the presence of CTH, increased with a maximum of 92.31 % at 2 g/L and the lowest percentage of tuberization of 38.46 % obtained at 3 g/L compared to that of the control of 61.54 % and a great significant difference of 5% between all the treatments and the control (Fig.5). In the presence of CCC, the percentage of tuberization increased with the concentration with a maximum of 61.54 % at 15 mg/L, same obtained with the control hence no significant difference between 15mg/L and the control. The lowest percentage of tuberization of 15.39 % was obtained at 5 mg/L (Fig.6).

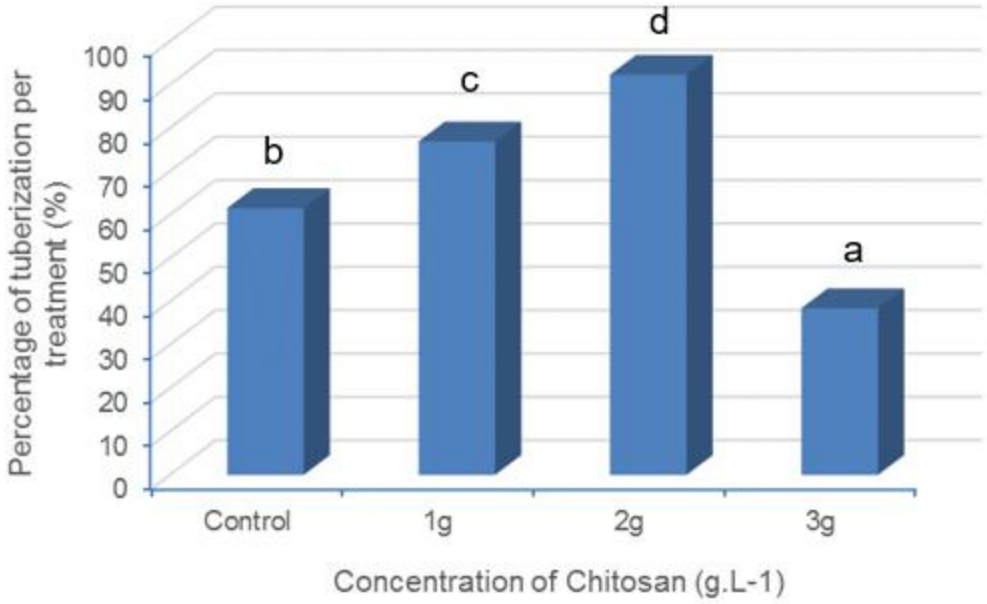

Fig.5: Percentage of tuberization per treatment of chitosan applies

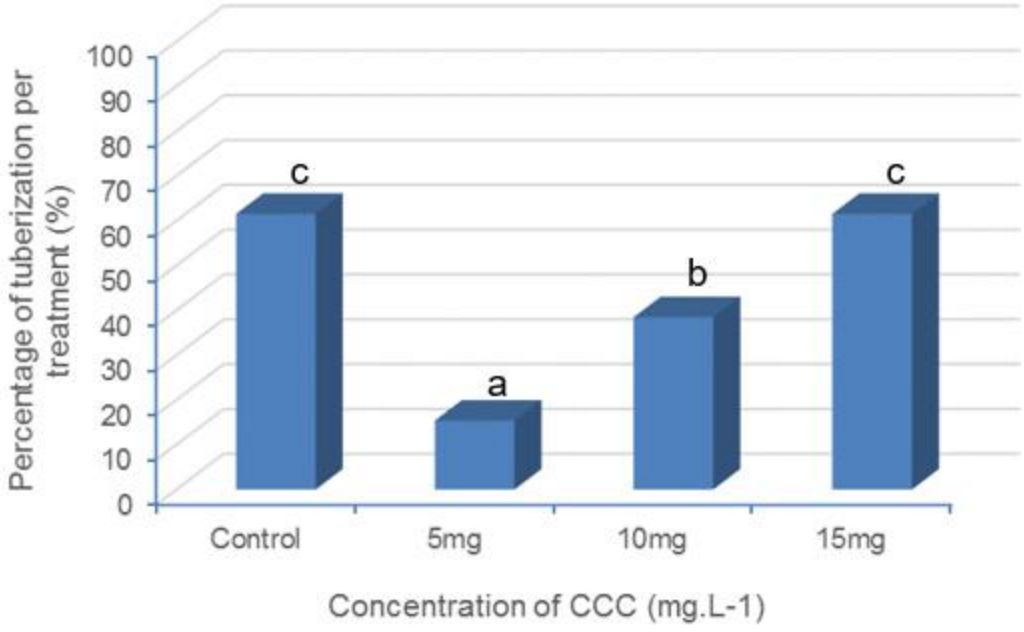

Fig.6: Percentage of tuberization per treatment of chlorocholine chloride apply

3.2.3. Size of minitubers per treatment

There exist great significant differences between all CTH treatments with a maximum size obtained at 3g of 2.6±0.26cm and a minimum of 1.43±0.82cm compared to the control of 0.76±0.48cm (Table.2). There also exist a great significant difference between all treatments of CCC with a maximum size obtained at 10mg of 0.56±0.27cm and a minimum at 5mg of 0.24±0.24cm compared to the control (Table.2).

3.2.4. Mass of the minitubers per treatment

For the average mass of minitubers harvested from plants treated with CTH showed a significant difference compared to the control with a maximum mass obtained at 3g of 2.8±0.84g and a minimum at 1g of 1.47±0.42g (Table.2). There also exist significant differences between all treatments of CCC compared to the control with a maximum value obtained at 10mg of 0.20±0.36g and a minimum at 5mg of 0.06±0.03g.

Table.2: effect of chitosan and chlorocholine chloride on the size and the mass of minitubers harvested.

Parameters	Treatments						
		CTH ($g.L^{-1}$)			CCC ($mg.L^{-1}$)		
	Control	1	2	3	5	10	15
Average size of minituber (cm)	0,76±0,48^{d}	1,43±0,82^{e}	1,96±0,41^{f}	2,6±0,26^{g}	0,24±0,24^{a}	0,56±0,27^{c}	0,50±0,22^{b}
Average mass of minituber (g)	0,51±0,95^{d}	1,47±0,42^{e}	1,80±0,56^{f}	2,8±0,84^{g}	0,06±0,03^{a}	0,20±0,36^{c}	0,12±0,13^{b}

Data sharing the same letter in the same line are significantly different at 5% level (Duncan's multiple range tests)

IV. DISCUSSION

The morphological analysis shows that plants treated with chitosan compared to plants treated with CCC permitted to obtain best performances on the quality and quantity of minitubers with relation to those obtained from traditional cuttings. This may be due to the fact that the plantlets are from the origin, exempted from all contaminations which are contrary to traditional cuttings which carry pathogen microorganisms, susceptible of limiting the production of minitubers. This result is in concordance with the work of [20],who showed that the quality of the plant materials influence the production of yam (*Discorea alata* L.). Their works stipulates that, to obtain minitubers from plantlets, it takes twenty weeks which is in concordance with our work where by the harvest of minitubers took place 171days after planting.

From this work, the average number of leaves, average height of the plants and average leaf surface, increased with the concentration of Chitosan, but decreased with time, this is in agreement with the works of [21],who realized the application of Chitosan solution ranging from 4-250 ppm significantly enhanced the vegetative growth, yield and quality of okra. [22]also remarked that chitosan increased the growth and yield of coffee and had a highly positive correlation with chlorophyll and carotenoid accumulations in the leaves and additionally, may increase mineral uptake and stimulate the coffee growth rate. It was confirmed that chitosan is a second source composed not only of carbon and nitrogen but, some other elements in the chain which are essential minerals for the growth of the plant[23].They also noticed that chitosan may also be attributed to the promoting effects on nutrient uptake and nutritional status: nitrogen, potassium and phosphorus especially result in higher plant growth. Work on soybean in 2010, also noted that even though the mineral composition of the soil that was mixed with chitosan before and after cultivation of soybean, was unchanged [24]. He explained it was because the content of nitrogen, potassium and phosphate significantly increased with the application of chitosan.

Results also shows that the average number of leaves of plants treated with CCC decreased while average height and leaf surface increased with the concentration and time. These results are in agreement with those of [25] on gaur cultivars who reported that exogenous application of CCC significantly increased the leaf surface area per plant contrary to this report, some reports says that exogenous application of CCC, significantly reduced the total leaf area in plants like soybean cultivar [26]and *Brassica juncea*[27]On the other hand, the beginning of the decrease in number of leaves, height and leaf surface of the plants also marks the beginning of minituberization of cocoyam.

The number of tubers increased with the concentration in the plants treated with chitosan of concentration 1 g/L and 2 g/L. The best tuberization with chitosan was obtained with 2 g/L (92.31 %) and 15 mg/L (61.54 %) for CCC. These results are in line with that obtained by [28] on *Solanum tuberosum* L. which showed

most effective improved acclimatization of plantlets in the greenhouse as expressed by significance in the number of minitubers and yield of potatoes with 500 mg/L. This can also be explained by the report of chitosan known as a growth promoter in various crops such as soybean sprouts [29].With CCC, the number of minitubers as well as the percentage of tuberization increased with continuous increase of the concentration, this is similar to the results obtained by [30]on potatoes, who realized increasing the rate of CCC increased the number and average weight of microtubers recorded at 500 mg/L CCC. The weight and height of the minitubers also increased with the concentration which is in line with the results obtained by [31]on potato, they found that the maximum number of microtubers per plants with 500 mg/L CCC and the weight of microtubers, decreased with the rate of CCC concentration. The highest tuber weight was recorded in the absence of CCC while the minimum at 500 mg/L (145.7mg) which is in agreement with those of [32]but disagree with those of [33]with relation to the weight. The height of the minitubers treated with CCC are smaller compared to the control and plants treated with chitosan. These results were obtained because according to [29],CCC produces a reduction in the height without malformation by reducing cell elongation and also by lowering cell division.

V. CONCLUSION

The general objective of this work was to study the effect of chitosan and chlorocholine chloride on the minituberization of cocoyam (*Xanthosoma sagittifolium* L. Schott). The results obtained shows that the plantlets which were treated with different concentrations of chitosan (1 g/L, 2 g/L and 3 g/L) are more productive with best results compared to plants supplied with different concentrations of CCC (5 mg/L, 10 mg/L and 15 mg/L). The morphological studies shows that the best results were obtained from plants treated with chitosan of concentration 2 g/L. It produced the greatest average number of leaves, height of plants, number of minitubers and weight of minitubers whereas in CCC, the best result was obtained with 15 mg/L.

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Evaluation of Salicylic acid Pre-Hardening Treatments of Cowpea for Resistance against the Flea Beetle, *Podagrica fuscicornis* Linn. (Coleoptera: Chrysomelidae)

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***Abstract*—** *The experiment was conducted to evaluate the potential of SA-treatment in the control of Pod sucking bug of cowpea. The mean annual rainfall was within the range of 865-1250mm with mean annual temperature of 22-38°C and relative humidity of 65-90mmHg. Four different cowpea varieties (IT97K-1069-6, IT98K-205-8, IT89KD-288 and Dan'ila) pre-hardened with Salicylic acid were established in various replicated field cages in completely randomized design. Five-pairs each of fresh pre-mated bugs were introduced into the various cages, allowed for 2-weeks to mate and oviposit after which all adult insects are removed. The different cowpea varieties screened showed variable response to the bug attack. IT97K-1069-6 and IT89KD-288 recorded lower indices of susceptibility and leaf damage to flea beetle attack, delayed development time of larvae with few progeny emergence. Pre-treatment effects also varied significantly (P<0.001) with higher concentrations (10ppm) of Salicylic acid (SA) being more effective in reducing leaf damage than the lower (5ppm) concentrations and the controls. The interaction of treatments and varieties was also found significant (P<0.002). Of the four Cowpea varieties screened, IT97K-1069-6 and IT89KD-288 pre-hardened with 10ppm of SA were found tolerant owing to their low susceptibility indices (7.09 and 9.09) and percentage leaf damages (18.58% and 25.93% respectively while Dan'ila and IT98K-205-8 treated from the same concentrations were however susceptible. Phytochemical analysis of treatments showed high concentrations of eugenol and terpenes in the tolerant varieties. These relations should be explored extensively toward sustainable plant protection.*

***Keywords*— Cowpea, Flea beetle, Management, Phytochemicals, Salicylic acid.**

I. INTRODUCTION

Cowpea leaf damage by flea beetles is increasingly becoming a threat to photosynthesis and production of essential nutrients by the plant at early stage of establishment. Although the pests has been classified as an occasional pests of legumes, it has become one of the major cowpea leaves pest which re-currently appear at early planting stage causing characteristic pores on cowpea leaves in the absence of its other host. This significantly retards development of cowpea which may decrease its recovery potential against other leave insect pests such as Aphids. Management approach using Chemical insecticides have posed detrimental effects to humans and the environment, and are also not affordable to majority of peasant farmers (Alebeck, 1996). Alternative approach towards efficient and cost effective means of production of cowpea is very desirable. The development and use of resistance legume varieties offer a simple, cheap and attractive approach to the reduction of pest damage (Ofuya, 2001). Host plant resistance is the easiest, most economical and effective way of managing insect pests and diseases. It requires low technological inputs, create low environmental hazard and is generally compatible with other methods of pest control. Inducible defenses are reported to improve plant fitness and may be more durable, than constitutive defense mechanisms (Agrawal, 1998; Agrawal, 1999).

1.1. The Flea Beetle, *Podagrica fuscicornis*

Flea-beetles, *Podagrica spp*. are classified into family leaf beetles (Chrysomelidae) and subfamily Alticinae (Aslan *et al*., 1996) and occur in all vegetation habitats. *P. fuscicornis* belongs among oligophagus herbivores and it induces damage on plants from genera *Althaea*, *Malva* and *Lavatera* (Cmoluch, 1988; Rotrekl, 1996; Brelih *et al*., 2003). It can cause damage also on

plants from families Lamiaceae, Urticaceae and leguminosae (Petitpierre, 1985). Flea beetles have been described as small bugs with a size from 1.5 to 4 mm. They can jump due to the enlarged backside organ (hind legs). They also have the ability to fly. When plant is heavily attacked by flea beetles, small round holes (up to 1 mm) caused by an individual flea beetles feeding may coalesce into larger areas of damage (Gruev and Doberl, 1997). Adult beetles of *P. fuscicornis* are 3.0 to 6.0 mm in size. Head and neck shield are coloured red. Front wings (sheath-wings) are dark blue to blue green and rarely are detected metal coloured. Sheath wings contain hollows which are deep, very abundant and spread scarced. Hollows on neck shield are even more compacted. Legs are characteristically yellowish to bright brownish, and upper labium is also yellow (Hubble, 2010).

1.1.1. Life cycle of Cowpea Flea Beetle

Flea beetles are insects with complete metamorphosis, adult females lay eggs, which hatch into larvae of various shapes. After feeding and moults several times, larvae mature, pupate and later emerge as adult beetles; the length of time it takes to complete the life cycle varies greatly from species to species and is also dependent on weather and other environmental conditions (RakCizej *et al*., 2001). Cmoluch (1988) reported that the female lays its small yellow eggs into the soil at the base of the host plant. The egg stage took 7-13 days to hatch and larvae hatch after 7–11 days and feed for a period of 11-28days on the rootlets. Pupation takes place in the soil and the adults emerge after 10-17 days from the pupa. The adult remains after rain on the host plant for as long as they can find suitable food (Brelih *et al*., 2003).They always prefers young growth. Physical and chemical factors of plant defence many times have influence on the survival ability of larvae. The latter can also be affected by the appearance of natural enemies and unfavourable weather conditions (RakCizej *et al*., 2001). Larvae molt three to four times and then pupate. After one to two weeks, the adults emerge. The Chrysomelid has only one generation per year (Cmoluch, 1988). Adults overwinter usually in the soil under the grass sward, where often enough organic matter or nearby host plant (RakCizej *et al*., 2001). Very known members of genus *Podagrica* cause most damage on plants from family Malvaceae.

Numerous control measures, including physical, chemical, biological and cultural have been employed by farmers to combat many devastating pests in cowpea seed production and storage. However, the continuous uses of insecticides at increasing dosage in order to compensate its ineffectiveness due to insect resistance are also potential threats to aquatic fauna, environmental and human health. Alternative approach towards efficient and cost effective means of production of cowpea is very desirable. Host plant resistance is the easiest, most economical and effective way of managing insect pests and diseases. Inducible defenses are reported to improve plant fitness and may be more durable, than constitutive defense mechanisms (Agrawal, 1999). Previous attempt to use plant growth hormones such as salicylic acid (Stout *et al*., 1999; Alvarez, 2000; Walling, 2000; Aviv *et al*., 2002; Brodersen *et al*., 2002) to induce resistance in some plants have been reported. However, little was found on how Salicylic acid (SA) affects resistance of cowpea to insect pests. While most research uses plant growth hormones exogenously as surface spray to control insect pests, this research investigates to test the potential of salicylic acid (SA) to induce resistance against insect pests using pre-sowing hardening treatments. Salicylic acid have been classified as safe and environmentally friendly, practically non-toxic and biodegradable with low potential for bioaccumulation (EU regulation on chemicals: 1272/2008 (EEC, 2008). The aim of the research is to assess the physiological effect of salicylic acid in Cowpea against the Leaf feeding beetle, *Podagrica fuscicornis* (L)

II. MATERIALS AND METHODS

The experimental field trials were conducted at the University Research farm, Faculty of Agriculture Bayero University Kano (11°58'N, 8°25' E and 457m above sea level), From June-November, 2014. The mean annual rainfall was within the range of 865-1250mm with mean annual temperature of 22-38°C and relative humidity of 65-90mmHg (Remote sensing unit Geography Dept, BUK).

2.1. Pre-sowing Hardening Treatments

Different concentrations (5ppm and 10ppm) each of the growth substances of salicylic acid was prepared in the laboratory by dissolving 1gram of each of Salicylic acid granules in 1ml of 75% ethanol for dilution in distilled water to make the stock solution (1000ppm). These were subsequently diluted to various concentrations 5ppm and10ppm of salicylic acids which were transferred from the reagent bottles into clearly labelled 250mls conical flasks according to the concentration of the growth substances to be used in the pre-hardening treatments. The seeds of the cowpea varieties (IT97K-1069-6, IT98K-205-8, IT89KD-288 and Dan'ila) were soaked in the various concentrations (5ppm and 10ppm) of Salicylic acids for a period of 6 hours. These were air dried in the laboratory before sowing (Darra *et al*., 1973; Audi and Mukhtar, 2009). Distilled water was also used for soaking and to also

serve as control (Darra *et al*., 1973) so that the effect of seed pretreatment on plant growth should not be affected by the differences in seed development along with untreated seeds for comparing the effect of various pre-treatments.

2.2. Sowing of Cowpea Seeds

Four different cowpea varieties (IT97K-1069-6, IT89KD-288, IT98K-205-8, and Dan'ila) Pre-hardened with salicylic acid were grown both in exposed field (normal unblocked/caged) and various replicated field cages. The field cages were for the screening against pod sucking bugs while the exposed field grown cowpea were used to monitor infestation and also serve as a source of the insects used for the various field cage experiments. The seeds were sown into four replicates on a split plot designed in a completely randomized block design, with level of hormone treatments representing the main plots as well as the controls while the four varieties stands as sub plots (field cages) in each main plot. A spacing of 75x20cm inter and intra row were used respectively and 4-seeds were placed per hole and thinned to 2 seedlings per stand at 2-weeks after sowing (Tanzubil, 2000).

2.3. Insect Collection and Method of Infestation

Five pairs of adult males and females of freshly mated flea beetle, *Podagrica fuscicornis* were collected from the exposed field while mating using glass jars. This was carried out between 7.30 am and 9.00 am, when the insects were relatively less active and would not readily separate from copulation or take flight when disturbed. Infestation was made during the late July (4-5 weeks after planting) at pre-booting stage. Each set up was established in 2-m by 4m screen cage with a door opening to the outside above a 0.5-m sill (which helped to contain larval beetles and the adults). In each cage, 6-stand of cowpea were planted in rows prevented from entrance by other insects from the main exposed planted cowpea crops this ensure accurate assessment of infestation due to the test insects under study but prevent multiple infestation from other pests (Underwood *et al*., 2002).

2.3.1. Effect of Pre-hardening treatments on Progeny emergence and Developmental time of flea beetle, *Podagrica fuscicornis*

To determine preference for insects' fecundity, on the different cowpea varieties grown in the various field cages, four-weeks old seedlings were infested with five-pairs adult males and females flea beetles, *P. fuscicornis*. The insects were allowed to mate and oviposit for one week after which they were removed from each plant. Clutches of yellowish eggs and egg masses were observed on surface soil at the undergrowth. These were maintained undisturbed for larval development but observed regularly until adult beetle emerged. Insects were counted and discard daily until no further progeny emergence observed. The total number of insects that emerged over a developmental period was determined by count for each treatment (Underwood *et al*., 2002).The mean developmental period of *P. fusicornis* was estimated as time from the middle of the oviposition period to the emergence of 50% of the F1 generation.

2.3.2. Effect of Pre-hardening treatments on Leaf Damage of *P. fuscicornis*

A similar set up was maintained unperturbed until all adults insects emerged but allowed to feed over 2 weeks periods beyond which few adults survived. Visual leaf damage caused by larval beetles during period of exposure (2 weeks) was determined based on scale rating by Panda and Kush (1995), Jackai and Singh (1988).Percentage leaf damage was calculated using the formular;

$$\text{Percentage of Damaged Leaves} = \frac{\text{No. of Damaged Leaves Per Plant}}{\text{Total No. of Leaves Per Plant}} \times 100$$

2.3.3. Effect of Pre-hardening treatments on leaves Susceptibility to *P. fuscicornis*

Based on the cumulative emergence of F_1 insect progeny and mean development time, the susceptibility indices of the different cowpea cultivars to flea beetle attack was evaluated (Dobie, 1974) using the following formula;

$$SI = \frac{\text{Log}_e Y}{D} \times 100$$

Where

SI = Susceptibility index

Y = total number of emerged adults

D = Mean development period of the progeny

2.4. PHYTOCHEMICAL ANALYSES

Fresh Leaves and pods of the different cowpea varieties were washed and shade dried at room temperature. The dried and grounded plant part were weighed and extracted and using 80% cold aqueous methanol (MeOH) supplemented with butylated hydroxy-toluene (BHT) as an extracting solvent. Extracts were further subjected to Quantitative analysis at National Research Institute for Chemical Technology (NARICT) Zaria, using Gas Chromatography Mass Spectrometry GC-MS (QP 2010 Plus Shimadzu, Japan). The relative % proportion of each

component was determined by comparing its average peak area to the total areas. Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were determine by comparing spectrum of the known component with that of the known components stored in the NIST library (Valls *et al.*, 2009).

2.5. STATISTICAL ANALYSIS

All data collected by counting were subjected to square root transformation while percentages were arcsine transformed prior to analysis. Transformed data were subjected to Analysis of Variance ANOVA using the Genstat Statistical Software (2011) version10.3DE, Rothamsted Experimental Station.

III. RESULTS

The effect of the different hormones (5 ppm and 10 ppm of SA) on infestation of flea beetle is shown in Table 1. There was significantly less progeny emergence in the Salicylic acid pre-hardened cowpea seeds with 5ppm SA showing considerable reduction in the number of progeny emergence (18.50) than with other treatments. But progeny emergence was significantly higher in both the distilled water treatment and the control (49.42 and 55.08) respectively (Table 1).

Table.1: Effect of Salicylic acid (SA) Pre-hardening Treatments of Cowpea on Developmental time (days) of Podagrica fuscicornis

Hormone treatments	**Cowpea varieties**				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	15.33	20.00	15.67	23.00	18.50
10ppmSA	11.67	17.00	21.00	27.33	19.25
DIST. H2O	47.33	49.33	51.00	50.00	49.42
CONROL	53.67	54.00	65.33	47.33	55.08
MEAN	32.00	35.08	38.25	36.92	35.56
LSD5%	11.96	10.69	13.22	7.612	10.76

Data are means of three replications, mean values with differences less than the least significant Differences (LSD) at 5% are not significantly different, P<0.001.

Developmental time of the beetle also varied significantly (P<0.001) with variety type and Salicylic acid hormone concentrations. The interaction of treatments and varieties was also found significant (P<0.001). Developmental was observed to be significantly longer (P<0.001) in all the hormone treatments when compared with the controls (Table 2). Larval development time was however longer in the 10ppmSA treatments (34.17days) than the lower concentration (5ppmSA).

Table.2: Effect of Salicylic acid (SA) Pre-hardening Treatments on Progeny emergence of Podagrica fuscicornis

Hormone treatments	Cowpea varieties				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	30.67	35.00	29.67	30.00	31.33
10ppmSA	37.00	38.00	32.00	29.67	34.17
DIST. H2O	29.67	28.67	27.33	26.00	27.92
CONROL	25.67	30.00	30.00	26.33	28.00
MEAN	30.75	32.92	29.75	28.00	30.36
LSD5%	2.602	2.411	1.061	1.179	1.663

Data are means of three replications, mean values with differences less than the least significant Differences (LSD) at 5% are not significantly different, P<0.001.

Table 3 shows the response of the different pre-hardened cowpea varieties to attack by Flea Beetle, *P. fuscicornis*. The tolerance capacity interms of susceptibility index and leaf damage levels also varied significantly (P<0.001) with

variety type and Salicylic acid hormone concentrations. The interaction of treatments and varieties was also found significant (P<0.001). The susceptibility index was remarkably lower in the entire hormone treated cowpea seeds compared with the distilled water treatment and the control. Treatment with 10ppmSA resulted in significantly (P<0.001) lower susceptibility indices (9.88) to leaf feeding beetles when compared with 5ppmSA (10.18) and the controls. Varietal response shows that IT97K-1069-6 and IT89KD-288 were tolerant (9.45 and 10.22 respectively) compared with IT98K-205-8 and Dan'ila with mean response of 11.01 and 12.52 respectively.

Table.3: Effect of Salicylic acid (SA) Pre-hardening Treatments on Cowpea Leaves Susceptibility (SI) to P. fuscicornis

Hormone treatments	Cowpea varieties				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	9.12	9.23	10.02	11.15	9.88
10ppmSA	7.09	9.09	12.56	11.99	10.18
DIST. H2O	10.60	10.78	10.37	13.11	11.21
CONROL	10.98	11.76	11.09	13.84	11.92
MEAN	9.45	10.22	11.01	12.52	10.85
LSD5%	0.978	0.711	0.623	0.659	0.521

Data are means of three replications, mean values with differences less than the least significant Differences (LSD) at 5% are not significantly different, P<0.001.

Percentage leaf damage was significantly (P<0.001) lower in the hormone treated seeds compared with the distilled water treatment and the control in which the percentage damage was greater than 60%. Comparison of the hormone treatments indicated that the least Percentage leaf damage was obtained in cowpea seeds treated with 10ppmSA (32.55%) than the lower concentration. Varietal response to treatments shows that IT97K-1069-6 and IT89KD-288 recorded significantly (P<0.001) lower percentage leaf damages (42.42% and 43.42% respectively) than the other varieties in which more than 50% leaf damages were observed (Table 4).

The relative proportion of secondary metabolites produce from different treated cowpea plants varied significantly (P<0.001) with hormone concentrations Pre-hardening treatment of cowpea with SA elicited synthesis and production of chemical compounds with insecticidal effect that enhance tolerance capacity in some of the treated seeds (Appendix 1).

Table.4: Effect of Salicylic acid (SA) Pre-hardening Treatments of on Cowpea leaves Damage(%) by P. fuscicornis

Hormone treatments	Cowpea varieties				Treatment Effects
	IT97K-1069-6	IT89KD-288	IT98K-205-8	DAN'ILA	
5ppmSA	27.42	27.77	57.02	54.65	41.72
10ppmSA	18.58	25.93	38.49	47.19	32.55
DIST. H2O	67.73	62.74	61.15	77.36	67.24
CONROL	55.89	57.22	67.13	79.05	64.82
MEAN	42.42	43.42	55.95	64.56	51.58
LSD5%	12.87	10.69	6.852	8.907	9.498

Data are means of three replications, mean values with differences less than the least significant Differences (LSD) at 5% are not significantly different, P<0.001.

IV. DISCUSSIONS

Pre-hardening treatments of cowpea in various concentrations of salicylic acids resulted in varied responses to flea beetle attack. Cowpeas treated with 10 ppm SA were more tolerant to flea beetle attack than at lower concentrations (5 ppm). The population of the beetles was considerably high in control of IT98K-205-8 and Dan'ila due to high emergence but was low in the less susceptible varieties. The development time of the beetle was longer in the 10 ppm SA treatments of IT97K-1069-6 and IT89KD-

288 than the other treatments and controls in which the shortest development time was observed in Danila variety. This delayed developmental time and fewer progeny emergence are consequent of inducible defense elicited by salicylic acid treatment which may have effect on the nutritive value of the diet or indirectly the effect of antifeedants detected in the leaves, mainly tannins and phenolic derivatives (2-hydroxyethoxyethyl and Quinolines respectively) in the less damage varieties. This may be related with the reports of Barbehenn and Peter (2011) who revealed that, ingested tannins reduce the digestibility of the proteins to insect herbivores thereby decreasing the nutritive value of plants and systemically induced neighboring leaves of the damaged plants. A similar report have also show that higher production of salicylates compounds may considerably affect the available nutrients or could reduce protein digestibility in insects (Barbehenn and Peter, 2011).

The susceptibility index (SI) of also varied significantly with varietal type and hormone- treatments. Pre-hardening treatments of IT97K-1069-6 and IT89KD-288 in 10 ppm SA confer resistance to the flea beetles attack than with other treatments in which Dan'ila variety was more prone to infestation and damage by the beetles owing to their high susceptibility index and damage levels. Varieties with low susceptibility indices were less damaged and are therefore tolerant to the flea beetle attack. Characteristic leaves windowing which extended towards the margin were irregularly made to varying degrees. Crops with such damage show reduce flowering and pod productions due to impaired photosynthesis and movement of nutrient across the leaves palisades. Susceptibility index is a measure of crop resistance; a high susceptibility index implied that more progeny developed from a variety over a shorter time. The low susceptibility of IT97K-1069-6 and IT89KD-288 treatments could be attributed to higher levels phenolics (Octadecadienol) detected in the varieties which may have adversely affected the utilization of nutrients by the leaves feeding beetle (Table 3). This may be related to the findings of Duffey and Stout (1996) and Bhonwong *et al.*, (2009) who reported that, Quinones formed by oxidation of phenols bind covalently to leaf proteins, and inhibit the protein digestion to insect herbivores. Reports in the same vein have similarly show that, changes in defensive constituents of a plant on account of insect attack develop unpredictability in the plant environment for insect herbivores, which in turn affects the fitness and behaviour of the herbivores (Miranda *et al.*, 2007).

V. CONCLUSION

Pre-hardening treatments of cowpea with Salicylic acid significantly influenced various regulatory responses to leaf feeding beetle *Podagrica fuscicornis*. Cowpea varieties IT97K-1069-6 and IT89KD-288 recorded low indices of susceptibility and leaf damage to flea beetle attack, delayed development time of larvae with few progeny emergence. Pre-hardening treatments with higher concentrations (10ppm) of Salicylic acid (SA) was effective in reducing leaf damage than the lower (5ppm) concentrations and the controls. Of the four Cowpea varieties screened, IT97K-1069-6 and IT89KD-288 pre-hardened with 10ppm SA were found tolerant to flea beetle attack owing to their low susceptibility indices and damage levels while Dan'ila and IT98K-205-8 of the same treatments were however susceptible.

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Appendix 1.

Table.5: Relative proportion of Phytochemicals Detected from different Pre-hardened Cowpea Varieties using Gas Chromatography Mass Spectroscopy(GC-MS)

Hormone Treatments	**Cowpea Varieties**											
	IT97K-1069-6			IT89KD-288			IT98K-250-8			DAN' ILA		
	RT	MC	Phytochemicals	RT	MC	Phytochemicals	RT	MC	Phytochemicals	RT	MC	Phytochemicals
5ppm SA	19.64	15.49	Dimethylbenze (monoterpenes)	3.51	17.83	Methyl hexane	3.54	7.12	o-Methyltoluene	4.95	6.64	Isobutyl cyclohexane
	19.64	9.17	Dihydrogeraniol	3.51	9.83	Phenylethane	3.51	8.03	Pseudocumol	4.95	6.67	Octadecadienol
	19.64	7.49	2-hydroxyethoxyethyl (Phenolic)	3.51	6.83	Ethylbenzol	3.51	9.03	Pentadecanecarboxylic acid, ethylbenzol	4.95	6.15	Ethylbenzol
10ppm SA	22.36	24.71	Phytol (Diterpene),	23.78	28.28	n-Hexadecanoic acid	22.16	7.23	o-Methyltoluene	3.58	4.09	Cyclohexane
	22.36	13.68	2-hydroxyethoxyethyl (Eugenol)	23.78	13.74	Pentadecanecarboxylic acid, ethylbenzol	22.16	8.09	Pentadecanecarboxylic acid	3.51	6.50	Ethylbenzol
	22.36	15.55	Quinolines (Tannin) Iodomethylbenzoic.	23.78	14.88	Octadecadienol, quinoxalin (Phenolic)	22.16	8.09	Phenylethane	3.58	7.19	Octadecadienol
D.H_2O	22.30	3.57	Trimethyl benzene	3.82	3.49	Methyltoluene,	22.38	5.96	o-Methyltoluene	3.66	2.82	Dimethylbenzene
Untreated	22.33	4.69	Methyltoluence	26.55	2.11	Methyleicosane	22.36	4.54	o-Methyltoluene	21.23	2.91	1, 2-Xylene

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The Effect of Mulch types with Dose of N, P, and K Fertilizer in Summed Dominan ratio and biomass weed Arabica (*Coffea Arabica* L.) Coffee in Indonesia

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Abstract**—** *This research was compiled based on Factorial Randomized Group Design (RBD) consisting of 2 factors with 3 groups to obtain 36 experimental units. Each unit consists of 12 plants, 3 plants are taken as samples, so that 432 plants in total with 144 plants as samples. The first fator is the treatment of various types of mulch with 4 levels, namely A1 = no mulch, A2 = mulch rice husk, A3 = mulch banana stem and A4 = black silver plastic mulch. The second factor is the treatment of N, P and K anoragnik fertilizer with 3 levels, namely B1 = 15 g N, 15 g P2O5, 15 g K2O, B2 = 20 g N, 20 g P2O5, 20 g K2O and B3 = 25 g N, 25 g P2O5, 25 g K2O. The results of the study that the treatment of various types of mulch showed the dominant weeds in the cultivation of Arabica coffee in Indonesia was Borreria alata, followed by Elausine indica and Ageratum conyzoides. While the lowest weed biomass was obtained in black silver plastic mulch or N, P and K 15g: 15g: 15g.*

Keywords**— Weed, SDR, Biomass Weed.**

I. INTRODUCTION

Coffee is one of the plantation commodities that has its own appeal, various processed coffee products create a taste and aroma that is attractive to fans. Coffee is also one of the mainstay commodities for export-producing estates and a source of income for farmers in Indonesia. Many factors support the cultivation of coffee plants and the development prospects for the future, but also many challenges and obstacles in the development of these commodities from various aspects. One of the obstacles is the low productivity of the coffee. The optimal productivity of coffee is caused by not using quality seeds, farming techniques that still use traditional methods, fertilization and maintenance of plants are not yet done. To overcome this problem, an action to increase the productivity of Arabica coffee is needed. In the short term, the choice of approach to increase crop productivity is intensification by fertilizing and giving mulch.

According to research conducted by Haryono (2009) that the benefits obtained when the land is given mulch is modification of soil temperature, conservation of soil moisture, reducing loss of nutrients, suppress weed growth and increase crop yields. However, low nutrient availability and availability takes a long time so mulch administration must be accompanied by fertilizer application. One of the cultivation activities is to ensure the availability of nutrients by fertilizing which is useful in supporting plant growth and production. Growth, development and continuity of good production, then the condition of the soil as a growing medium must be improved quality or ability in the supply of nutrients, both in number and type. This is the reason for the author to see the type of mulch and how many doses of N, P, K that can be able to suppress the growth of Arabica coffee weeds so that it is expected to increase productivity.

II. MATERIALS AND METHODS

The materials used in this study were rice straw, rice husk, banana midrib, 8 months old Arabica coffee seedlings of Sigararutang variety with plant height 10-15 cm, Urea, SP36 and KCL fertilizer and manure. Observation of weeds after one week of treatment. Each treatment group consisted of 12 plots measuring 10 m x 7.5 m, the distance between plots was 0.5 m. In the treatment plot, 12 planting holes were made with a spacing of 2.5 m x 2.5 m so that there were 432 planting holes on the land. Planting holes measuring 60 cm x 60 cm x 40 cm. The next activity after making the planting hole is to apply mulch. Mulch to be used is mulch of rice husk, mulch of banana midrib and black silver mulch. Black silver plastic mulch was spread over a planting hole of 1 m2, while for rice husk mulch and banana midrib sprinkled with an area of 1 m2 leaving a hole for the

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coffee planting site Observations were made on the amount of each weed at the end of the study to observe weed biomass and SDR.

III. RESULTS AND DISCUSSION

3.1. Summed Dominan Ratio (SDR) Weeds

Table 1 shows that the three types of weeds that have the highest SDR value are Borreria alata weeds, then followed by Elausine indica and Ageratum conyzoides These three types of weeds belong to the dangerous weed species for coffee cultivation because these weeds have very fast proliferation both vegetative and generative, broad leafy type and lush canopy so that it can cover coffee plants in the early phase of growth. Another thing that can be described by the value of SDR is the level of mastery of weeds on biotic and abiotic factors that exist on the land.

According to Meilin (2006), Borreria alata is a group of broadleaf weeds that are dominant in coffee plantations or also called weed groups that need a lot of light (weeds are not shade resistant). Whereas according to Sembodo (2010) this weed has a high adaptability and is one type of broadleaf weed that disrupts the growth and yield of cultivated plants.

Borreria alata produces seeds reaching 9953 of the seeds of its perennial crops and a long level of seed dormancy (Sastroutomo, 1990). Dormancy is a reproductive weed strategy to survive in unfavorable conditions. Dormancy intensity is influenced by the environment during seed development. Dormancy in certain types causes the seeds not to germinate in the soil for years. This explains the presence of weeds in continuously cultivated agricultural land. Weed seeds in the soil have different levels of dormancy, so the germination of a population of weed seeds does not occur simultaneously. This situation causes weed seeds in the soil to remain a problem as long as the seeds are still there.

Ageratum conyzoides is a broadleaf weed with leaf formation and rapid stem lengthening. This will greatly affect the process of taking light. Competition for light is an important factor in determining the rate of plant growth. The leaves of Ageratum conyzoides are able to protect other plants and inhibit photosynthesis. According to Tjitrosoepomo et al (1987), the Asteraceae family is a broadleaf weed group that likes to be slightly moist and able to produce as many as 40,000 seeds per year.

In addition to weeds Borreria alata and Ageratum conyzoides also found weeds Crassocephalum crepidiodes. This weed habitus is in the form of upright plants, soft-wooded trunk, stem height can reach ± 1 m and have many branches. These weeds include the Asteraceae family. According to Reader and Buck (2000), weeds of the Asteraceae family can breed through seeds and have the ability to adapt to the environment and flower throughout the year. If it is associated with the type of mulch, the growth of these weeds will be hampered if you get the treatment of black silver plastic mulch.

In general, the best treatment to suppress the value of weed SDR is the use of black silver silver mulch while the value of SDR weeds will also decrease if the N: PK fertilizer dosage is lower, ie 15g: 15g: 15g. The lower the dose of fertilizer N: P: K, the lower the weed biomass. Low weed biomass at N: P fertilizer dosage: K 15g: 15g: 15g has to do with the number of individual weeds, weed population and type of mulch. The use of black silver plastic mulch gives a decrease in the number of individual weeds. The fewer the number of individuals and the lower the number of population, the inhibition of weed growth in giving black silver plastic mulch.

Table.1: SDR value of weeds after treatment.

Weed Type	SDR Weed After Treatment											
	Without mulch			Rice straw mulch			Banana mulch			Black silver plastic mulch		
	N:P2O5:K2O			N:P2O5:K2O			N:P2O5:K2O			N:P2O5:K2O		
	15g	20g	25g	15g	20g	25g	15g	20g	25g	15g	20g	25g
	%											
Boreria alata	40	39	49	28	37	39	22	34	38	10	23	32
Ageratum conyzoides	12	31	3	15	19	45	19	7	19	9	30	
Crassocephalum crepidiodes	7		16	4	17	13	7	17	9	3		
Sida acuta	3	5	20			9		9		6	5	31
Elausine indica	13	14		8	6		24		9	4		
Imperata cilindrica	10	4	3		5		10	10		13	4	
Axonopus compresus		6		5	12	8					8	
Bidens pilosa	3	4				6	5	5	14			

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Mikania micrantha	5		4		3		6						6				7		3					
Brachiaria reptans																								33
Blumea lacera					6										11				15					
Polygala paniculata	7		4								3										5			
Leptochloa chinensia	3		4				4								8									
Galinsoga parviflora cav			6				5		4		3													
Mimosa Infisa			7		4																			
Casia tora					3								6											
Centella asiatica			4																3					
Cinodon dactylon																			6					
Stachytarpheta jamaicensis			5																					
Nephrolepis biserrata					4																			
Cyperus rotundus					3																			
Physalis angulate											3													
Thitonia diversifolia													1											

3.2. Biomass Weed

Based on the results of statistical analysis of coffee plants aged 8 Weeks After Plan (WAP) through a 5% F test showed that various types of mulch and N: P: K fertilizer dosages affect weed biomass, while the interaction of various types of mulch and fertilizer dosage N: P: K gives effect not real to weed biomass.

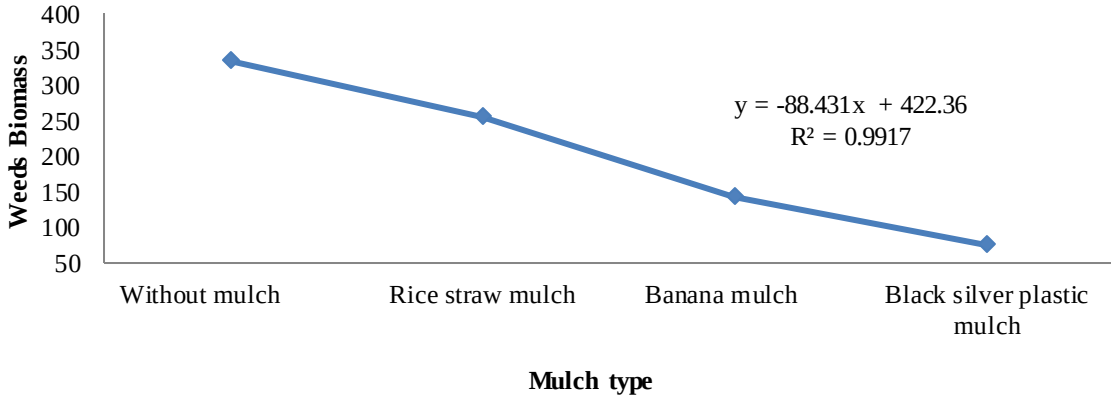

Fig.1: Relationship between Types of Mulch to Weed Biomass in the Age of Coffee Plants 8 WAP

Figure 1 shows the type of mulch increase weed biomass with the equation y = -88.43x + 422.3. The treatment that gave the highest effect for decreasing weed biomass in the treatment of black and silver plastic mulch was the value of weed biomass of 75 g / m3. The administration of banana stem mulch and rice straw mulch gives weed biomass values of 143 g / m3 and 255 g / m3. While the treatment which gave the lowest effect on the decrease in weed biomass in the treatment without giving mulch was the highest weed biomass value of 332 g / m3.

This is due to the use of various types of mulch which can suppress weed growth compared to the use of mulch. The use of black silver plastic mulch has a high influence compared to other types of mulch because black silver plastic mulch can regulate sunlight that reaches the ground surface. In general, all sunlight that hits the surface of black silver plastic, then some of the light will be reflected back into the air, in small amounts absorbed by plastic mulch and continued to reach the surface of the ground covered in black silver plastic mulch. The light reflected by the black silver mulch surface into the atmosphere will affect the top of the plant while the light that is passed down the surface of the black silver plastic will affect the physical, biological and chemical conditions of the covered phosphorus.

Sunlight is continued to melt the surface of the mulch trapped on the surface of the soil it is covering and forms a 'greenhouse effect' on a small scale, reinforced by statements (Wagoner, 1960; Tanner, 1974; Mahrer et al., 1979) in Fahrurrozi (2009) This trapped heat will increase soil surface temperature, modify groundwater balance, soil carbon dioxide, suppress weed growth, and increase

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the activity of microorganisms. Dark plastic mulch is very effective in controlling weeds (Fahrurrozi and Steward, 1994). This happens because weed seeds under black silver plastic mulch do not have access to sunlight to photosynthesize so that weeds that grow will experience etiolation and grow weak

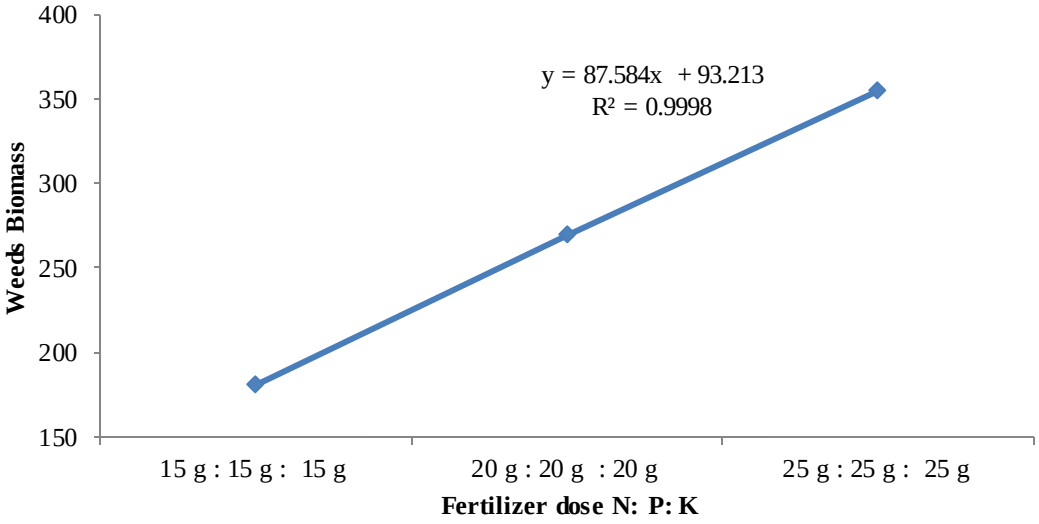

Fig.2: Relationship of N: P: K Doses to Weed Biomass in Coffee Crop Ages 8 WAP

Figure 2 shows the dosage of fertilizer N: P: K can increase weed biomass with the equation y = 87.38x + 93.21. The treatment that gave the highest influence for the reduction of weed biomass in the treatment of N: P: K 15g: 15g: 15g fertilizer was the value of weed biomass of 75 g / m3. Provision of N: P fertilizer: K 20g: 20g: 20g provides weed biomass values of 143 g / m3. While the treatment that has the lowest effect on decreasing weed biomass in fertilizer treatment N: P: K 25g: 25g: 25g is the highest weed biomass value 255 g / m3.

It is assumed that the higher the dosage of N: P: K fertilizer given, the more nutrient content and organic matter in the soil, which will increase soil fertility. The more fertile the soil, the more optimal plant growth will be. This means that fertilization will increase the carrying capacity of the land but will not reduce the composition of plant yields or disturbance of weeds still exist and detrimental even though the soil is fertilized (Sukman & Yakup, 2002).

Table 2 also shows the N: P fertilizer treatment: K 25g: 25g: 25g (88.79 g / m3) significantly different weed biomass values with fertilizer treatment N: P: K 15g: 15g: 15g (45 g / m3) but different not significant with fertilizer treatment N: P: K 20g: 20g: 20g (67.49 g / m3). While the value of weed biomass was not significantly different between fertilizer N: P: K 20g: 20g: 20g with fertilizer dosage N: P: K 15g: 15g: 15g and 25g: 25g: 25g. This is presumably due to the increase in nutrients given by a high N: P: K fertilizer dose which will cause an increase in the number of weeds. According to Harjadi (1993), at a higher level even though symptoms of deficiency do not appear, plants will respond to fertilization with increasing yield. With the availability of nutrients that are complete with the amount of each nutrient according to the needs of the plant will be able to stimulate the growth and development of the vegetative part of the plant.

Table.2: Weed Biomass in the Type of Mulch and Dosage Treatment N: P: K Age 8 WAP

Mulch type	**Fertilizer Dose N: P: KT**			**Effect of Mulch Type**
	15g :15g :15g	20g :20g :20g	25g :25g :25g	
Without mulch	84.45	117.57	130.16	110.73 A
Rice Mulch Rice	46.76	81.38	126.99	85.04 A
Banana Mulch	32.13	42.45	68.49	47.69 B
Black Silver Plastic Mulch	16.66	28.56	29.54	24.92 B
Effect of Fertilizer Dosage	45.00 b	67.49 ab	88.79 a	

The numbers followed by the same large letter in the same column and the numbers followed by the same small letter on the same line show different effects that are not significant according to the DNMRT level of 5%.

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IV. CONCLUSION

Based on the experiments that have been done, some conclusions are obtained, namely

1. The dominant weed in the cultivation of Arabica coffee in Indonesia is *Borreria alata*, followed by *Elausine indica* and *Ageratum conyzoides*.
2. The lowest weed biomass is obtained in black silver plastic mulch or N, P and K 15g: 15g: 15g.

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Grouper Fish Fisheries in Bays of Kwandang, Gorontalo Province are reviewed from Economic Social Aspects

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Abstract**—** *Socio-economic information is needed in the formulation of responsible grouper management. This study aims to determine the socio-economic conditions of grouper fishermen in Bays of Kwandang. This research was conducted from January to July 2018. Primary data was collected through surveys and direct interviews with fishermen and fishermen figures.. The Collecting data using questionnaire assistance on 35 people respondents. Grouper fishermen in Bays of Kwandang were still in the category of traditional fishermen. The income of fishermen is below of Rp. 200,000/trip with the catch per trip less than 25 kg. To improve welfare, fishermen need subsidies from the local government. The subsidies are in form of fishing fleets and capital to develop business diversification in the fisheries sector.*

Keywords—Grouper fish, socio-economic, and bays of kwandang.

I. INDRODUCTION

Bays of Kwandang is one of the main bases of fishing activities in the Sulawesi Sea (Faizah et al., 2014). There are three fishing efforts, namely large (Rahmat, 2007)small pelagic (Faizah et al., 2014), and demersal (Fahmi and Adrim, 2002) in the area . Of the various types of fish caught, groupers are one of the most dominant fish and have high economic value. Market demand for live groupers, both at domestic and abroad is very high (Kristanto et al., 2015).

To meet domestic and export market needs, the fishing of grouper is carried out on a large scale (Mujiyanto and Sugianti, 2014). More intensive exploitation of groupers occurs because of the high economic benefits (Ramadhani et al., 2017). The intensive fishing leads to over-exploitation (Santoso, 2016). Sustainable management is needed to anticipate problems. Regarding sustainable management, the socio- economic conditions of grouper fishermen need to be known. Therefore, a study was conducted to determine the socio-economic conditions of grouper fishermen in Bays of Kwandang.

II. MATERIALS AND METHODS

The research was conducted from January to July 2018, in Bays of Kwandang. Sample collection areas consisting of three islands, namely Pulau Malambe, Dudepo, and Ponelo. Primary data collected through surveys and direct interviews with fishermen and fishermen figures. Questionnaires arranged to collect data as modifications of Lorwens & Wouthuyzen (Lorwens and Wouthuyzen, 2015) (Table 1). The distribution of respondents in this study were 15 people in Malambe Island, 10 people in Dudepo, and 10 people in Ponelo.

Table 1. Questionnaires

1	Name:			Age:			
2	Education	a.	Elementary school	b.	Junior high school	c.	High school
3	Catching tool	a.	Fishing rod	b.	Speragun	c.	Combined
4	Fishing season	a.	Transition west to	b.	Transition east to west season		

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		east season				
5	Arrest fleet	a.	Without engine	b.	Machine	
6	Target of arrest	a.	Pelagic fish	b.	Coral fish	c.Combined
7	Long sail	a.	One day fishing	b.	> 1 day	
8	Range collaps	a.	Far	b.	Permanent	c.Close
9	Mileage	a.	<5 miles	b.	5-10 miles	c.> 10 miles
10	Traveling time	a.	<1 hour	b.	1-2 hours	c.> 2 hours
11	Size of fish	a.	Smaller	b.	Permanent	c.Big
12	Catch	a.	<25 kg	b.	25-50 kg	c.> 50 kg
13	Subsidy	a.	Really need	b.	Not really needed	c.No need
14	Handling	a.	Consumption	b.	Sell	c.Processed
15	Expected assistance	a.	Catching tool	b.	Capital	c.ship
16	Income / trip	a.	<Rp. 200,000	b.	Rp. 200,000-400,000	c.> Rp.400,000
17	Amenities	a.	Ice factory	b.	First time	c.Cooperative
18	Expenditures / month	a.	<1.5 million	b.	1.5-2.5 million	c. > 2.5 million

III. RESULTS AND DISCUSSION

Bays of Kwandang is one of the centers of capture fisheries in North Gorontalo Regency. At the beginning of 2011, the Kwandang Fishery Port has been built at whose position on the edge of the Sulawesi Sea. The port is the main base of reef fish fisheries. The economically important reef fish that are caught in Bays of Kwandang are five species. The species are mud grouper fish (Epinephelus coioides), tiger grouper (Epinephelus fuscoguttatus), rat grouper (Cromileptis altivelis), sunu grouper (Plectropomus leopardus), and moon tail grouper (Variola albimarginata). The fish were caught by stretching, fish trap, and speargun.

Based on the survey and interviews of social aspects, the highest percentage of grouper fishermen age is 30- 40 years (49%). Then followed by more than 40 years (29%) and under 30 years (23%) (Figure 1a). The majority of fishermen's education only elementary school with a percentage of 57% followed by junior high school with a percentage of 29%, and high school only 14% (Figure 1b). The low an educational level of fishermen is caused by two factors. The factors are the lack of awareness of the community island about the importance of education and the difficult access to schools that very far from the fishermen's settlements.

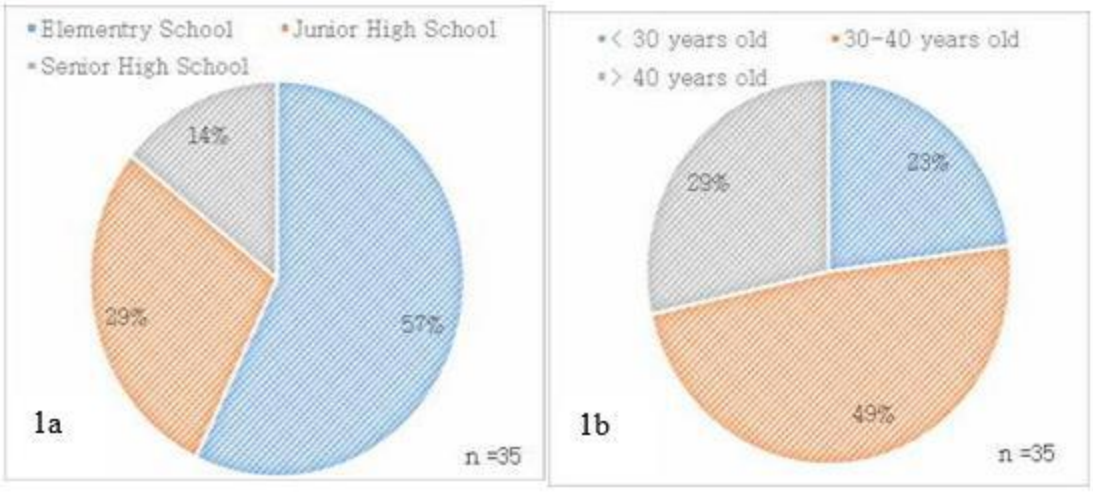

Fig.1: The age (a) and educational level of fishermen in Bays of Kwandang, Gorontalo Province

Based on the of fishing gear and fishing area aspects, fishermen in Bays of Kwandang are traditional fishermen category. The fishing gear used in the area are fishing rods with a percentage of 54%, speargun 40%, and a combination of the two fishing gear at 6% (Figure 2a). District Marine and Fisheries of North Gorontalo reported in 2017 that the catch of mud groupers used stretch fishing rods is 74, 2 tons, sunu grouper 61, 8 tons, and rat grouper

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24, 7 tons. Thus, the catch of speargun fishing gear is mud grouper fish of 47.5 tons. All grouper fishermen in Bays of Kwandang have used boats with 5.5-15 HP engine capacity (Figure 2b). The engine is a small engine capacity category that use to cover a distance to the fishing area (fishing ground) as far as below of 5 miles (Figure 2c).

The grouper fishing area in Bays of Kwandang is concentrated in several locations such as around Mohinggito Island, Malambe Island, Saronde Island, and the most remote one is Lampu Island. The areas are directly next to the Sulawesi Sea. As many as 91% of fishermen said that the travel time needed to get to the farthest fishing ground (Pulau Lampu) was around 45 minutes to 1 hour. Thus, only 9% of fishermen needed 1-2 hours to get to fishing ground (Figure 2d). Therefore, the operation length of grouper fishermen is only around 12 hours/trip (one-day fishing) (Figure 2e). Furthermore, 94% of grouper fishers in Bays of Kwandang make arrests in the eastern season and only 6% also make arrests in the western season (Figure 2f). The fishermen decision based on oceanographic conditions such as strong currents and bad weather and the limitations of adequate fishing facilities (Achmad et al., 2017; Yulianto et al., 2013).

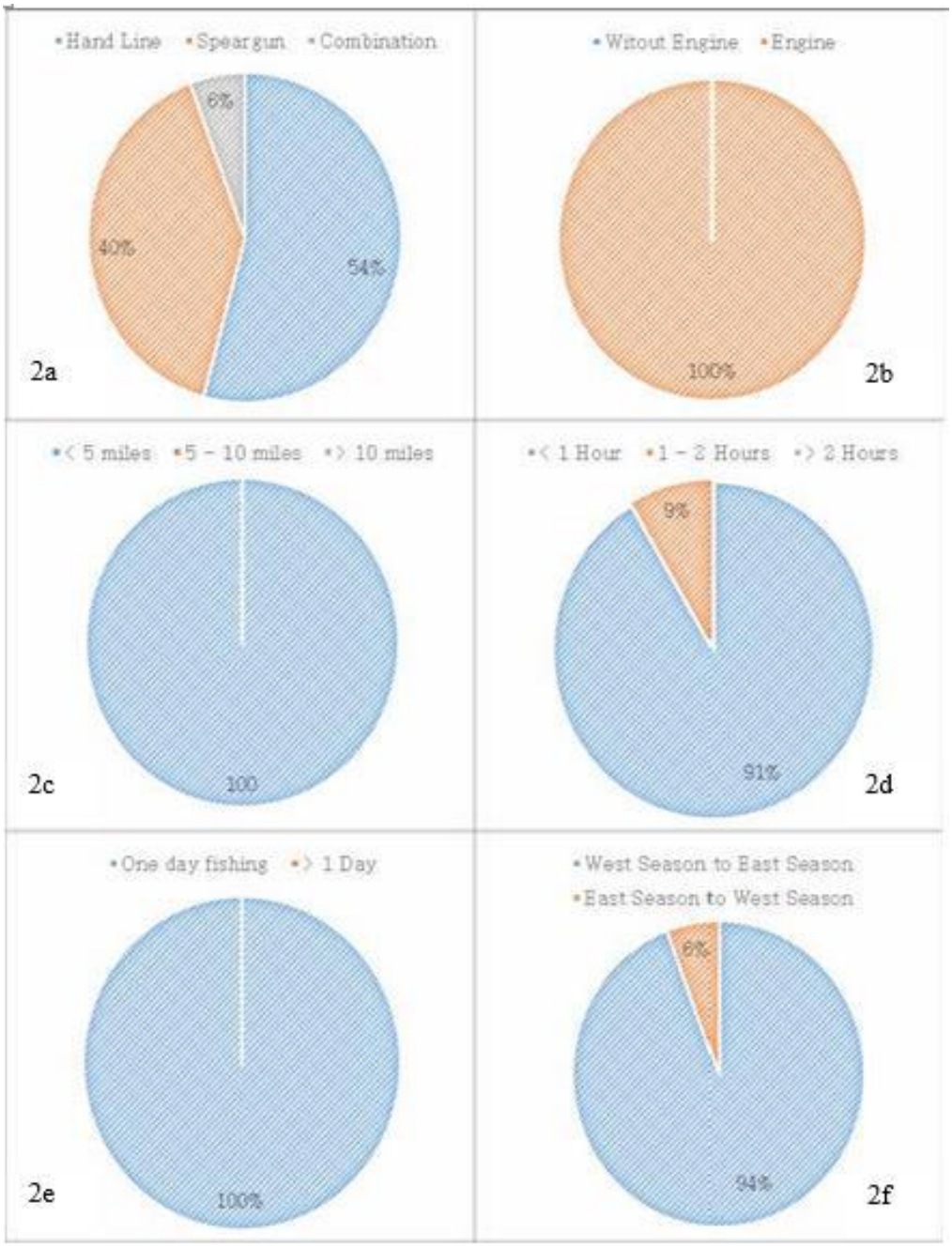

Fig.2: The Types of fishing gear (a), Capture fleet (b), Distance to fishing ground (c), Travel time to fishing ground (d), Length of sea (e), and Season of grouper fishing (f) in Bays of Kwandang, Gorontalo Province.

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The survey and interview revealed that 86% of the distance of fishing ground was increased while only 14% said it remained (Figure 3a). In the last few years, the fishing ground has shifted somewhat to the outer islands. Before that time, catch activity of fishermen only in around of Mohinggito Island and Malambe Island. The islands are close to their settlements. However, it is currently shifting far to Saronde Island and Lampu Island. The shift in the fishing ground was also followed by the increasing number of small-sized fish caught (71%) while only 29% of fishermen said the size of the caught fish did not change (Figure 3b)

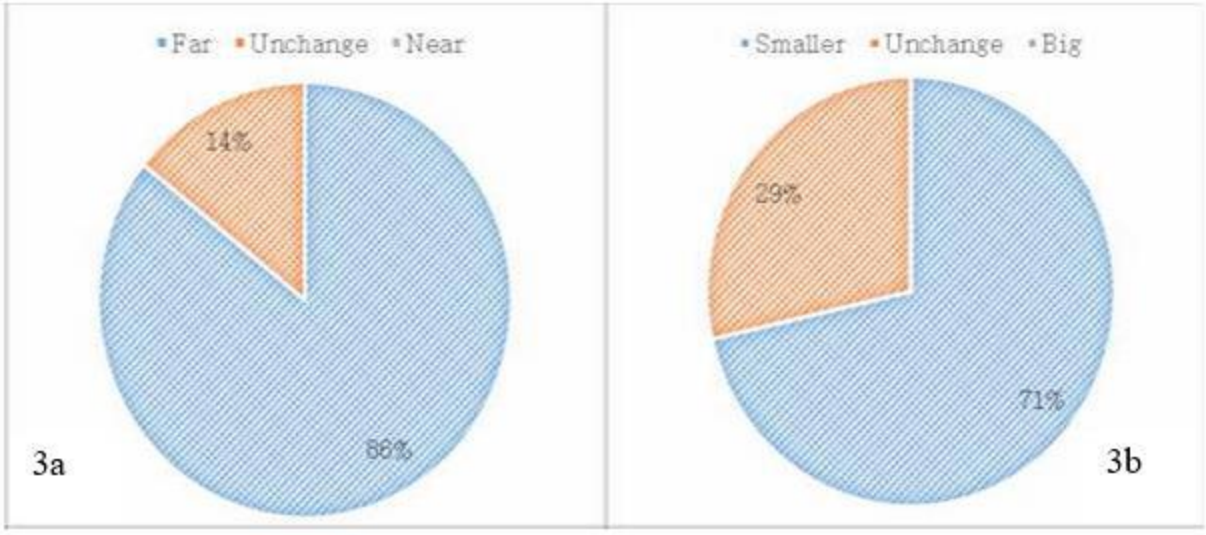

Fig.3: The Range collaps (a) and (b) Fish size in Bays of Kwandang, Gorontalo Province

The results of surveys and interviews for economic aspects show that the majority of grouper fishermen have income below Rp. 200,000/trip (54%), Rp. 200,000-400,000/trip
(40%), only a small portion of the income is above Rp. 400,000 trip (6%) (Figure 4a). While the monthly expenditure of fishermen is mostly above Rp. 2,500,000 (66%), and other fishermen have in the range of Rp. 1,500,000- Rp.2,500,000 (34%) (Figure 4b). The high expenditure of fishermen is sometimes not accompanied by the amount of catch obtained. Percentage of catch per trip shows that 69% of fishermen obtained catches below 25 kg/trip, 26% in the range of 25-50 kg/trip, and only 6% above of 50 kg/trip (Figure 4c). The catch of 96% of fishermen is directly sold to collectors and 6% for self-consumption (Figure 4d).

The survey results also revealed that fishermen need subsidies from the local government (Figure 4e). The subsidies needed are capture fleets (86%), fishing gear (3%), and capital assistance (11%) (Figure 4f). So far, many fishermen have spent funds to repair their fleet catches. The capture fleet that has a more distance travel capacity enables fishermen to expand into the open sea which has been exploited so far. Besides, the capital assistance help to develop business diversification in the fisheries sector.

Facilities needed by fishermen in Bays of Kwandang are ice factories (51%), Fuel stations (46%), and cooperatives institutions (3%) (Figure 5). The access collectors away from fishing ground cause the fish catches sometimes not so fresh that it affects the price of fish. With the existence of an ice factory on the islands around the fishing ground, the quality or freshness of the fish can be maintained. Fishermen also need the fuel stations around the island to guarantee the availability of fuel. So far the fuel for fishing boats has been obtained from traders around the Kwandang Fishery Port and the price reaches Rp. 10,000/liter while the normal price is only Rp. 8,000/liter.

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Fig.4: The Fishermen's income per trip (a), monthly fishermen's expenditure (b), one-trip catch (c), handling of catches (d), fishermen who need subsidies (d), and assistance expected by fishermen (f), in Bays of Kwandang, Gorontalo Province.

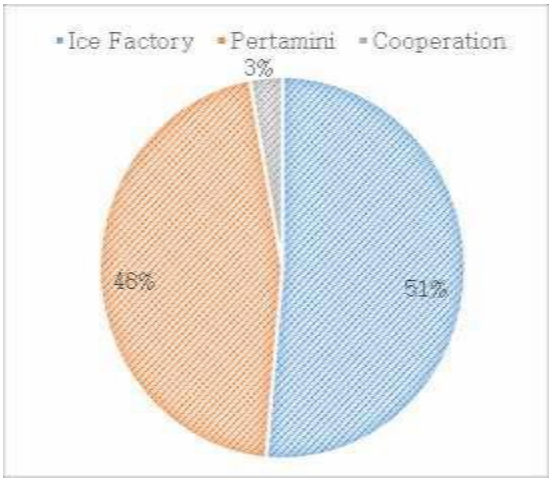

Fig.5: facilities needed by fishermen in Bays of Kwandang, Gorontalo Province.

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IV. CONCLUSIONS AND RECOMMENDATIONS

Grouper fishermen in Bays of Kwandang are a traditional fishermen category who income below Rp. 200,000/trip and less than 25 kg catch/trip. To improve welfare, fishermen need subsidies from the local government like fishing fleets and capital to develop business diversification in the fisheries sector.

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Effect of some Bacterial Bioagents against Root-Knot Nematode (*Meloidogyne incognita* race2)

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Abstract-*The effect of culture filtrate of different bacterial isolates on egg hatching and juvenile mortality of root-knot nematodes* (*Meloidogyne incognita race 2*) *was studied in vitro and the effective strains were selected. The per cent mortality and hatching inhibition was proportional to the concentration of culture filtrate and the duration of exposure period. Culture filtrate of all the isolates of bacteria significantly induced mortality and inhibition of egg hatching of M. incognita juveniles. The highest percentage of inhibition of egg hatching was recorded for Bacillus thuringiensis followed by Bacillus sp. and Pseudomonas fluorescens whereas the highest percentage of mortality of juvenile was recorded for Bacillus thuringiensis followed by Pseudomonas fluorescens and Pseudomonas sp.*

Keywords: Rhizospheric bacteria, egg hatching, juvenile mortality, culture filtrate, duration of exposure period, root-knot nematode.

I. INTRODUCTION

Root-knot nematode *Meloidogyne incognita* race 2 is one of the major constraints in crop production in Assam. Present strategies for nematode management largely depend on cultural practices such as crop rotations, use of resistant varieties and use of nematicides which has several disadvantages. Utilization of biocontrol agent is of advantage over the above mentioned nematode management strategies. Many species of *Pseudomonas* and *Bacillus* have been reported as plant growth promoting rhizobacteria (PGPR) which produces iron-chelating siderophores, antibiotics or hydrogen cyanide, and these compounds have been implicated in the reduction of deleterious and pathogenic rhizosphere microorganisms, creating an environment more favourable for root growth[1] .It has been demonstrated that bio-agents produce different metabolites and antibiotics which directly or indirectly stimulate plant growth [2] . A number of bacterial species has been used as biocontrol agents against *Meloidogyne* spp. [3-12]. However, few biocontrol products are currently commercially available and it is necessary to find different strains of antagonistic bacteria for controlling plant-parasitic nematodes more efficiently. Therefore, a laboratory bioassay was carried out to test some of the isolated strains of *Bacillus* and *Pseudomonas* for their nematicidal activity.

II. MATERIAL AND METHODS

Isolation of bacteria

A survey was conducted during Feb 2018 to May 2018 in different localities of the district Jorhat, Assam comprising an area approximately 500 ha, in order to identify the nematodes infection and to isolate the bacterial antagonists from crops rhizosphere viz. cucurbits, tomato, brinjal, okra, cabbage, banana, citrus, and tea. A total of 50 soil (500 g) sample were collected from the rhizosphere of each crop. Serial dilution technique was used for the isolation of bacterial strain. One g of rhizosphere soil was dispensed in 9 ml sterile water, from the 10^{-6} dilution, 50 µl were inoculated over Petri plates containing Nutrient Agar and King's B agar media (autoclaved at 121°C for 15 min) separately. The plates were incubated at room temperature 28±2°C for 48-72h.The colonies obtained on plates were picked and streaked on more plates and purified by re-streaking. The isolates were initially categorized into two broad groups based on Gram staining by Hucker's modified method [13]. The isolated strains were identified by up to generic level by Bergey's manual and using standard protocol [14, 15].Two isolates namely *Pseudomonas* sp. and *Bacillus* sp. were collected from soil rhizosphere of cowpea and banana respectively. *Pseudomonas fluorescens, Bacillus thuringiensis, B.brevis* were procured from the Department of Plant Pathology, AAU, Jorhat, Assam. All the strains were evaluated for nematicidal properties against nematodes.

Nematode inoculum and mass culturing

The inoculum of root-knot nematode *M. incognita* race 2 was collected from naturally infested tomato crop in field and single egg mass was used to raise pure culture. Mass culturing of nematodes was done on tomato variety Sel 7, in

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order to get regular supply of the inoculums for the experiments. One month old tomato seedlings were inoculated with small volume of egg suspension approximately consisting of 2000 eggs of *M.incognita* race 2. These pots were watered and kept in glasshouse at temperature 28-35°C.

Preparation of culture filtrate and test for nematoxicity of bacterial culture filtrate

The bacterial isolates were inoculated in the respective medium (40 ml). *Pseudomonas* on King's B broth and *Bacillus* on Nutrient broth were separately incubated at 30°C on a shaker for 48 hrs. The liquid culture was filtered through Whatman No.1 filter paper and passed through bacterial filter[16] .Filtrates were centrifuged at 6000 rpm for 15 min. The supernatant was taken and the suspended residue was discarded.

Juvenile mortality test

One ml of S/2, S/8, S/32 dilutions of each cell-free culture filtrates was mixed with 1 ml nematode suspension (containing ca. 100 *M.incognita* J2) in cavity block to obtain S/4, S/16 and S/64 dilutions and tested for per cent mortality of nematodes. There were 3 replications for each treatment. Observations were taken after 24 h, 48 h and 72 h. The immobile J2 were confirmed for mortality by randomly disturbing with a needle. Sterile water and medium alone served as control. The per cent juvenile mortality was calculated by the following formula.

$$\text{Percent mortality} = \frac{\text{Total number of dead juveniles}}{\text{Total number of juveniles}} X\ 100$$

Egg hatching test

To determine the effect of culture filtrate on the hatching of eggs of J_2 of *M. incognita* eggmasses of *M.incognita* were dissolved in NaOCl to release individual eggs [17]. Sterilized Petri dishes of 5 cm dia were separately pipette 1 ml of culture filtrate with dilution S/2, S/8 and S/32. Hundred eggs of *M.incognita* in 1 ml water suspension were transferred to each dish. Medium alone and water alone served as control. All Petri dishes were kept at 28±2°C, replicated thrice. Observations were recorded on after 5, 10 and 15 days. The per cent egg hatch was calculated by the following formula.

$$\text{Hatching \%} = \frac{\text{No. of hatched juveniles}}{\text{No. of hatched} + \text{unhatched eggs}} X\ 100$$

Statistical analysis

Per cent egg hatch and per cent mortality data was subjected to statistical analysis using the three factorial completely randomized design statistical package. The critical differences in main effects i.e. isolates, concentration, and time of exposure as well as in their interactions were tested at P=0.05.

III. RESULTS

Two numbers of isolate of bacteria were isolated from the root rhizosphere of *M.incognita* race 2 infected plants. *Bacillus sp.* and *Pseudomonas* sp. were isolated from rhizospheric soil of banana and brinjal, respectively. The results presented revealed significant differences in juvenile mortality of *M.incognita* among isolates (biocontrol agent) (T), concentration of culture filtrate(C) and exposure period (t).

The data showed in the Table 1 revealed that all the culture filtrates of bacterial isolates were having nematicidal effect of varying degree on *M.incognita* race 2. Per cent mortality of nematodes was directly proportional to the concentration of culture filtrate and the period of exposure. Irrespective of concentration of culture filtrate(C) and duration of exposure (t), six isolates namely *Bacillus* sp., *B.brevis B.thuringiensis*, *Pseudomonas* sp., *P.florescence* and *P.aeruginosa* were exhibited nematicidal effects on *M. incognita* J_2. Irrespective of concentration of culture filtrate and exposure period the mortality rates of *M. incognita* J_2 caused by *B.thuringiensis* reached 75.77% at which is at par with *P.florescence* (75.44%).All the new isolate namely *Bacillus* sp, and *Pseudomonas* sp displayed more than 40% juvenile mortality during 24 h exposure time at S/16 concentration of culture filtrate. On the other hand, *P.aeruginosa* showed the lowest toxicity, caused only 48% juvenile mortality S/64 at concentration of culture filtrate during 48 h exposure time. Irrespective of isolate and period of exposure the mortality rate of *M. incognita* J_2, at lowest concentration (S/64) is 43.62% whereas at highest concentration (S/4) mortality rate is 51.66%.Similarly irrespective of isolate and culture filtrate concentration the mortality rate of *M. incognita* J_2 is highest (63.07%) at 72 h exposure period and lowest (34.85%) at 24h exposure period. *B.thuringiensis* and *P.florescence* showed highest toxicity (100% J2 mortality) at S/16 concentration of culture filtrate during 72 h exposure period. There was least effect of media on juvenile mortality of *M. incognita*.

The results presented in Table 2, revealed significant differences among isolates (T), concentration of culture filtrate(C) and exposure period (t) in egg hatching of *M. incognita*. Irrespective of concentration of culture filtrate (C) and time of exposure period (t), the culture filtrate of *B.thuringiensis* followed by, *Bacillus* sp., *P. fluorescens* and *Pseudomonas* sp. adversely affected the larval hatching of

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M.incognita race 2. Similarly, irrespective of isolate (T) and concentration of culture filtrate (C), time of exposure (t) also affected the larval hatching. With increase in exposure period up to 15 days there was a correspondingly increased in egg hatching. Hatching of *M. incognita* J_2 is highest (57.03%) at 15 day exposure period and lowest (40.00%) at 5 day exposure period. With increase in the dilution of culture filtrate, the cumulative hatching was increased irrespective of isolate (T) and time of exposure period (t). Highest inhibition in hatching was obtained in S/4 concentration of each bacterial culture filtrates. The percentage hatching of *M.incognita* was 38% for 15 day of exposure period in the S/64 concentrations of culture filtrates of *B.thuringiensis* followed by *Pseudomonas* sp. with percentage hatching of 48. *B.brevis* and *P.aeruginosa* showed negligible effect on inhibition of egg hatching of *M.incognita*. There was negligible effect of culture media on hatching of *M.incognita*.

IV. DISCUSSION

Culture filtrates of several soil borne microbes are known to exhibit nematicidal action besides inhibiting larval emergence of plant parasitic nematodes. Antibiotics, extracellular enzymes and other toxic compounds present in metabolites of rhizobateria are probably involved in the increase of J_2 mortality and the inhibition of egg hatching[18]. The lethal effect of the cultural filtrates of these bacteria may be attributed to the production of nematicidal metabolites i.e. lytic enzymes (gelatinase, protease and chitinase) and volatile compounds in the cultural media. Similar results were reported by Ali *et al.* [19] who found that cultural filtrates of *Pseudomonas* sp. caused juvenile mortality of *M. javanica*. The nematicidal volatile products that were produced by *Bacillus* and were characterized to include mainly the benzene acetaldehyde, 2-nonanone, decanal, 2-undecanone and dimethyl disulphide, which were active against *M. incognita* juveniles and eggs at the concentration of 0.5 mmol [20]. Ahl *et al*., [21] proposed the mechanism of reduction of nematode population which was due to premature egg hatching and reduction in viability and mortality of juveniles induced by secondary metabolites such as 2,4 Diacetylphloroglucinol and lytic enzymes [22], antibiotics and hydrogen cyanide produced by *Pseudomonas* spp. and non cellular extract and toxic metabolites like bacillopeptidase, subtillin E and a lactamase from *Bacillus* spp. Regina *et al*. [23] and Hanna *et al*. [24] have reported that mortality of *M. incognita* increased with increase in exposure time as well as the concentration. The effects of rhizobacterial toxins include the suppression of nematode reproduction, egg hatching and juvenile survival, as well as direct killing of nematodes by causing paralysis and convulsive movements [4]. Dhawan *et al.* [25] evaluated four strains of *Bacillus thuringiensis*, and found that the mobility of *M.incognita* juveniles completely ceased after 24h exposure in S and S/10 dilutions. However, all dilutions above S/25 were ineffective. Production of surfactin by *Bacillus* is a characteristic that supports their persistence under extreme conditions [26, 27]. El-Hamshary *et al.* [28] found that *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* affected *M. incognita* juvenile survival *in vitro* study, and the mortality percentages of the nematode were dependent on the bacterial concentration and exposure time. Terefe *et al.* [29] found that an aqueous suspension of *Bacillus firmus* at 2.5 and 3% concentration caused 100% inhibition of mobility *M. incognita*, 24 hrs after treatment. The mortality percentage of *Bacillus circulans* (KSB2) at dilutions of 1/100 and 1/1000 were the highest inhibitors comparing with the other tested bacteria being 97.8 and 40.3%, respectively [30]. Xia *et al.* [31] reported that the culture supernatant of *B. subtilis* strains caused a higher mortality of *M. incognita* J_2. Nagesh *et al.* [32] observed that, cell-free culture filtrates of *B. cereus* reduced egg hatching (90%) and caused 100% mortality of juveniles. Bin *et al.* [33] mentioned that culture filtrates of rhizobacterium are heat stable and resistant to extreme pH values, which suggested that the antibiotic action might be responsible for the nematicidal activity**.** The exposure of *M. incognita* to various concentrations (5-100%) of cultural filtrate of *Paenibacillus polymyxa in vitro* conditions significantly reduced egg hatching and caused substantial mortality of its juveniles [34]. These results are similar with the findings of Ying *et al.* [35] who reported *Bacillus* spp. culture could significantly inhibiting the hatching of eggs and increases the mortality of second stage juveniles and reduce infection of the nematode through production of nematicidal volatiles. Antibiotic production viz., 2, 4 DAPG, phenazine and pyocyanine were also well pronounced in the *Pseudomonas* strain, Pf 128 through thin layer chromatography [36] .The failure to regain its activity of *M. incognita* after separating from culture filtrate of *Pseudomonas* spp. placed over sterile water demonstrates the numbers of metabolites that had longer systemic activity [37,38].

V. CONCLUSION

It is clear from this work that, there are many bacteria which have potentialities for controlling root-knot nematode in plant rhizosphere region. Among six bacterial isolates *Bacillus thuringiensis and Pseudomonas fluorescens* were

exhibited the highest production of nematicidal activities against root-knot nematode *M. incognita* race 2.

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Table.1: Effect of culture filtrate of some bacterial bioagent on juvenile mortality of Meloidogyne incognita race 2.

Treatment	Culture filtrate concentration	Period of exposure(h)			Treatment (T)Mean	Culture filtrate concentration (C)Mean
		24	48	72		
Bacillus sp.	S/64	36(36.85)	55(47.87)	85(67.21)	62.00(52.79)	43.62(39.61)
	S/16	40(39.20)	58(49.62)	86(68.08)		48.85(44.26)
	S/4	48(43.85)	60(50.79)	90(71.62)		51.66(46.20)
B.brevis	S/64	38(38.04)	60(50.79)	70(56.79)	63.44(53.81)	
	S/16	42(40.38)	62(51.94)	90(71.94)		
	S/4	50(44.99)	66(54.33)	93(75.05)		
B.thuringiensis	S/64	55(47.86)	74(59.35)	75(60.00)	75.77(63.74)	
	S/16	60(50.76)	77(61.38)	100(90.00)		
	S/4	62(51.94)	79(62.74)	100(90.00)		
Pseudomonas sp.	S/64	46(42.70)	60(50.76)	72(58.11)	66.44(55.87)	
	S/16	50(44.99)	62(51.94)	93(74.34)		
	S/4	52(46.14)	68(55.64)	95(75.07)		
P.florescence	S/64	58(49.60)	74(59.35)	76(60.67)	75.44(63.52)	
	S/16	58(49.60)	75(60.00)	100(90.00)		
	S/4	60(50.76)	78(62.08)	100(90.00)		
P.aeruginosa	S/64	38(38.04)	48(43.85)	82(64.91)	59.55(51.13)	
	S/16	42(40.39)	52(46.14)	84(66.44)		
	S/4	48(43.85)	56(49.02)	86(68.05)		
Culture media(NA)	S/64	6(14.04)	10(18.37)	20(26.54)	14.00(21.49)	
	S/16	8(16.34)	12(20.7)	20(16.50)		
	S/4	10(18.37)	18(25.07)	22(27.94)		
Culture media(KB)	S/64	8(16.34)	10(18.37)	16(23.54)	14.00(21.75)	
	S/16	12(20.22)	12(20.22)	18(25.07)		
	S/4	14(21.93)	16(23.46)	20(26.54)		
Distilled water	S	0(0.00)	2(8.13)	4(11.27)	1.77(6.12)	
	S	0(0.00)	2(8.13)	4(11.27)		
	S	0(0.00)	2(8.13)	2(8.13)		
Period of Exposure (t)Mean		34.85 (33.60)	46.22 (41.38)	63.07 (55.09)		
CV=4.47,CD(P=0.05): Treatment(T):1.03; Concentration(C):0.59; Period of exposure (t): 0.59;T×C: 1.79; T×t: 1.79; C×t:1.50;T×C×t : 3.10						

Figures in the parentheses are Arc-Sine transformed values

Table.2: Effect of culture filtrate of some bacterial bioagent on hatching of Meloidogyne incognita race 2.

Treatment	Culture filtrate concentration (%)	Period of exposure(days)			Treatment Mean	Culture filtrate concentration Mean
		5	10	15		
Bacillus sp.	S/64	32(34.43)	38(38.04)	52(46.14)	35.33 (36.32)	52.25(46.61)
	S/16	26(30.65)	34(35.66)	44(41.54)		48.70(44.35)
	S/4	22(27.94)	32(34.43)	38(38.04)		45.74(42.47)
B.brevis	S/64	39(38.63)	48(43.85)	56(48.44)	43.11 (41.01)	
	S/16	30(33.19)	45(42.70)	52(46.14)		

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	S/4	28(31.93)	40(39.22)	50(44.99)		
B.thuringiensis	S/64	22(27.95)	30(33.19)	38(38.04)	26.55 (30.77)	
	S/16	18(25.07)	26(30.64)	36(36.86)		
	S/4	15(22.71)	22(27.95)	32(34.43)		
Pseudomonas sp.	S/64	30(33.19)	40(39.22)	48(43.85)	37.77 (37.87)	
	S/16	36(36.86)	38(38.04)	44(41.55)		
	S/4	32(34.43)	32(34.43)	40(39.22)		
P.florescence	S/64	34(35.66)	42(40.39)	50(44.99)	36.66 (37.09)	
	S/16	24(29.32)	38(38.04)	46(42.70)		
	S/4	22(27.95)	30(33.19)	44(41.55)		
P.aeruginosa	S/64	34(35.66)	44(41.54)	50(44.99)	38.66 (38.32)	
	S/16	28(31.93)	38(38.03)	48(43.85)		
	S/4	24(29.31)	36(36.85)	46(42.70)		
Culture media(NA)	S/64	64(53.12)	78(62.03)	82(64.91)	72.33 (58.47)	
	S/16	63(52.53)	74(59.35)	80(63.44)		
	S/4	60(50.76)	72(58.05)	78(62.03)		
Culture media(KB)	S/64	67(54.93)	78(62.03)	82(64.93)	73.55 (59.24)	
	S/16	64(53.12)	76(60.66)	80(63.44)		
	S/4	62(51.94)	75(60.00)	78(62.03)		
Distilled water	S	70(59.02)	79(62.72)	84(66.49)	76.11 (61.19)	
	S	67(54.93)	78(62.16)	82(64.91)		
	S	67(54.95)	78(62.03)	80(63.44)		
Period of Exposure (t)Mean		40.00 (38.97)	49.66 (44.98)	57.03 (49.47)		
CV=3.33,CD(P=0.05): Treatment(T):0.78; Concentration(C):0.45; Period of exposure (t): 0.45;T×C: 1.35; T×t: 1.35; C×t:0.78;T×C×t : 2.34						

Figures in the parentheses are Arc-Sine transformed values

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The Effect of 2, 4 D (*Dichlorophenoxyacetic acid*) and BAP (*Benzyl Amino Purine*) Concentration on the Establishment of Patchouli (*Pogostemon cablin Benth*) in Vitro

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Abstract**—** *The aim of this study was to determine the concentration of 2,4-D growth regulator and the most effective BAP for the formation of callus for endemic patchouli plants in western markets, namely Akasesi Situak in vitro. The research has been carried out at the Andalas University Faculty of Agriculture Tissue Culture Laboratory, in September November 2018. The method used is Random Trap Design (RAL) with 2 treatment factors, namely 2,4-D (0, 0.5, 1, 1.5, and 2 mg). / l) and BAP (0, 0.5, 1, 1.5, and 2 mg / l), the total consists of 25 treatments with 3 replications with codes namely A1S1, A1S2, A1S3, A1S4, A1S5, A2S1, A2S2, A2S3, A2S4 , A2S5, A3S1, A3S2, A3S3, A3S4, A3S5, A4S1, A4S2, A4S3, A4S4, A4S5, A5S1, A5S2, A5S3, A5S4, A5S5. Data were statistically analyzed by F test at 5% real level. If F count is greater than F table 5%, then proceed with Duncan's New Multiple Range Test (DNMRT) at the level of 5%. The results of the study show that on the treatment of A3S3 (1.0 mg / l 2.4-D + 1.0 mg / l BAP), A3S4 (1.0 mg / l 2.4-D + 1.5 mg / l BAP), A3S5 (1.0 mg / l 2.4 -D + 2.0 mg / l BAP) and A4S1 (1.5 mg / l 2.4-D + without BAP) produce explants. whereas in other combination treatments up to 30 days of observation did not produce patchouli plants in the Situak Accession plant.*

Keywords**— Patchouli (Pegestemon cablin Benth), 2,4-D, BAP, Tissue Culture.**

I. INTRODUCTION

Patchouli oil (*Pogostemon cablin Benth*) is one of the types of plants producing essential oils known as patchouly oil. In the world of flavor and fragrance, especially for the perfume and aroma therapy industries, patchouli plants contribute to the country's foreign exchange and farmers' income. Before the Second War, patchouli oil produced from Indonesia reached 90% of the world's needs. Even Indonesia has earned the nickname of the Manufacturer of Patchouli Sumatra because most patchouli plants are produced from several regions in Indonesia, which are found in East Java, West Sumatra, Aceh, Central Java, Jambi, South Sulawesi, and followed by West Java, North Sumatra, West Sulawesi, South Sumatra, Central Sulawesi, Gorontalo, Lampung, Yogyakarta, Bali, East Nusa Tenggara, and East Kalimantan. Patchouli cultivation in West Pasaman is spread in 10 sub-districts, each patchouli plant cultivated in each region has its own peculiarities. Based on Rahmad (2017) research related to the exploration and characterization of the local patchouli phenotype in West Pasaman Regency, it was explained that there were 7 (seven) types of patchouli plants which were scattered and had different characteristics and morphology. The names of the seven types of accessions are Aia Maruok, Bukik Nilam, Rimbo Binuang, Tombang, Tanjung Durian, Situak and Lubuk Godang. From these data, Situak Accession is the highest patchouli plant reaching 117.2 - 129 cm (Appendix 7). The high range of these plants also beat the height of patchouli plants from the Research Institute for Medicinal and Aromatic Plants in 2006, the superior varieties of Lhokseumawe, Tapaktuan, Sidikalang, Patchoulina 1 and Patchoulina 2 with a range of plant height sequentially 61.07 - 65.97 cm, 50.57 -82.28 cm, 70.70 - 75.69 cm, ± 112.34 cm and 117.50 cm. Strengthened by Linda's research (2017), it was shown that patchouli of Situak Accession contained higher PA (Pathouli Alcohol) than six (six) other accessions from West Pasaman, reaching 28.04% with AV 2.58%. Patchouli oil is one type of essential oil that has properties that are difficult to wash, difficult to

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evaporate, can dissolve in alcohol and can be mixed with other essential oils. The need for patchouli oil will continue to increase in line with the increase in consumption of perfume, cosmetics, soaps and so on, which leads to the prospect of patchouli oil exports becoming more promising in the future. Patchouli oil can be obtained conventionally through direct extraction from plants. However, this method requires large-scale patchouli cultivation so that it has difficulty providing land. Yuhono and Suhirman, (2006) also added that the low quality of patchouli oil was partly due to the possibility that the seeds planted were not of superior varieties, so the yield of patchouli oil produced was relatively low.

The quality of patchouli oil is very important to note. Paul et al., (2010) reported that Pathouli Alcohol levels of patchouli plants in vitro were higher at 56.30% compared to patchouli plants in vivo which was 44.35%. The higher the content of PA (Pathouli Alcohol), the better the quality of patchouli oil will be. Efforts to increase Pathouli Alcohol content in patchouli oil continue. One solution is tissue culture. Tissue culture techniques can overcome obstacles that are often encountered in problems surrounding the supply of seeds, for example the provision of uniform seeds, in a relatively short time, not dependent on the season, free of disease. Besides that it is also able to increase the production of secondary metabolites such as those contained in patchouli plants. To stimulate the production of sekundet metabolites can be done by callus culture. The success of culture techniques using explants depends on the factors possessed by the explants themselves (size, physiological age, source and explant genotype), aseptic conditions, proper media selection, and environmental factors (Kartikasari et al., 2013) Selection of appropriate media with a combination of growth regulator substances is a determining factor in inducing secondary metabolites.

Growth regulating substances that are often used in tissue culture for callus initiation and increasing secondary metabolite production (organogenesis) are auxin and cytokinin. Auxin is usually used to induce callus formation, suspension culture, and roots, by stimulating cell lengthening and division in cambium tissue. The relatively high concentration of auxin will refer to embryogenic callus formation and somatic embryo structure. Addition of auxin and cytokinin to culture media can increase the concentration of endogenous growth regulating substances (fitohormones) in cells, thus becoming a trigger factor in the process of growth and development of tissues (Lestari, 2011). This can be proven in Palupi's study (2004) that the combination of 1.0 mg / L 2,4-D.

II. MATERIALS AND METHODS

This study uses an experimental method conducted in August to November 2018 at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University, Padang. The material used in this experiment is the patchouli of Situak Accession (Jorong Situak, Lembah Melintang Sub-district). 2,4-D (Dichlorophenoxyacetic acid), BAP (Benzyl Amino Purine), MS media (Murashige and Skoog), agar agar (Pure) 7 g / L, fungicide 300 mg / L, bactericidal (Agrept 20WP) 30 mg / L , Tween 20, sterile aquades, 70% and 96% alcohol, 3% sucrose, 1 mol / L HCL, 1 mol / L NaOH, digital pH meter, plastic, rubber band, plastic wrap, tissue, spritus, masking tape (clear tape)), disinfectants (formalin), aluminum foil, micropipette tips, and label paper. The tools used in this experiment are Laminar Air Flow Cabinet (LAFC), autoclaves, analytical scales, hot plate magnetic stirers, ovens, scalpell blades, tweezers, erlenmeyer 1000 mL, 50 mL goblets, culture bottles, bunsen, petridisk, measuring cups 10 mL, glass bottles, culture shelves, micropipets, handsprayer stationery, cameras. This research was conducted in 2 stages. The first stage is the quarantine process of the Situak Accession patchouli plant, the second stage is the induction of patchouli callus. Callus induction was arranged in a Completely Randomized Design (CRD) consisting of two factors. The first factor was the administration of 2,4-D concentration with 5 levels of treatment and the second factor was the administration of BAP concentrations with 5 levels of treatment. Thus 25 treatment combinations are obtained with codes namely: A1S1, A1S2, A1S3, A1S4, A1S5, A2S1, A2S2, A2S3, A2S4, A2S5, A3S1, A3S2, A3S3, A3S4, A3S5, A4S1, A4S2, A4S3, A4S4, A4S5, A5S1, A5S2, A5S3, A5S4, A5S5. Each treatment with 3 replications and 3 bottles per replicate. So that there are 225 bottles of explants (Appendix 2). The data obtained were analyzed using the F test at the level of 5%, if F count is greater than F table then the analysis is continued with the DMNRT test at the level of 5%.

III. RESULTS AND DISCUSSIONS

1. When Appearing Callus (HST)

The time when callus appears on each plant has a very diverse variation. Changes in explants that are characterized by tissue swelling and explant color become brownish yellow is a sign that callus begins to appear. explant swelling is a response from plants which results in the majority of existing carbohydrates and proteins accumulating in the injured tissue (Merlin et al., 2012). Average when appearing callus can be seen in Table 1.

Table 1 shows the diversity of time when callus appears. The average when the fastest callus starts on the A1S3 treatment is 8 HST. A1S3 is a combination of 2,4-

D 0 mg / l and BAP 1 mg / l. Sugiharto et al. (2007) concluded from the results of his research regarding invitro patchouli plant propagation with a combination of cytokinin and auxin that the effective concentration for in vitro propagation of patchouli plants is BAP 1 ppm with no 2.4 D. Excessive cytokonine dosage or the types of cytokines that do not fit the needs of plants can be the cause of epigenetic diversity. The use of cytokinin that is very strong / excessive can cause a bad influence on the next micropropagation stage (Acram Taji et al., 2005). From the results of the Princess study (2016) stated that the combination of Auxin NAA 0.1 mg / l with Cytokinin BAP (0.1, 0.3 and 0.5 mg / l) had a significant effect on the emergence of the average patchouli shoots of Aceh 6.33 HST. Strengthened by the results of Rozalina et al. (2013) study, the combination of NAA and BAP significantly affected the parameters of the best initiation time, 7 days with treatment of 0.6 mg / l NAA + 1.5 mg / l BAP.

George and Sherrington (1984) state that cell division leads to the formation of callus, which is after the explant changes with the removal of leaves. In line with the research conducted by Gunawan (1988), that cell division does not occur in all cells in the original tissue, but only cells in the periphery layer that divide continuously. The initiation of cell division which is only limited to the outer layer of tissue can be caused by the availability of higher oxygen, the release of more nutrient availability of CO2, phenolic inhibitors that evaporate faster, and light. . Budiarti (2017) research results on the treatment of 2,4 –D concentrations (2, 4, 6 ppm) and BAP (0, 0.5, 1, 2 ppm) showed that callus emergence was seen at 14 HST (days after planting).

2. Explanation Life Percentage (%)

Based on Table 2 shows that of the 25 treatment combinations of 2,4-D and BAP in the Situak patchouli patchouli plants there were 18 treatments with live ekplan. The A1S1 treatment is the treatment with the smallest percentage of life, which is only 11%. This treatment is a treatment that only uses MS without any growth regulator 2,4-D and BAP. Followed by treatment A1S2 44%, A1S3 67%, A2S3 67% and A4S2 89%. Apart from these treatments, all of them show 100% live explants. Although up to 4 (four) weeks of observation have not been seen. The results of Rozalina's study (2013) related to the percentage of explant life of patchouli plants with NAA and BAP treatment showed that the administration of BAP concentrations of 1 mg / l and BAP 1.5 mg / l was treated with the highest percentage of live explants reaching 93.75% . And the lowest treatment was 0.5 mg / l BAP, which was 81.25%. Callus growth is influenced by several factors related to explants such as the availability of energy sources, the environment and growth regulators, especially the balance between cytokinins and auxin in tissue culture (Sumardi, 1996). Wattimena et al. (1992) in in vitro culture, morphogenesis of explants always depends on the interactions between auxin and cytokinin given and those contained in explants. The concentrations of these two ZPTs often control the shape and amount of growth of a culture, both in callus growth and organogenesis (Wulandari et al., 2004).

3. Percentage of Explants Forming Callus (%)

Table 3 shows that of the 25 treatment combinations given to patchouli explants, only 4 treatments formed callus. The media used in this initiation is MS, because MS is a standard medium that will meet plant nutrient needs. 2.4 D and BAP concentrations on MS media prove that explants can grow into callus. Although it can be seen in the previous table, 80% of explants begin to appear callus. However, until the 4th week (four) after planting, only 4 treatments showed callus growth, namely on the treatment of A3S3 (2,4-D 1 mg / l + BAP 1 mg / l), A3S4 (2,4-D 1 mg / l + BAP 1.5 mg / l), A3S5 (2,4-D 1 mg / l + BAP 2 mg / l), A4S1 (2,4-D 1,5 mg / l + BAP 0.

Figure 1 Callus can appear because of the opening on the plant tissue that comes from the incision. As Merlin et al. (2012) stated, the stimulation of injured explant tissue triggers callus formation. This stimulation causes the dinsing of the cell to change direction, where a portion of the protoplast flows out and a callus is formed. Budiarti (2017) study results on the treatment of 2,4 -D (2, 4, 6 ppm) and BAP (0, 0.5, 1, 2 ppm) concentrations showed the percentage of patchouli plant callus formation occurred in a combination treatment of 2 ppm 2, 4 –D BAP (0, 0.5, 1, 2 ppm) is 100% which is the best combination of other combination treatments. Auxin 2,4-D is likely to affect P. cablin's callus oil metabolic pathway via phosphoenol pyruvate acid. This occurs because endogenous auxin is formed from phosphoenol pyruvate acid, so the presence of 2,4-D (exogenous auxin) will affect the work of enzymes in the metabolic pathways of patchouli essential oils. The influence of 2,4-D on phosphoenol pyruvate acid is thought to affect the enzymes that work in the metabolic pathway to form phenylpropanoid compounds and terpenoid compounds which are compounds that make up patchouli oil. Whereas Benzyladenine (BA) is likely to affect the metabolic pathway of essential oils of P. cablin callus through isopentenyl pyrophosphate. This occurs because endogenous cytokinins (isopentenyl adenine) are formed from isopentenyl pyrophosphate, so that with the administration of BA (exogenous cytokinins) it will affect the work of enzymes in the metabolic pathways of patchouli essential oils. The effect of BA on isopentenyl pyrophosphate is thought to affect the metabolic pathway

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of the formation of terpenoid compounds which are the main component compounds of patchouli essential oils (Palupi, 2004).

IV. FIGURES AND TABLES

Table.1: At the time of emergence of situak patchouli callus in various treatments.

Sample Code	Treatment	Appearing Callus
A_1S_1	Without concentration 2,4 + Tanpa BAP	0,0
A_1S_2	Without concentration 2,4 + BAP 0,5 mg/L	14,5
A_1S_3	Without concentration 2,4 + BAP 1 mg/L	8,0
A_1S_4	Without concentration 2,4 + BAP 1,5 mg/L	17,0
A_1S_5	Without concentration 2,4 + BAP 2 mg/L	0,0
A_2S_1	0,5 mg/L 2,4-D + Tanpa BAP	15,0
A_2S_2	0,5 mg/L 2,4-D + BAP 0,5 mg/L	14,0
A_2S_3	0,5 mg/L 2,4-D + BAP 1 mg/L	15,6
A_2S_4	0,5 mg/L 2,4-D + BAP 1,5 mg/L	16,3
A_2S_5	0,5 mg/L 2,4-D + BAP 2 mg/L	11,0
A_3S_1	1 mg/L 2,4-D + Tanpa BAP	15,7
A_3S_2	1 mg/L 2,4-D + BAP 0,5 mg/L	12,0
A_3S_3	1 mg/L 2,4-D + BAP 1 mg/L	8,2
A_3S_4	1 mg/L 2,4-D + BAP 1,5 mg/L	8,7
A_3S_5	1 mg/L 2,4-D + BAP 2 mg/L	9,2
A_4S_1	1,5 mg/L 2,4-D + Tanpa BAP	9,6
A_4S_2	1,5 mg/L 2,4-D + BAP 0,5 mg/L	9,0
A_4S_3	1,5 mg/L 2,4-D + BAP 1 mg/L	12,3
A_4S_4	1,5 mg/L 2,4-D + BAP 1,5 mg/L	14,0
A_4S_5	1,5 mg/L 2,4-D + BAP 2 mg/L	16,0
A_5S_1	2 mg/L 2,4-D + Tanpa BAP	0,0
A_5S_2	2 mg/L 2,4-D + BAP 0,5 mg/L	0,0
A_5S_3	2 mg/L 2,4-D + BAP 1 mg/L	20,0
A_5S_4	2 mg/L 2,4-D + BAP 1,5 mg/L	16,5
A_5S_5	2 mg/L 2,4-D + BAP 2 mg/L	0,0

Table.2: Percentage of life of explants in situ patchouli plants in various treatments.

Sample Code	Treatment	Life Percentage (%)
A_1S_1	Without concentration 2,4 + Tanpa BAP	11%
A_1S_2	Without concentration 2,4 + BAP 0,5 mg/L	44%
A_1S_3	Without concentration 2,4 + BAP 1 mg/L	67%
A_1S_4	Without concentration 2,4 + BAP 1,5 mg/L	100%
A_1S_5	Without concentration 2,4 + BAP 2 mg/L	100%
A_2S_1	0,5 mg/L 2,4-D + Tanpa BAP	100%
A_2S_2	0,5 mg/L 2,4-D + BAP 0,5 mg/L	89%
A_2S_3	0,5 mg/L 2,4-D + BAP 1 mg/L	67%
A_2S_4	0,5 mg/L 2,4-D + BAP 1,5 mg/L	100%
A_2S_5	0,5 mg/L 2,4-D + BAP 2 mg/L	100%
A_3S_1	1 mg/L 2,4-D + Tanpa BAP	89%
A_3S_2	1 mg/L 2,4-D + BAP 0,5 mg/L	100%
A_3S_3	1 mg/L 2,4-D + BAP 1 mg/L	100%
A_3S_4	1 mg/L 2,4-D + BAP 1,5 mg/L	100%
A_3S_5	1 mg/L 2,4-D + BAP 2 mg/L	100%
A_4S_1	1,5 mg/L 2,4-D + Tanpa BAP	100%
A_4S_2	1,5 mg/L 2,4-D + BAP 0,5 mg/L	89%
A_4S_3	1,5 mg/L 2,4-D + BAP 1 mg/L	100%
A_4S_4	1,5 mg/L 2,4-D + BAP 1,5 mg/L	100%
A_4S_5	1,5 mg/L 2,4-D + BAP 2 mg/L	100%
A_5S_1	2 mg/L 2,4-D + Tanpa BAP	100%
A_5S_2	2 mg/L 2,4-D + BAP 0,5 mg/L	100%
A_5S_3	2 mg/L 2,4-D + BAP 1 mg/L	100%
A_5S_4	2 mg/L 2,4-D + BAP 1,5 mg/L	100%
A_5S_5	2 mg/L 2,4-D + BAP 2 mg/L	100%

Table.3: Percentage (%) of explants forming calluses of situak patchouli plants in various treatments

Sample Code	Treatment	Forming Callus (%)
A_1S_1	Without concentration 2,4 + Tanpa BAP	-
A_1S_2	Without concentration 2,4 + BAP 0,5 mg/L	-
A_1S_3	Without concentration 2,4 + BAP 1 mg/L	-
A_1S_4	Without concentration 2,4 + BAP 1,5 mg/L	-
A_1S_5	Without concentration 2,4 + BAP 2 mg/L	-
A_2S_1	0,5 mg/L 2,4-D + Tanpa BAP	-
A_2S_2	0,5 mg/L 2,4-D + BAP 0,5 mg/L	-
A_2S_3	0,5 mg/L 2,4-D + BAP 1 mg/L	-
A_2S_4	0,5 mg/L 2,4-D + BAP 1,5 mg/L	-
A_2S_5	0,5 mg/L 2,4-D + BAP 2 mg/L	-
A_3S_1	1 mg/L 2,4-D + Tanpa BAP	-
A_3S_2	1 mg/L 2,4-D + BAP 0,5 mg/L	-
A_3S_3	1 mg/L 2,4-D + BAP 1 mg/L	100 ± 0,1
A_3S_4	1 mg/L 2,4-D + BAP 1,5 mg/L	100 ± 0,1
A_3S_5	1 mg/L 2,4-D + BAP 2 mg/L	100 ± 0,1
A_4S_1	1,5 mg/L 2,4-D + Tanpa BAP	100 ± 0,1
A_4S_2	1,5 mg/L 2,4-D + BAP 0,5 mg/L	-
A_4S_3	1,5 mg/L 2,4-D + BAP 1 mg/L	-
A_4S_4	1,5 mg/L 2,4-D + BAP 1,5 mg/L	-
A_4S_5	1,5 mg/L 2,4-D + BAP 2 mg/L	-
A_5S_1	2 mg/L 2,4-D + Tanpa BAP	-
A_5S_2	2 mg/L 2,4-D + BAP 0,5 mg/L	-
A_5S_3	2 mg/L 2,4-D + BAP 1 mg/L	-
A_5S_4	2 mg/L 2,4-D + BAP 1,5 mg/L	-
A_5S_5	2 mg/L 2,4-D + BAP 2 mg/L	-

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Figure 1. Patchouli callus with 1 mg / L and BAP 1 mg / L A3S3 2,4-D treatment;

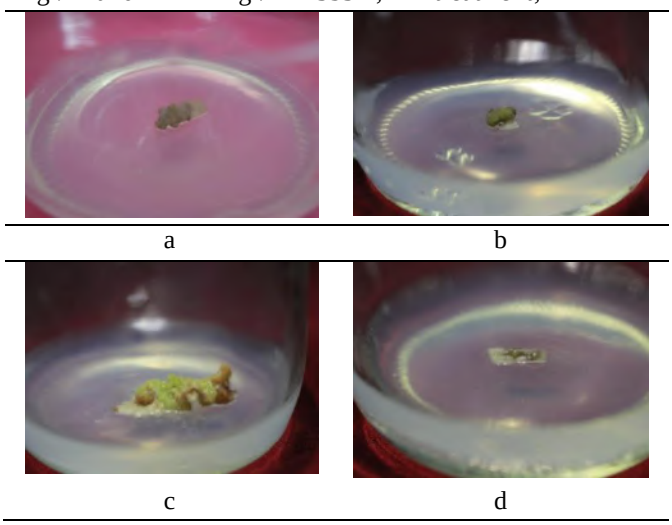

Fig.1: Patchouli callus with 1 mg / L and BAP 1 mg / L A3S3 2,4-D treatment; (a), Callus Nilam with the treatment of 1 mg / L A3-4 2,4-D and 1.5 mg / L BAP; (b), Callus Nilam with the Treatment of A3S5 2,4-D 1 mg / L and BAP 2 mg / L; (c), Patchouli Callus with 1.5 mg / L and 2.4 mg / L

V. CONCLUSION

The results of the study show that on the treatment of A3S3 (1.0 mg / l 2.4-D + 1.0 mg / l BAP), A3S4 (1.0 mg / l 2.4-D + 1.5 mg / l BAP), A3S5 (1.0 mg / l 2.4 -D + 2.0 mg / l BAP) and A4S1 (1.5 mg / l 2.4-D + without BAP) produce explants. Whereas in other combination treatments up to 30 days of observation did not produce patchouli plants in the Situak Accession plant. Further research is needed regarding the type of auxin and cytokinin appropriate for patchouli callus formation in Situak Accession and longer observation time.

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Utilization Strategy and Management of Green Waste to Improve Wasteland Fertility in Australia

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***Abstract*—** *This paper covers production of food waste in Australia, including to find the best solution to address the food waste issue by collective understanding of what we mean by food waste and where it happens. Recognizing what we are starting at now do well can empower us to take in and use from prevailing exercises and moreover development. Moreover, the point of administration and recycling of food waste in header of biomass preparation can build soil fertility of wasteland in Australia. With the aim of considering natural composts are infinite, biodegradable, reasonable, and ecologically attractive. This thought can be proposed as reusing of waste as a natural fertilizer to enhance soil readiness of wasteland in Australia.*

Keywords—food waste, management, organic, environment, economic, natural resource management.

I. INTRODUCTION

The definition of food waste differs worldwide it depends on from where food waste occurs into the food supply chain to consumption. For example, whether it, contains or discounts inedible waste of food.

Food waste is simply defined as 'the quantitative or qualitative reduction of food. Definition of food waste also covers solid or liquid kinds of food that is occurred from human consumption during the complete consumption and supply chain. It is part of food loss, refers to avoidance of alternative use and disposal of food. Moreover, Food is key component in whole food supply chain, from primary production to the level of household consumer. Because, it is a significant source of nutrition for human consumption. However, majority of food waste is generating by the consumers (FAO, 2014).

II. BACKGROUND

According to FAO (Food and Agriculture Organization of the United Nations) report (2011) the estimated global annual food waste was approximately one-third from total production of food that means every year, we lost 25 % consumable food world-wide. This report also indicated that the per capita food waste is in Europe and North-America as compare to Africa and South/Southeast Asia. It is 95 to 115 and 6 to 11 kgs/year respectively. Moreover, if we look at Australia the generation of food waste is higher than others because, in the year of 2014-2015 Australia produced around 64 million tons food waste that means individual person food waste is 2700 kgs/ year (Australian National Waste Report, 2016).

In Australia, government supporting to reduce food waste in AgriFutures Australia industry, CSIRO (Commonwealth Scientific and Industrial Research Organization) and Cooperative Research Centers by investing more than $ 10 million annually. To encourage the developmental research on higher-value products from recovered or converted food waste such as, kitchen, organic and green waste management (e.g. vegetables, non-veg., and garden waste, leaf cutting, trimming etc.) by developing alternative markets and advance digital technologies.

What's more, Australia has a strong dependence on landfill as a sort of waste organization. Most of the waste that isn't reused or re-used in Australia is disposed of in the nation's landfills. While, as indicated by, the administration report (2016) states that Australia creates approximately 64 million tons of waste a year, apart from around 40 % isn't reused.

III. CHALLENGES FOR FOOD WASTE

Australia has complex challenges for management of the food waste. Because, there are large numbers of outlet units are engaged in supply chain throughout producing, moving, selling, redistributing and disposing food. Firstly, there are several challenges in procurement for appropriate quantities and feedstock to have access market for the end products. Secondly, the Australian local, territory and state governments has an additional layer of complexity as a role of legislative requirements, environmental and human health policy. Thirdly, households are also important part of food waste in Australia. Finally, global Greenhouse gas

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(GHG) emissions have twisted eight per cent due to more use of water in agriculture (National Food Waste Strategy Report, 2017). This scope of waste needs to encourage a large amount of initiatives to address food waste issue across the world.

IV. SOCIAL ASPECTS

1. WASTE GENERATION ANALYSIS

The drivers of sustenance squander are changed and complex and happen at each point along the supply and utilization chain. Models of how sustenance waste can happen are plot beneath.

Primary Production

At initial stage, food waste occurs when item misfortune because of irritations and illnesses or climate, stock or harmed or disposed of amid creation, pressing or dealing with fall in market costs making it unrewarding to gather. Moreover, failure to meet contracted create, determinations, for example, quality or size. Changes in purchaser tastes and inclinations.

Processing and manufacturing

This kind of waste occurs when food product harmed amid taking care of, due to deterioration because of tainting or deficient. Over the top trimming of vegetables for handling and changes underway because of buyer request. Spillage on transport lines and exchange focuses, harm to bundling bringing about sustenance unfit as well.

Retail Stores

Retailers are also included in waste generation, there are several reasons because of poor stock administration, including over requesting, inappropriate stock pivot, stockpiling and taking care of practices. Furthermore, deliver never again meets quality models and very late request changes that can leave providers with overabundance item. Constrained access to offices to reuse or repurpose nourishment squander.

Household

Family squander happens amid disarray over 'use-by' and 'best-previously' date marking and over-obtaining of sustenance that is then discarded. Constrained information of how to securely repurpose or store sustenance remains and restricted access to nourishment squander accumulation frameworks.

2. HOW MUCH FOOD WASTE PRODUCED BY AUSTRALIA?

Australia is one of the most elevated makers of waste per head of populace on the planet. In 1999, Australia positioned second, behind the USA, as far as local waste age. Generally, Australians discarded $2.9 billion of crisp sustenance, $630 million of uneaten takeaway nourishment, $876 million of scraps, $596 million of incomplete beverages and $241 million of solidified sustenance, an aggregate of $5.3 billion on all types of sustenance in 2004.

Looks at the aggregate waste created, arranged and recouped per capita in chosen OECD countries. Vitality recuperation was incorporated where information was accessible. (Figure 1) The way that they contrast implies the correlations ought to be utilized with the alert.

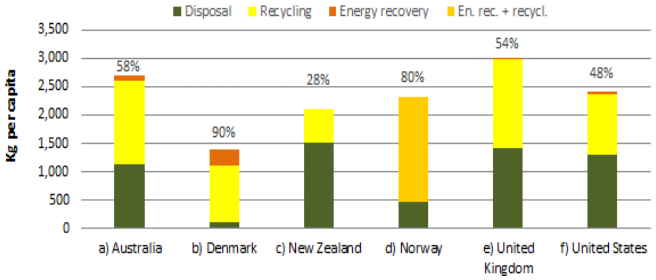

Fig.1: Comparison of waste generation and fate per capita, Australia and selected OECD countries (Pickin and Randell, 2017).

V. ECONOMIC ASPECT

1. WASTE HIERARCHY

This technique embraces a roundabout economy approach that considers the sustenance squander pecking order and looks to catch nourishment squander as an asset, so it isn't sent to landfill. The waste progression organizes squander administration rehearses in favoring sustenance squander shirking over asset reuse, reusing, reprocessing, and vitality recuperation, trailed by waste transfer. Moreover, the pecking order perceives the natural estimation of nourishment squander in giving direction on the most asset proficient and earth sound ways to deal with managing waste.

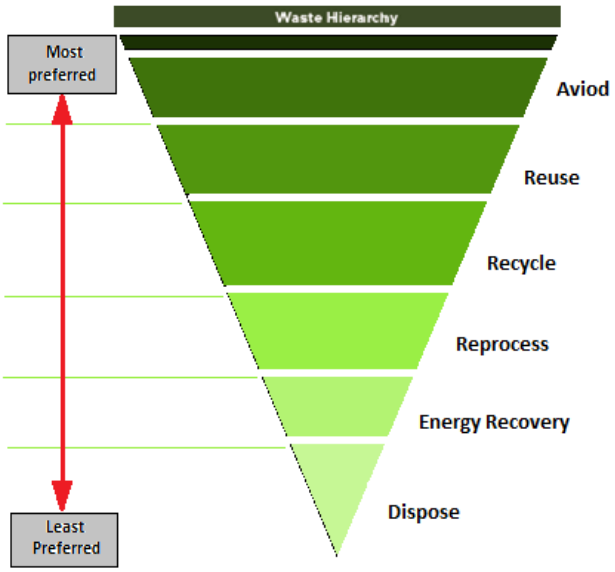

Fig.2: Waste hierarchy (Modified from NFWS, 2017)

The utilization of round economy approaches and the waste chain of command to address sustenance squander

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requests a more vital and synergistic approach. This will move us to discover arrangements over the whole nourishment framework as opposed to proceeding to work inside single, straight supply and utilization chains. Moreover, the structure and healthy benefit of sustenance squander is essential to figure out where esteem including can be connected. Access to data on the volume, type and wholesome piece of nourishment squander helps with supporting venture choices on item improvement. For instance, sustenance squander that can be utilized for treating the soil (NFWS, 2017).

2. UTILIZATION OF GREEN WASTE OR ORGANIC WASTE

Treating the soil or aging sustenance waste can give cost sparing to neighborhood experts in waste administration and decrease cost spent in decline gathering and waste transfer. By delivering manures from waste materials, the expense of purchasing business composts that give supplements to plant and soil can be lessened. The manures created can be utilized as natural composts for soil molding and sustenance. The manure produces materials that can be utilized as moderate discharge compost in a manor. The fulfilment can be picked up from the enhanced development and improvement of plants in fertilizer revised soil.

Sustenance squander age and destiny by source segment. The lion's share – around 3.1 Mt or 133 kg for each capita – was from household sources. Around 6% (0.2 Mt) of gathered MSW nourishment squander was reused, principally through treating the soil (Figure 2).

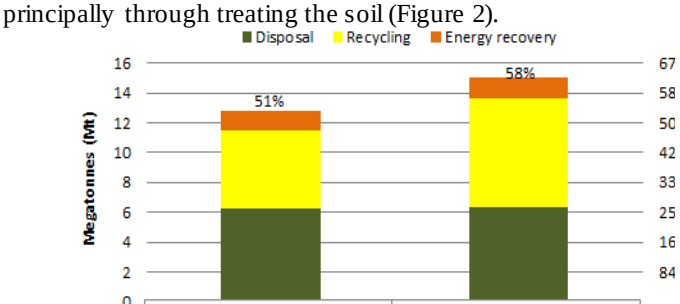

Fig.3: Non-hazardous and hazardous organic waste generation and fate, Australia 2014-15 (Pickin and Randell, 2017).

VI. ENVIRONMENTAL ASPECT

1. PREPARATION OF ORGANIC FERTILIZER FROM ORGANIC WASTE

The manure pile is developed of layers of materials, as in a major sandwich. The essential grouping is

Layer 1: A layer of dry plant materials or mix of dry plant materials with compost making helps like incredible soil, stool and in addition a couple of ashes. The layer should be 20– 25 cm thick, i.e. as significant as a hand. The compost influencing jars be mixed with the water to make slurry. Water or slurry should be scattered by hand or sprinkled with a watering can consistently over this layer making it saturated yet not sprinkling wet.

Layer 2: A layer of sticky (green) plant materials, either fresh or shriveled, e.g. weeds or grass, plants from clearing a pathway, stems and leaves left completed from gathering vegetables, hurt results of the dirt. Verdant branches from woody plants can moreover be used as long as the materials are tidied up. The layer should be 20– 25 cm thick. Water should NOT be sprinkled or scattered over this layer.

Layer 3: A layer of animal fertilizer assembled from new or dried cow-like excrement, horse, jackass or ass compost, sheep, goat or chicken droppings. The animal fecal matter can be mixed with soil, old excrement and a couple of slags to make a layer 5– 10 cm thick. In case there is only a little measure of animal dung, it is best to mix it with water to make slurry, and after that spread it over as a thin layer 1– 2 cm thick. At last, in the wake of treating the soil time of 5 two months it will be prepared with loaded with dietary parts to use as a natural fertilizer to enhance soil richness.

The transformation of food waste into natural composts by aging, strong state aging or fertilizing the soil is generally led to diminishing the measure of sustenance squander created day by day. Nourishment squanders that isn't dealing with legitimately can cause sullying of groundwater, discharge of harmful gas, a spread of scent and fascination of vermin. The best possible disintegration of sustenance waste can cause a decrease of pathogen and smell. Nourishment waste, for example, natural products, vegetables, grain, bread and eggshells can be made and changed over into natural manures. Nourishment waste, for example, red meat and bones likewise can be made however they take a more extended period to form.

Furthermore, nourishment squander is broadly reused into natural manure since sustenance squander has exceptional includes as crude manure specialist. Sustenance squander contains high vitality and is appropriate for vitality creation and waste adjustment. Moreover, the proportion of carbon to supplements of natural squanders is critical amid maturing and treating the soil procedure. These procedures rely upon a microorganism that utilizations carbon source to give vitality and nitrogen to assemble cell proteins. Vegetable and organic product squanders have C: N proportion of ≤27:1 is reasonably appropriate in maturation and fertilizing the soil (Ahmad et al. Cited in Min, 2015).

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2. INCREASE SOIL FERTILITY OF WASTELAND BY USING ORGANIC FERTILIZER

The primary components in treating the soil sustenance squander are physical and compound attributes of a substrate which incorporate the structure and molecule estimate. Sustenance squander has high dampness substance and low physical structure when contrasted with sewage muck and excrement. Nourishment squander is blended with building specialists, for example, yard waste and sawdust that contain high C: N proportion to assimilate more dampness and add structure to the blend along these lines upgrading treating the soil of sustenance squander.

VII. NATURAL RESOURCE STRATEGY

Australia is the 6th biggest nation on the planet by region (393,797,177 ha). According to, Australian Bureau of Statistics report (2011) emphasis that, roughly 53% of Australia's aggregate land zone was utilized for farming. Apart from southern hemisphere has big amount of wasteland in Australia including mountains, rocky area, bare lands or which can be not utilized in Agriculture purpose due to nutrition deficiency. To improve or balance nutritional deficiency of soil required tons of fertilizer. The fertilizer containing chemicals which have unaffordable prized or cost. To revel from cost re3duction problem the manure preparation from organic and kitchen waste will be a novel step towards improvement of non-fertile lands.

How much land can be productive by using organic compost?

In March 2011, a pilot venture began with a fertilizing the soil office in the surroundings of Kathmandu, Nepal. The plant gathers squander from vegetable markets creating manure through vigorous corruption and in the meantime decreasing methane emanations. Amid the pilot, altogether **140 tons** of new natural waste from neighborhood markets were gathered and **15 tons** of organic fertilizer was created (Gnirrep, 2018). So, that mean from total **64 million tons** organic waste can be useful to prepare **6.9 million tons** organic fertilizer.

1. CALCULATION AND ANALYSIS BY USING BASE STUDY

= 140 tons natural waste required to make 15 tons organic manure (As per base study)

= 64 million tons (64,000,000 tons) total organic waste generate Australia every year

= (140 tons organic waste = 15 tons organic manure)

= (140 / 15) = 9.3 tones organic waste

(.'. that means, to produce 1 ton organic manure, 9.3 tons waste is required)

= 140 ---------- 15

= 64,000,000 ---------- ?

= (64,000,000 x 15 / 140) = 68,57,142.8 (6.9 million tons)

(.'. available waste can be utilized, to produce 6.9 million tons organic manure)

According to Madison *et al*. (1995) states that, at a most extreme application rate of natural fertilizer is 25 tons/ 1 acer for every section of land every year without consolidation. It means, this fertilizer can be used to recover 274,285.6 acres of unfertile lands in Australia every year at very low cost.

= (68,57,142.8 x 1 / 25) = 274,285.6 acres (110 Million Meter Square)

Important for waste recycling and utilization

Unproper discarding of waste creates terrific contamination the environment by proper utilization of waste. This waste can be turns into pathogen fee manure, so it will be an eco-friendly procedure.

The manure prepared by using the waste will help to improve soil fertility. Moreover, this type of agricultural land can be utilized for ZBNF (Zero Budget Natural Farming) and organic farming.

Additionally, treated the dirt material is less awkward than the primary material and less complex and all the more beguiling to manage. In the midst of the treating the dirt technique, carbon dioxide and water are lost to the atmosphere and the proportion of the pile lessens by 30–60%. Likewise, many weed seeds and disorder causing animals may be killed by the high temperatures in the stack. Annoying scents are wiped out. Flies, a regular issue around waste products and other characteristic misuses, are generously less of an issue with composts. Treating the dirt declines or takes out the reduction in nitrogen availability that routinely happens when normal materials, for instance, sawdust or straw, are added particularly to the soil. Preparing the dirt is furthermore uncommonly supportive for reusing kitchen misuses, additional item developments, weeds, and manures. Various sorts of neighborhood normal waste, for instance, apple pumice, lake weeds, leaves, and grass clippings, can be dealt with the dirt.

VIII. BENEFITS FROM ORGANIC COMPOST

Manures are natural or inorganic, normal or engineered substances that additional to soil to improve plant development and creation. Plants rely upon the supplements in soil to complete metabolic responses since soil contains essential synthetic compounds for plant development (Turing *et al*., 2006). Be that as it may, the supply of essential synthetic concoctions in soil to plants is restricted. At the point when plants are being reaped, the supplement content decreases and causes the decrease of

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amount and nature of plants. Composts are connected to supplant the concoction materials in soil that are used by plants amid development and improvement (Miller Cited in Min, 2015).

Moreover, composts are utilized to upgrade the dirt's developing potential since manures can give a superior developing condition to plants when contrasted with common soil. (Turing *et al*. Cited in Min, 2015).

Composts give expansive measure of macronutrients, for example, nitrogen, phosphorous and potassium while regular soil may not contain adequate measure of this macronutrients. Manures likewise give follow components, for example, magnesium, calcium and copper that are essential in plant development. Notwithstanding in view of the natural issue present in natural compost, soil structure is enhanced and accordingly the dirt's capacity to clutch water and supplements increments.

Natural compost is wealthy in natural issue, which enables microorganisms to flourish. Natural manure contains carbon as a major aspect of its synthetic cosmetics; and it is the carbon, alongside nitrogen, phosphorus and potassium that feeds microorganisms and empowers them to make supplements accessible for plants in a normally happening organic process. Manufactured manures overflow into our conduits hurting marine life and water quality. Natural composts don't keep running off as effortlessly (if by any means) and are related with soil structure. As indicated by the Organic Trade Association, natural manure likewise expands species biodiversity by 30% contrasted and manufactured compost.

XI. CONCLUSION

From the above study it can be concluded that, 64 million tons of waste can be utilized by preparing organic manure annually, to improve soil fertility of 274,285.6 acres wasteland in Australia. Moreover, natural squanders can offer ascent to leachate, methane discharges, scent, vermin and precarious landforms. Alternately, they are a potential wellspring of soil conditioners or vitality. Redirection of organics, either through a division at a source or handling of lingering waste streams, is a noteworthy focal point of city squander administration and a developing number of committees crosswise over Australia give a different canister to natural squanders, including both garden and nourishment wastes. Here, it is recommended that, strategies are strong of sustenance squander shirking, decrease and repurposing with building up an intentional responsibility of government to diminish nourishment squander and empowering enactment to better help sustenance squander decrease and repurposing.

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The Impact of Farmer Field Schools (SLK) Program Implementation on the Cocoa Farm Business in Lima Puluh Kota Regency

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Abstract**—** *Cocoa industry in West Sumatra has high potential to increase the income of small farmers. However, cocoa production is still below production potential. The implementation of the cocoa field school program (SLK) is one of approaches to develop cocoa farm business. This field school is expected to improve the performance of smallholders' cocoa farming, both in terms of production and quality. To assess the extent to which this field school has helped farmers to increase their capacity to manage cocoa farming, research is needed to analyze the impact of the implementation of the farmer field school program on cocoa farming as measured by level of technology adoption and economic impact by comparing cocoa income between participant and non-participant farmers in farmer field school program. The study was conducted in Lima Puluh Kota Regency which involved a sample of 80 people, consisting of 40 SLK farmers and 40 non-SLK participants. The results showed that forthe impact of the SLK on cocoa farming, it was found thatfor the price variable there was a significant difference but for the variable production, farming costs and income between farmers and non-SLK participants there were no significant differences. In conclusion that the cocoa field school program has not significantly affected cocoa farming practices in Lima Puluh Kota. As a result, the expected increase in production and quality of cocoa has not been achieved.*

Keywords**— production and quality, farmers field school, cocoa.**

I. INTRODUCTION

Field School concept is not new in the field of agriculture Indonesia. Introduced in 1989 by the Food and Agriculture Organization (FAO) as an effort integrated pest management, farmer field schools invited to learn directly in the field, observed the problem and its causes, as well as to analyze the development of their crops. Implementation of a field school for many years in various regions in Indonesia is considered a major role in helping farmers reduce the use of pesticides and increase yields (worldagroforestry, 2013). The Field School is seen as one of the methods in the learning process is quite effective, because it is very suitable as a learning method for adults (andragogy) because it is not formal.

Schools field is already widely practiced in the agricultural sector in Indonesia. Implementation of a field school for many years in various regions in Indonesia is considered a major role in helping farmers reduce the use of pesticides and increase yields. Various agencies in Indonesia either government or private institution has conducted a field school in various agricultural sub-sector either sub-sectors of food, horticulture and plantation sub-sector.

Field school activities undertaken in the plantation sector sub one on the plantation of cocoa by name by Cocoa Field School Program (SLK). The importance of the cocoa field school activities is to increase the production and quality of the cocoa bean itself. Currently Indonesia is the world's third largest producer of cocoa after Evory Coast (Cote d'Ivoire) and Ghana. Indonesian cocoa plantation area was recorded of 1.4 million hectares with a production of approximately 500 thousand tons per year. Ivory Coast ranks first largest cocoa-producing country has a total area of 1.6 million hectares with a production of 1.3 million tons per year and Ghana amounted to 900 thousand tons per year (Ardhiyan, 2015).

Terms of the exploitation of the cocoa able to absorb the labor force large enough, since nationally nearly 87 percent of the development of cocoa grown by smallholder, while the remainder raised by the State Great Estates and Private Big Plantation. And the contribution of energy absorption kerjA for the overall cocoa around 7.8 percent. (Dirjenbun, 2016).

Nationally although cocoa plantation area tends to increase, but not accompanied by peningatan contrary cocoa production cocoa production has decreased. At the beginning of 2010, the production of cocoa plantations of people initially fell to 701 229 tonnes 837 918 tonnes in 2015 (Dirjenbun, 2016).

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Increased cocoa plantation area that is not accompanied by an increase in cocoa production is caused by many factors, one of which is for tarjadinya La Nina, rainfall hujan high so as to make the cocoa plant experienced a compounded interest kerontokoan cocoa plant shortlyjadi highly vulnerable to pests and diseases such as pest PBK (Cocoa Fruit Borer) and disease dieback /VSD (Vascular Streak Dieback) which eventually led to the production of cocoa shortlyjadi disturbed (Zulhefi, 2015).

Therefore, efforts need to be done in order to repair the national cocoa production can be maintained and even improved. Repair cocoa plantations can be done through the rehabilitation, rejuvenation and expansion with superior plant material and application of advanced technologies. In addition, efforts to control pests and diseases PBK VSD must be intensified (Pulsitbangbun, 2017).

All this has made various efforts to improve conditions such as the empowerment of farmers through the Field School of the cocoa (SLK) or Field School Integrated Pest Management (IPM FFS) and System Togetherness Economics (SKE), as well as the application of control technology by methods PsPSP (pruning, sanitation , harvest often andfertilization) for CPB and VSD control and the provision of seedssuperior. Given the partial implementation is still small in scale, the results are not optimal. Therefore, these activities need to be carried out simultaneously, comprehensive and integrated through a movement that involves all stakeholders and resources (Directorate General of Plantation of the Ministry of Agriculture, 2012).

In addition to government agencies, private organizations also intervened in the repair of cocoa in Indonesia such Governmental Organization Save The childern funded by Mondelez and NGO Swisscontact sustainable cocoa production program or The Sustainable Cocoa Production Program(SCPP).

Swisscontact is a Non Governmental Organization (NGO) International founded by private and educational institutions in Switzerland in 1959.The Sustainable Cocoa Production Program (SCPP) is the program of Swisscontact is a partnership Public-Private large that began in 2012 with the main focus on building a better capacity between the cocoa farmers to improve the quantity and quality of cocoa production (Swisscontact, 2014).

District Fifty town is located in the province of West Sumatra is one area that is a center of folk and cocoa plantation development has been fostered by Swisscontact in economic capacity building, gardens and surroundings cocoa farmers. This is supported by state of the climate and soil in accordance with the terms of plantation crops specifically grown for cocoa plantations.

Swisscontact main objective is to help the Indonesian cocoa farmers create sustainable cocoa products. With a program calledThe Sustainable Cocoa Production Program (SCPP), which is a program to create a cocoa plantation promising and sustainable both for the farmers, the environment and the market. The main focus of the program is to build capacity SCPP is better between the cocoa farmers in order to increase the quantity and quality of cocoa production.

SCPP program is run in the form of cocoa field school for cocoa farmers who joined as a participant. Various training in cocoa field school has been given by the NGO Swisscontact some of which are field schools on cocoa farming practices are good and true as materials about pruning, fertilizing, field sanitation, frequent harvesting, pest and disease control and post-harvest.

In the year of 2017 activities and assistance with cocoa field school program of non-governmental organizations Swisscontact has been done in District Fifty Cities and has trained farmer groups with a total of 16 groups of farmers 400 people with these types of programs are the activities carried out; (1) good agricultural practices and technology transfer system; (2) Integration of Nutrition and gender sensitivity; (3) The organization of farmers, access to market and certification; (4) an integrated financing facility; (5) Management of stakeholders and networking platform.

Identification of problems

Of the five programs over the study will focus on discussing the impact of the implementation of good agricultural practices and the transfer system electronic technology, so it appears some of the questions the research is "Is the Field School of Cocoa (SLK) are undertaken by NGOs Swisscontact able to increase the ability of farmers in cocoa farming? "

Research purposes

Based on the background and the problems above, this research aims to "analyze the impact of cocoa field school program provided by Swisscontact to assisted farmers in the District Fifty Cities in terms of production, sales price, the cost of farming and income?"

hypothesis

"Cocoa Field School Has Real Impact Of Cocoa Farming in the District Fifty Cities".

II. RESEARCH METHODS

Types of research

The method used in this research is descriptive method with case study. Descriptive method is a method in researching a group of people, an object is a system of thought or a class of events in the present. The purpose of

this descriptive study is to create a description, picture of systematic, factual and accurate information on the facts as well as the relationship between the phenomenon investigated. The case study research on the status of research subjects that relate to a specific phase or typical of the whole personality. The research subject can be individuals, groups, institutions and communities (Nazir 2005: 54-55).

Research variable

Based on the research objectives of variables - variables were observed in this study are:

1. Total production is the result obtained by farmers in the cocoa farming obtain cocoa field school program or not.
2. Cocoa bean prices received by farmers, is a monetary compensation received by farmers every cocoa bean sales in a matter of kilograms.
3. Cocoa farming costs, is the value of being sacrificed to obtain the factors of production used in farming.
4. Cocoa farm income, the difference between the total revenue with costs incurred tottal.

Sampling method

The sampling technique in this research using the technique of simple random sampling (simple random sampling techniques). Kasmadi and Sunariah (2013: 66) argues that "the simple random sampling technique is simple sampling technique is done randomly without regard to strata that exist in the population" as the basic principle of making members of the sample who expressed W.Gulo (2002: 84) that "that each member of the population has an equal chance to be drawn as members of the sample ".

According Sugiyono (2008: 116) "sample is part of the number and characteristics possessed by this population." Meanwhile, according Arikunto (2008: 116) "Determination of sample taking is when a population of less than 100 better taken all until the research is the study population. If a large number of subjects can be taken between 10-15% or 20-55% or more depending somewhat on:

1. The ability of researchers seen from the time, effort and funds
2. Narrow the breadth of observations from each subject area, because it involves a lot of lack of funds.
3. The size of the risk borne by researchers for researchers, the risk is great, of course if large samplenya result will be better

Thus, the researchers took a number of samples required as a source of data in this study is 10% of the total population or 10% x 400 = 40, so that the sample in this study amounted to 40 cocoa farmers get a field school program cocoa and 40 farmers do not get the cocoa field school program, so the total sample is keseluruahan numbered 80 people spread over three districts namely Payakumbuh District, District Guguak, District Mungka.

Method of collecting data

1. Primary data

Data is taken directly from the interviews with key informants namely Field Staff Coordinator along Swiscontact NGOs in the district Fifty Cities, and some people from Swiscontact assisted farmers and growers from outside the assisted farmers to be used as a control or comparison.

2. Secondary Data

Data is a form of writing or document relating to the problems of research, obtained from literature study, document organization, as well as the relevant literature such as books, journals and research reports related to the research. In this study, secondary data collected comes from Lemabaga Governmental Swisscontact and related agencies such as the Department of Plantations Kabupatrn 50 State, Department of Trade and Industry, literature and other written sources related to this research.

Data analysis method

For analyzing the impact of field school program cacao given by Swisscontact to assisted farmers in the District Fifty Cities in terms of production, prices and costs of farming using quantitative descriptive method by using analytical tools questionnaires and interviews, see the benefits received by the assisted farmers in terms of production and a reduction in farming costs. To test the hypothesis proposed in this study conducted a comparative analysis (t-test), comparing the condition of farmers get with the program that did not get the SLK SLK program.

According Sugiyono (2009: 264) to test samples correlated / paired t-test then used the samples related to the formulation as follows:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2} - 2.r\left(\frac{s_1}{\sqrt{n_1}}\right)\left(\frac{s_2}{\sqrt{n_2}}\right)}}$$

Where :

- t = The value of the region criticism (t_{count})
- $\bar{x}_1$ = The average value of production, prices, costs and incomes of cocoa farmers who do not get the program SLK

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- $\overline{X_2}$ = The average value of production, price, cost and income of cocoa farmers get SLK program
- s1 = standard deviation value of production, prices, costs and incomes of cocoa farmers who do not get the program SLK
- s2 = Standard deviation value of production, price, cost and income of cocoa farmers get SLK program
- S21 = Variance of production, prices and the cost of cocoa farmers who do not get the program SLK
- S22 = Variance of production, prices and the cost of cocoa farmers get SLK program
- r = Correlation between production, prices, costs and incomes of cocoa farmers are not getting to the get the program SLK
- n1 = Number of samples that did not get the program SLK
- n2 = Number of samples mendapatakan SLK program

Forms of statistical hypothesis is:

- H0: μ1 = μ2 (there are no significant differences between production, prices, costs and incomes of cocoa farmers are not getting to the get the program SLK)
- H1: μ1> μ2 (there are significant differences between production, prices, costs and incomes of cocoa farmers are not getting to the get the program SLK)

The data were processed using SPSS with hypothesis testing criteria as follows: 1) If the probability (p) <0.01 or 0.05 or by other means t> t table then H0 rejected 2) If the probability (p) ≥ 0.01 or 0.05 or otherwise t ≤ t table then H0 is accepted.

III. RESULTS AND DISCUSSION

Regional Overview Research

Geography and Regional Administration

District Fifty Town area of 3354.30 km2, located in the eastern part of West Sumatera, namely 00 22 'N and 00 23'LS and between 1100 16' - 1000 51'BT and the adjacent province of Riau. Government administrative region comprising 13 districts with 76 384 Jorong Nagari and has a population of 327 652 inhabitants in the area and boundaries as follows:

The North	:	Riau Province
Eastern section	:	Riau Province
South side	:	Tanah Datar and Sijunjung
West Region	:	Agam and Pasaman

Total population

District residents Fifty Cities in 2016 amounted to 372 568 people, with details of 184 995 inhabitants male and 187 573 female inhabitants. Judging by the number of villages in the district Fifty Cities as many as 79 villages, then with a population of 372 568 inhabitants, the average number of residents per village amounted to 4,716 people. District of highest average is the District Guguak by the number of 7,260 people per village.

Population by age group in the District Fifty Cities still dominated by young people aged. The age group is the most numerous group of 0 s / d 4 years with a total of 38 285 inhabitants, while the smallest number is a group of 60 s / d 64 years in the amount of 16 112 inhabitants.

Profile Cocoa Farming between Farmers Field School Getting the cocoa With Non Getting Farmers Field School in the District Cocoa Fifty Cities

Here is a table showing the amount of Production, Productivity, Price, Cost Farm and Farmers Income Respondents in the District Fifty Cities 2018:

Table.21. Number of Production, Productivity, Price, Cost Farm and Farmers Income Respondents in the District Fifty Cities 2018

Component	farmers SLK	Farmers Non SLK
Production (Kg / year)	21 402	19 537
Productivity (Kg / ha / year)	535	488
The average price Dried Cocoa Beans (per kg)	25 175	24,900

(Rp.)		
Average Cost Farming (per ha / year) (Rp.)	2883837	2579174
Average - Average income (per ha / year) (Rp.)	10,757,045	9.63563 million

Source: Primary Data Processing 2018

1. Farming costs

Production Costs According Wasis (1992), production costs are sacrifices that absolutely must be held in order to obtain a result. In this study a year for the maintenance of the average farmer only cost in the form of production, purchase organic fertilizers, chemical fertilizers, pesticides, herbicides and labor.

Most farmers only pay for the purchase of organic fertilizer, and labor for more emphasis on family workers, arguing that they lack of money to buy other needs in the treatment of cocoa. Only a small proportion of farmers who want to spend more money to purchase chemical fertilizers, pesticides and labor.

In table 15 can be seen average farm costs incurred by the farmer respondents averaged annually after the results obtained to farming costs incurred by farmers who get SLK Rp. 2,883,837 / ha / year and for farmers who do not get SLK Rp. 2,579,174, for a more detailed data can be found in appendix 12 s / d 15.

2. Production

Results obtained from the cacao plant is wet cocoa beans and farmers sell in the form of dried cocoa beans at random, where the cocoa beans have been dried 1-2 days (1 day = 2-5 hours drying). After drying, the water content contained in cocoa beans and cocoa beans decreases will experience a depreciation of 25% (100 kg wet cocoa beans will be 75 kg of dried cocoa beans at random).

Based on the results of this research is that the amount of the average production of cocoa every farmer in the district of Fifty Cities are getting SLK amounting to 21 402 kg / year with a productivity of 535 kg / ha / year, whereas for farmers who do not get SLK production amounted to 19 537 kg / year and a productivity of 488 kg / ha / year. Total production and productivity of cocoa in one year can be seen in the following table:

From table 15 it can be analyzed that there is little difference between farmers and non SLK SLK in terms of production and productivity but the differences are not too significant. When viewed as a whole both production and non-farmers SLK SLK (annex 12 s / d 15) exist that have high production and there is also low. Analysis by researchers in the field main factors that affect farmers' production is in terms of the intensity of the maintenance of the garden each farmer. If farmers diligently perform lawn care such as pruning and fertilizing the resulting production will also be high and vice versa if farmers rarely do care then the resulting production will be low, usually farmers who rarely perform maintenance go kekebun cocoa just to harvest.

3. Selling Price of Cocoa Beans

For the price of cocoa beans in the City District Fifty average farmer selling dried cocoa beans to a collector for the village / market. Most of the farmers who have the production of cocoa beans <50 kg per week were more likely to sell to a collector for the village and which has production> 50 kg prefers to sell directly to the market / wholesalers in the county because of the price between traders gatherer village with pedangan large in the county ranges from Rp. 1000, - s / d
Rp 2,000, -.

In this study, researchers will set the price farmers with farmers Non SLK SLK traders village level. The price of cocoa beans in each farmer will be different from one another, depending on the quality of cocoa beans are produced. Based on the research results at the same time each farmer to get a different price ranges between Rp. 24000, - s / d Rp.27.000 and the price evenly between peasant farmers with Non SLK SLK (annex 12 s / d 15) that sets it apart is if the cocoa produced by farmers good / high quality, the price obtained will be high and vice versa if the quality of cocoa produced by farmers ugly / poor quality, the price obtained is also low. Cocoa quality are generally grouped into three muti / grade (Attachment 5) and measured based on the number of moldy grain moisture content, attached, deflated, and trash (something other than beans).

4. Farmers' income

Cocoa farmers' income is the difference between total revenue with total costs incurred in cocoa farming activities. In this study, the majority of farmers only pay the cocoa farm just to purchase organic fertilizer (compost / manure), only a fraction is spend on the purchase of chemical fertilizers, pesticides, herbicides and labor. From the results of primary data processing for the income of farmers in the Ha / year can be seen in appendix 12 s / d 15.

Based on the attachment 12 s / d 15 in one ha / year farmers are able to earn an income between Rp. 4.000.000, - s / d Rp. 26,000,000, - both farmers and non SLK SLK there who have low income and there is also a high everything depends on the production produced and the prices received by each farmer. Farmers will have a high income and high production if otherwise farmers will get a lower price when production is low, while the

level of production is determined on the level of lawn care, and the price level is determined by the quality of the cocoa beans are produced.

5. Test T-Test

Analysis of this data aims to test the hypothesis of the research is to determine the difference production, prices, costs and incomes of farming cocoa farmers get with the program that did not get the SLK SLK program, Forms of statistical hypothesis is:

- H0: μ1 = μ2 (there are no significant differences between production, prices, costs and incomes of cocoa farmers are not getting to the get the program SLK)
- H1: μ1> μ2 (there are significant differences between production, prices, costs and incomes of cocoa farmers are not getting to the get the program SLK)

The hypothesis in this study were tested using the Test-T. The first step taken was to develop formulations. Through the preparation of such formulations formulated the t-test test formula used is bidirectional (two-tailed test) with menggunankan Microsoft Excel. Once it was determined the level of significance that is at the level of 10%. The provision in question is, if the significance value is smaller than the significance level of 10% and T-count is greater than the T-table at a significance level of 10% then H0 rejected and H1 accepted. Conversely, if the use values of T-count is smaller than the T-table at a significance level of 10% then H0 and H1 rejected.

2 different test average (Independent Samples T-Test) is used to determine whether there are differencesSignificant among production, prices, costs and incomes of cocoa farmers are not getting to the get the program SLK, Tests using a significance level of 0.1. Before the 2 different test performed on average, F test (Test Homogeneous) need to be done first. If the same variant, the Equal Variance t test using Assumed (assumed to be the same variant) and, if different variants using Unequal Variance Assumed (assumed different variants).

The results of hypothesis testing can be seen in appendix 17 and 18 for the F test each variable. variablesproduction obtained F-count = 1,20 <F-table = 1.70 thus to homogeneous production variants. Variable Price obtained the F-count = 2.56> F-table = 1.70 thus to the price variance is not homogeneous. Variable Cost Farming obtained F-hiutng = 0,89 <F-table = 1.70 thus to variant variables Farming fee is homogeneous. Variable Income earned F-count = 1,41 <F-table = 1.70 thus to variant Revenue variable is homogeneous. So to test variable T production, farming costs and revenues using*Equal Variance Assumed* and for variable prices using Unequal Variance Assumed.

Table.22: Results of Testing Test F

Component	F-count	F-table	result	test T
Production	1.20	1.70	F arithmetic <F table	Equal Variance Assumed
Price	2.56	1.99	F count> F table	Unequal Variance Assumed
Farming costs	0.89	1.99	F arithmetic <F table	Equal Variance Assumed
Income	1.41	1.99	F arithmetic <F table	Equal Variance Assumed

Source: From Primary Data Processing 2018

From the results of hypothesis testing T test in the table (annex 17 and 18) result for the variable production of T-count value = 0.83, for the variable price of T-count value = 1.69, for the variable costs of farming the value of T-count = 1 , 12 and for the income variable value of T-count = 0.96, while for T-table = 1.99. T-count for each variable <T-tables mean H0 rejected and H1 accepted so that it can be concluded that there are no significant differences between production, prices, costs and incomes of cocoa farmers are not getting to the get the program SLK. The following table T test of hypothesis testing:

Table.23: T test Hypothesis Testing

Component	T-count	T-table	result	Decision
Production	0.83	1.66	T arithmetic <T table	H0
Price	1.69	1.66	T arithmetic> T table	H0 is rejected
Farming costs	1.12	1.66	T arithmetic <T table	H0
Income	0.96	1.66	T arithmetic <T table	H0

Source: From Primary Data Processing 2018.

The above table shows that there are significant differences in terms dar cocoa selling prices received by farmers and farmers Non SLK SLK. This means that farmers SLK has been able to create quality cocoa beans

that better so that when sales are also rewarded with a higher price. But in terms of production, Farming Costs and Revenues. there was no significant difference between the farmers who mendapakatkan SLK (SCPP Swisscontact) to farmers who did not get the SLK. From the results of the review discussion with farmers in the field and it happened because:

- Most of the farmers who are members of the SCCP is a woman farmer program while the more intensive work in the garden is a male farmer making the transfer of technology was not optimal.
- Most cocoa farmers who are members of SCPP program is farmers who have land area under ¼ Ha cocoa and cocoa plantation is not a primary means of livelihood. Dikabupaten Fifty Cities for farmers especially its main business of respondents majority of farmers are farming rice paddy, pulses, gambier plantations and farms.
- The majority of routine activities diperkebunan cocoa farmers only harvest and pruning melakukukan light.
- Judging from the level of technology adoption only a small proportion of farmers who are able to adopt all the technology components remaining only a few components are adopted when all components are interlinked with each other to support the production and quality of cocoa beans produced, especially those components PsPSP (Harvest often, pruning, Sanitas and Fertilization).

IV. CONCLUSIONS AND RECOMMENDATIONS

Conclusion

The results showed thatthere are significant differences in terms of selling prices of cocoa beans received by farmers This means that the farmers participating in the SLK has been able to create quality cocoa beans are better than non-participant farmers SLK. But in terms of production, Farming Costs and Revenues. there was no significant difference between the farmers participating in the SLK (SCPP Swisscontact) with a non-participant farmers SLK. Soit can be concluded that cocoa field school program provided by Swisscontact no significant impact on cocoa farmers in the district Fifty Cities.

Recomendation

From the above conclusions, it is recommended that farmers can continue to be guided and monitored in a given application of the technology component. For farmers who receive coaching to be more active doing garden maintenance in order to get more production with a minimum of routine applying the core technology that is PsPSP (Harvest often, pruning, and fertilizing the garden Sanitation), and can share their knowledge with other farmers.

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Cassava Processor's Awareness of Improved Processing Technologies in Oyo State

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***Abstract*—** *The cassava production is most carried out by smallholder farmers in the rural areas using low-level production techniques which have left them with production of cassava tubers without much value addition. Therefore cassava processors' awareness of improved processing technologies in Oyo State was investigated.*

Simple random approach was employed through questionnaire and interviews to collect information from 176 Cassava processors. Descriptive and inferential statistics such as frequency counts, percentages, mean, Chi-square were used in data analysis at 0.05 level of significance.

Results of analysis revealed that majority (77.2%) of the respondents fell within the age range of 21 years to 50 years with mean age of 41.23 and greater population of females (75.6%) than the males (24.4%). Over 45.5% had secondary and below as their educational qualification with most (75.6%) married, with fairly large household size 4-6 persons.

The respondents' level of awareness of improved processing technologies among most need respondents low (54.5%) and this may accounted for low level of utilization of improved processing technologies among majority (56.8%) of the respondents. Chi-square analysis revealed that, among socio-economic characteristics of the respondents, religion(x^2= 6.805, p= 0.033), educational qualification (x^2 = 10.572, p= 0.032), mode of processing (x^2 = 14.015, p= 0.001) and mode of Labour (x^2=11.960, p=0.003) were significantly related to respondents' awareness of improved processing technologies. The result revealed clearly that respondents' awareness of improved processing technologies does not depend on marital status, sex, household size and experience.

It is therefore recommended that Government and NGO's should encourage extension agents by giving them motivation as at when due to improve their services of dissemination of information to the cassava processors on awareness of improved processing technologies toward enhancing higher productivity, income and better standard of living of the respondents in the study area.

Keywords*— *Cassava processors, awareness, Improved processing technologies.

I. INTRODUCTION

In the area of cassava production, the recent Agricultural Transformation Programme of the Federal Government which has facilitated the establishment of Cassava processing plants, exportation of Cassava chips to countries like China has led to increase in the interest of the rural famers including women to increase their level of Cassava production and processing. In the domestic parlance the rural women processing cassava into various products such as cassava flour, chips, and starch among other products.

In Nigeria, women cassava utilizers constitute more than 60% of the adult population resident in the rural areas (Odebode, 1997). However, their traditional contribution to agricultural production has been rendered inefficient by the crude and inappropriate form of agricultural technologies frequently used (Olawoye, 1988), the result is a relatively low agricultural productivity, which is inversely proportional to the enormous labour intensive input. It is against this backdrop that this research investigated the extent of the cassava processors' awareness of improved processing technologies in Oyo State.

Specific objectives

The specific objectives of this study include to:

i. Identify socio-economic characteristics of the cassava processors in Oyo state.
ii. Determine the level of awareness of improved processing technology by cassava processors in Oyo State.
iii. Find out attitude of respondents towards utilization of improved processing technology in the study area.

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Hypothesis of the study

The hypothesis stated in the null form is tested:

Ho_1 – There is no significant relationship between awareness of the respondents and their utilization of improved processing technology in the study area.

II. MATERIALS AND METHODS

The study was carried out in Oyo state, which is one of the six states in south west Nigeria. The economy of the state is based on the agriculture and the major occupation of the rural people is farming. The climate in the state favour the cultivation of crops like maize, yam, cassava, millet, rice, plantains, cocoa, oil-palm, cashew etc. They engage in other activities such as transport operation, blacksmithing, tailoring, and carpentry and so on. The state is homogenous and comprises the Oyos, the Ibadans and the Ibarapas, all belonging to the Yoruba family and speaking the same Yoruba language. People within and outside the country trade and settle in the urban areas. The capital, Ibadan is reputed to be the largest city in African, south of the sahara.

Sampling Procedure and Sample size

A Multi-stage sampling procedure was used to select respondents for the study. Random selection of 50% of the two ADP zones (Ogbomoso and Ibadan/Ibarapa), Purposive sampling Technique was used to select Local Government and Communities that are predominantly noted for cassava production and processing from the selected two zones: Ogbomoso South Local government (Ogbomoso zone) and Ibarapa East Local government (Ibadan/Ibarapa zone). List of registered cassava processors in selected communities was collected from the Department of Agric. and Health at the Local Government Headquarters in Arowomole and Eruwa respectively. 50% of registered Cassava Processors in selected communities was randomly selected and this gives a total of one hundred and seventy six (176) respondents that was used for the study.

Analysis of data

Data Collected were subjected to descriptive and inferential Statistical analysis using Statistical Package for the Social Sciences (SPSS). Descriptive statistical tools used included frequency counts, mean and percentage while inferential statistical used is Chi-square.

III. RESULTS AND DISCUSSION

Socio-economic characteristics of respondents

The mean age of all the respondents was 41.23. This implies that most of the respondents are in their active ages and this may urge them favourably to utilize improved processing technologies for their cassava processing. This finding is in line with that of Akinbile (2007) and Adedeji et al (2013) who reported a mean age of 41.42 with age-range (20-50years) that constitutes the active workforce of the population.

Results of analysis on respondents' sex in Table 1 revealed that 24.4% are males while 75.6% were females in the study area. This implies that females are more predominant in cassava processing than males in the study area. Results further showed that most 60.8% of the respondents were Christians, 36.4% were Muslim and few ones 2.8% were traditional believer. Religion believe is one of the major identities of Nigerians. Oyesola and Ademola (2011) asserted that religion institution can assist in making information available most especially in rural areas on awareness and utilization of improved processing technologies among cassava processors. This finding is in agreement with the work of Torimiro *et al.,* (2006) that majority (70.9%) of the rural dwellers are Christians.

Results in Table 1 also revealed that most respondents (75.6%) were married. This implies that marriage institution is still highly esteemed in our society because it is evidence of being responsible. This result is also in tandem with report of Ewebiyi and Arimi (2013) who submitted that marriage institution plays a key role in the livelihood of rural dwellers. The results further showed that most (58.0%) of the respondents had four to six persons household size. This implies that the household size among the rural dwellers is fairly large because most of the respondents rely on family labor. This result collaborates with findings of Oluwatayo and Aliyu (2007) that the household size among rural farm families is usually large. The results also revealed that majority of the respondents (33.0 %) had six to ten years of experience in processing while (2.3%) of the respondents still stay till twenty-one to twenty-five in the study area. Results indicated that (54.0%) of the respondents using improved processing technologies (modern) in the study area. Also, the monthly income earned by the respondents in the study area range between #20,100- #40,000 per month, (34.1%) had less than #20,000 per month, (13.1%) had income range between #40,100-#60,000 while 1.7% had between #60,100-#80,000 and only (0.6%) of the respondents did not state their income per month. The implication is that respondents need to engage themselves in another income generating activities in order to increase their economic status, so as to acquire more improved processing technologies. This agreed with the finding of FAO (2001) that rural dwellers characterized with meager income.

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Table.1: Socio-economic characteristics of the respondents

Variable	Frequency	Percentage	Mean
Age (years)			
11-20	3	1.7	41.23
21-30	27	15.3	
31-40	65	36.9	
41-50	44	25.0	
51-60	28	15.9	
Above 60	9	5.1	
Sex			
Male	43	24.4	
Female	133	75.6	
Religion			
Christian	107	60.8	
Muslim	64	36.4	
Tradition	5	2.8	
Marital status			
Single	16	9.1	
Married	133	75.6	
Divorce	4	2.3	
Separated	7	4.0	
Widowed	16	9.1	
Educational background			
No formal	20	11.4	
Vocational education	2	1.1	
Primary school	62	35.2	
Secondary school	80	45.5	
Tertiary school	12	6.8	
Household size			
1-3	41	23.3	
4-6	102	58.0	
7-9	28	15.9	
10-12	1	0.6	
No states	4	2.3	
Year of experience			
1-5	52	29.5	
6-10	58	33.0	
11-15	31	17.6	
16-20	31	17.6	
21-25	4	2.3	
Method of processing			
Traditional	37	21.0	
Modern	95	54.0	
Traditional & modern	44	25.0	
Income earn (#)			
<20,000	60	34.1	
20,100-40,000	89	50.6	
40,100-60,000	23	13.1	
60,100-80,000	3	1.7	

Not stated	1	0.6
Total	**176**	**100.0**

Source: Field survey, 2017.

Respondents' awareness on improved processing technologies

By categorization of level of awareness in table 2b showed that the level of awareness of improved processing technologies is low (54.5%) and high (45.5%) in the study area. The implication of this is that those who claimed to be awared got the information through educational background or exposure to social network. This is in line with Bamikole et al (2016) that reported that apart from grater, presser, fryer machines that have their awareness rate above 50% awareness rates of processing is low.

Table.2a: Respondents' awareness on Improved Processing Technologies

Improved processing technologies	Aware		Not Aware	
	F	%	F	%
1. Mechanical grater	161	91.5	15	8.5
2. Screw Press hydraulic jack	160	90.9	16	9.1
3. Motorized Peeler	67	38.1	109	61.9
4. Granulator	128	72.7	48	27.3
5. Kiln Dryer/Drum Dryer	154	87.5	22	12.5
6. Improved Pulverized	66	37.5	110	62.5
7. Sifter	81	46.0	95	54.0
8. Aluminum Fermentation tank	72	40.9	104	59.1
9. Hammer mill	157	89.2	19	10.8
10. Washing Machine	81	46.0	95	54.0
11. Dewatering	123	69.9	53	30.1
12. Rotating Sieve	121	68.8	55	31.3

Source: Field survey, 2017.

Table.2b: Level of awareness

Category	f	%	Mean	SD	Minimum	Maximum
Low	96	54.5	7.76	3.62	0.00	12.00
High	80	45.5				

Source: Field survey, 2017.

Respondents' attitude on the improved processing technologies.

Table 3b revealed that (56.8%) of the respondents had positive attitude to utilize improved processing technologies while (43.2%) had positive attitude towards utilization of improved processing technologies. Also table 3a showed that (69.9%, 66.5% and 58.0%) agreed strongly to modern cassava processing method increase production, modern processing save time and also ready to recommend the techniques to their friends respectively. The implication is that the respondents may eager to utilize the improved processing technologies due to the benefits cum the improvement of their standard of livings.

Table.3a: Respondents Attitude on the improved Processing Technologies (N= 176)

Attitudinal Statement	SA	A	D	SD	Mean
Modern cassava processing method save time	117 (66.5)	46 (26.1)	12 (6.8)	1 (0.6)	3.52
Spare parts of improved methods are not readily available	4 (2.3)	35 (19.9)	81 (46.0)	56 (31.8)	1.77

Modern cassava processing method increase production	123 (69.9)	40 (22.7)	13 (7.4)	0	3.55
Fund is not a problem to acquire the improved techniques	14 (8.0)	7 (4.0)	19 (13.6)	131 (74.4)	2.06
In operation of modern techniques injuries are sustained	44 (25.0)	109 (69.9)	19 (10.8)	4 (2.3)	3.01
I'm not feeling comfortable using the method	8 (4.5)	10 (5.7)	73 (41.3)	85 (48.3)	1.73
Instability of electricity prevents the use of improved method	16 (9.1)	17 (9.7)	42 (23.9)	101 (57.4)	2.04
Use of modern method reduces the use of hired labour	14 (8.0)	34 (19.3)	121 (68.8)	7 (4.0)	1.67
I will recommend modern methods of cassava processing to any friend	102 (58.0)	51 (29.0)	21 (11.9)	2 (1.1)	3.33
I prefer used of hand grating as traditional method techniques	29 (16.5)	23 (13.1)	92 (52.3)	32 (18.2)	1.94
Stone pressing as traditional techniques is cheaper	125 (71.0)	38 (21.6)	11 (6.3)	2 (1.1)	3.57

Source: Field survey, 2017.

Table 3b: Categorization of respondents by their attitude

Category	F	%	Mean	SD	Minimum	Maximum
Unfavorable	76	43.2	28.20	5.03	11.00	41.00
Favorable	100	56.8				

Source: Field survey, 2017.

Hypothesis: test of relationship between awareness of the respondents and their utilization of improved processing technologies.

This hypothesis was tested with the aid of PPMC. The result of the analysis in Table 4 revealed that there is a significant positive relationship between awareness (r=0.524, P= 0.000) of the respondents and their utilization of improved processing technologies This implies that the more the respondents aware of improved processing technologies, the more they will utilize it.

Table 4: Pearson Product Moment Correlation (PPMC) analysis between Awareness of improved processing technologies and their utilization

Variable	r	p	Decision
Awareness	0.524	0.000	Significant

** Correlation is significant at the 0.01 level (2- tailed)

IV. CONCLUSION AND RECOMMENDATIONS

More females than males that married with secondary education alternative involves in cassava processing in the study area. Awareness of the respondents of improved processing technologies was low.

The study thus, concludes that

Based on the empirical findings of this study, the following recommendations are made;

Government should create or build factory or industry where improved processing technologies would be fabricated at low cost. Also fund Research adequately and have MOU (Partners) with private Organizations to come up with quality/ standard improved processing technologies for increased productivity. Workshops and seminars should be periodically organized through extension by the government and NGOs for cassava processors on effective use of improved processing technologies. This will go a long way in easing effective use of improved processing technologies.

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Production and Growth of Soybean on Water Hyacinth Bokashi Giving with Two Activators Types

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Abstract*—The study aimed to determine the production and growth of soybean on water hyacinth bokashi giving with two activators types. The study was conducted in Leweng Village, TakkalallaSubdistrict, Wajo District. The method used was Randomized Block Design (RBD), consisting of three (3) treatments, namely Bokashi (b_0), Bokashi 2 tha^{-1} with Activator EM4 (b_1), and Bokashi 2 tha^{-1} with Activator Tadabur (b_2). Each treatment was repeated three times so that the number of treatment combinations was 9 combinations (9 plot treatment). The results show that the average soybean production per hectare application of water hyacinth bokashi with EM_4 activators is 0.80 tons per hectare, while the application of water hyacinth bokashi with Tadabur activists is 0.79 tons per hectare. The application of water hyacinth bokashi with EM_4 activators is much better, compared to water hyacinth bokashi with activist Tadabur.*

***Keywords*— Bokashi, Water Hyacinth, EM_4, Soybean, Tadabur.**

I. INTRODUCTION

The use of organic materials is currently very important considering the level of organic matter in low agricultural soils. Land has a very important meaning for the life of mankind and the nature of the soil is very dependent on its constituent components. Soil organic matter is one of the most important components, which is the result of the decay of plant / plant remnants mixed with soil minerals in the topsoil (Suhardjo et al., 1998).

Soybeans are short-day plants, which will not flower if the length of irradiation (day length) exceeds the critical limit. The duration of irradiation is a function of latitude. Light intensity above 1,076 lux (100 foot candles) for 8 hours has been able to stimulate flowering, but flowering does not occur if the light intensity is less than 1,076 lux (Asrijal, 2004).

Water hyacinth has the potential to be made as compost (bokashi) because it turns out that this water plant has some organic fertilizer content. According to Fryer and Matsunaka (1988), water hyacinth is a material that is very potential to be used as organic fertilizer because based on the results of analysis in the laboratory it contains, among others: 1.681% N, 0.275% P, 14.286% K, 37.654% C, with a ratio C / N 22,399.

According to Murbandono (2003), compost is organic materials (organic waste) that have undergone a weathering process because of the interaction between microorganisms (decomposing bacteria) that work in it. Organic materials such as leaves, grass, straw, the remnants of twigs, and branches, animal feces, fall flowers, urine. This composting process takes a long time, so Yovita (2003) suggests one activator that can accelerate the composting process by using effective microorganisms 4 (EM4) compost produced through the fermentation process with the provision of EM4 called Bokashi. The word bokashi is taken from Japanese

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which means fermented organic matter. By the Indonesian people the word bokashi is extended to "a rich source of organic matter".

EM4 activators are agricultural cultivation technologies to improve soil and plant health and fertility. EM4 contains lactobacillus, photosynthetic bacteria, yeast, actinomicetes, and decomposers which can be used as inoculants to increase the diversity of soil microbes to ferment organic materials into simple organic compounds that are easily absorbed by plant roots, so that the quality and quantity of crop production increases (Wididana and Teruo , 1996).

TadaburBiofertilizer Activator is one of the biological fertilizer products that are produced and marketed by PT. Quantum Innovation. Tadabur is a multi-use liquid organic biofertilizer which is highly concentrated by plants to stimulate growth and strengthen plants. Tadabur biological fertilizers enriched with organic phytohormones, complex aminino acids and enzymes (Anonim, 2013).

The use of bokashi is one way to improve the fertility of physical, chemical, and biological soil. However, the type and dose of bokashi needed by plants varies greatly, depending on the variety, availability of materials, and soil conditions. The advantage of using bokashi is high efficiency, does not interfere with nutrient balance in the soil, improves the physical, chemical, and biological properties of the soil so as to increase land productivity. Therefore the use of organic fertilizers needs to be applied as a result of expensive inorganic fertilizer prices and to create more sustainable agricultural land (Farid, 2003).

II. MATERIALS AND METHODS

The study was conducted in Leweng Village, TakkalallaSubdistrict, Wajo District. The method used was Randomized Block Design (RBD), consisting of three (3) treatments, namely Bokashi (b_0), Bokashi 2 t ha^{-1} with EM_4 (b_1), and Bokashi 2 t ha^{-1} activator with Tadabur Activator (b_2) Each treatment was repeated three times so that the number of treatment combinations was 9 combinations (9 plot treatment).

III. RESULTS AND DISCUSSION

3.1. Research result

3.1.1. Plant height

The average plant height at the age of 60 days after planting and its variance is presented. Sidik Variety shows that plant height 60 days after planting on soybean plants on water hyacinth bokashi with two types of activators, showed a very significant effect on plant height 60 days after planting. The test results can be seen in **Table 1**.

Table 1. Average plant height 60 days after planting on soybean plants (strands).

Treatment	Average	Different Test Results*	LSD (level of 5%)
b_0	47,20	b	7,59
b_1	65,31	a	
b_2	64,09	ab	

**) Different letters indicate differences at the level of 5%*

The LSD Test Results (level of 5%) in **Table 1**, showed that the treatment of b_0 was significantly different from treatment b_1 and was not significantly different from the treatment of b_2, but between treatments b_1 and b_2 showed no significant difference in plant height 60 days after planting on soybean plants.

3.1.2. Number of Pods

The average number of pods of soybean at harvest and variance is presented. Sidik Variety shows that the number of soybean pods at the time of harvesting on the supply of water hyacinth bokashi with two types of activators, showed no significant effect on the number of soybean pods at harvest, which can be seen in **Fig. 1** below.

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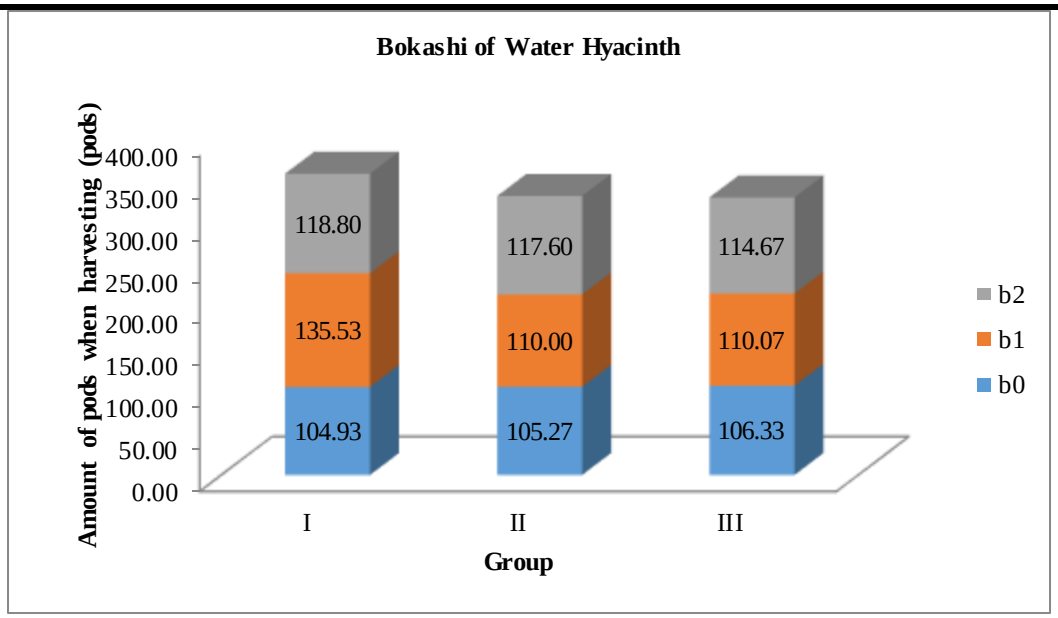

Fig. 1. Amount of Soybean Pods during Harvest (pods)

3.1.3. Production per hectare

The average production per hectare of soybean crops at harvest and variance is presented. Sidik Variety shows that the production per hectare of soybean at harvest time for the administration of water hyacinth bokashi with two types of activators, shows an unrealistic influence on the production per hectare of soybean plants at harvest, which can be seen in **Fig. 2** below.

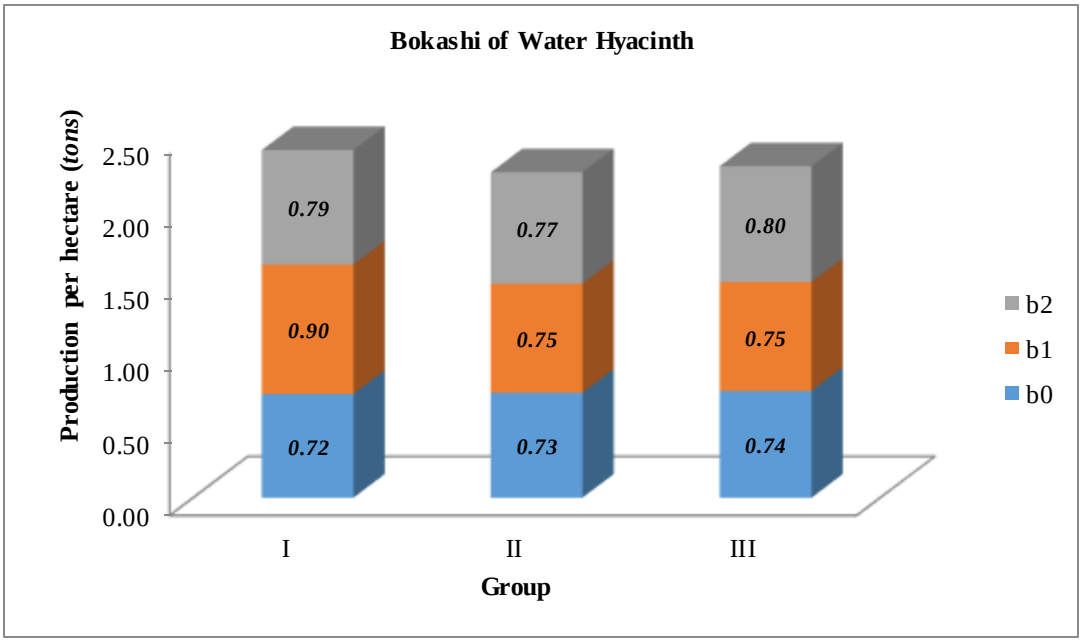

Fig. 2. Production per hectare of soybean plants during harvest (tons)

3.2. Discussion

The growth and development of a plant must be supported by the availability of nutrients. Especially macro nutrients, besides also other micro elements, to support the process of forming plant tissues. According to Situru (1998), that macro nutrients are needed by plants for growth until production in large quantities, especially in the process of fruit formation.

The relationship in the process of forming plant tissue, macro nutrients must support each other, so that the

formation of a strong network causes smooth process of absorption and utilization of nutrients for plants. In the opinion of Wolverton, (1978), that if one or several nutrients are not in sufficient quantities or one of them in excessive amounts, the plant will show symptoms such as lack of nutrients.

The results showed that administration of water hyacinth bokashi with EM4 activists and Tadabur activators showed a significant effect on parameters (plant height, number of leaves, and number of branches), but showed no significant effect on parameters (number of pods; number of empty pods; dry weight ; and production of samples / plot / per hectare). This is because the accumulation of nutrients available has not been able to support growth to production maximally, meaning that the nutrients available in this case are water hyacinth bokashi with both EM4 activists and Tadabur activators, not yet available in balanced conditions so that photosynthesis and metabolic processes support the development of plant organs shows results that are not optimal. Likewise, if viewed from the results of analysis through **Fig 2,** the average production per hectare is good without bokashi treatment (b_0), bokashi activator EM_4 (b_1), and bokashi activator Tadabur (b_2), respectively 0.73, 0.79, and 0.80 t ha^{-1}. The total average turns out to show low production yields of 0.77 t ha^{-1}. If compared with the average production in the Pacet experimental garden reaches 2.19 t ha^{-1}, while in Citayam it is only 0.99 t ha^{-1}(Asrijal, 2004).

IV. CONCLUSIONS

Based on the results and previous discussion, it can be concluded that the application of water hyacinth bokashi with activator EM4 is much better, compared with water hyacinth bokashi with activist Tadabur. With an average production per hectare of 0.80 tons per hectare.

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Induction of Systemic Resistance by Rhizobacterial and Endophytic Fungi against Foot Rot Disease of *Piper nigrum* L. by Increasing Enzyme Defense Activity

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***Abstract*—** *Two strains of rhizobacteria, Bacillus subtilis and Pseudomonas fluorescens and two strains of endophytic fungi Trichoderma viride and Trichoderma asperellum were selected as inducers of systemic resistance against Phytophthora capsici and were tested individually for biological control of foot rot disease of pepper. Black pepper vines were grown under greenhouse and challenge inoculated with P. capsici were selected for analyzing the disease index, chitinase, peroxidase, phenylalanine ammonia lyase activity, polyphenol oxidase, ß-1, 3-glucanase assay and estimation of phenol content. Treatments with B. subtilis and T. viride isolates provided protection to pepper plant against P. capsici infection as observed with reduced per cent disease incidence showed 20 and 18% as compared to untreated control. Increases in the enzyme level were detected in different strains of pepper vines at time intervals after challenge inoculation. The study reveals the potential of B. subtilis and T. viride as biocontrol agent for prevention of P. capsici infections in black pepper.*

***Keywords*— *Endophytic fungi, Foot rot disease, Piper nigrum L., P. capsici, Rhizobacteria*.**

I. INTRODUCTION

Black pepper (*Piper nigrum* L.) is a commercial spice crop cultivated in India. It ranks first in the world in terms of production, consumption and exports. In India, it is grown in an area of 1,31,230 ha with an annual production of 55,500 tones (Indian Spice Board, India, Feb 2017). Foot rot disease of black pepper is caused by *Phytophthora capsici*. It was known to affect 90% of the yield in India (Nair and Gupta, 2003; Krishnamoorthy and Parthasarathy, 2011). The control of foot rot disease has been almost exclusively based on the application of chemical pesticides that effectively kill the *Phytophthora capsici*. Although, several effective pesticides have been recommended for use against this pathogen, they are not considered to be long-term solutions due to concerns of expense, exposure risks, fungicide residues, toxicity to non-target organisms and other health and environmental hazards. Therefore, recent efforts have been focused on developing eco-friendly safe, long lasting and effective against many plant pathogens for the management of plant diseases.

Now a day's many chemical fungicides are being used to control this disease. The control of plant diseases using antagonistic bacteria is now considered as a promising alternative method that could reduce the use of hazardous chemical fungicides or bactericides. Rhizobacteria, saprophytic bacteria that live in the plant rhizosphere and colonize the root system, have been studied as plant growth promoters for increasing agricultural production and as biocontrol agents against plant diseases (Kloepper and Beauchamp, 1992; Liu *et al*., 1995; Chen *et al*., 1996; Silva *et al*., 2004). Colonization of the plant root system can lead to reduced pathogen attack directly through the production of antimicrobial substances or competition for space, nutrients and ecological niches and indirectly through induction of systemic resistance (Liu *et al*. 1995; Kloepper and Beauchamp 1995). They are eco-friendly in nature, have a high cost-benefit ratio and do not pose the risk of the pathogen developing resistance. Many root-rot colonizing bacteria are known to promote plant growth by

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producing gibberllins, cytokinin and indole acetic acid. Several plant growth promoting rhizobacteria (PGPR) strains have been reported to control various fungal (Van Peer *et al*., 1991), bacterial (Liu *et al*., 1995) and viral diseases (Maurhofer *et al*., 1994; Raupach *et al*., 1996). The mechanisms of biological control by PGPR strains generally involve the production of siderophores (Kloepper *et al*., 1980), hydrogen cyanide (Ahl *et al*., 1986) and lytic enzymes (Sneh *et al*., 1984; Jones *et al*., 1986), the biosynthesis of antibiotics (Howie and Suslow, 1991) and competition for substances (Elad and Chet, 1987).

Accumulating evidences suggest that the organisms under most scrutiny for potential use in biological control of pest and diseases are bacteria belonging to the genera *Pseudomonas* and *Bacillus* (Ramamoorthy *et al*., 2001). Further, plant growth promoting endophytic (PGPE) bacteria, especially *Bacillus subtilis* and PGPR, especially *Pseudomonas fluorescens* (Pf1) strains have been developed commercially as a talc based formulation and tested against several crop diseases (Vivekananthan *et al*., 2004; Rajendran *et al*., 2007; Kavino *et al*., 2007; Harish *et al*., 2008). Sundaramoorthy *et al*. (2012) reported that combination of *P. fluorescens* strains and *B. subtilis* strain together resulted in significant growth promotion that was correlated with induced resistance in *Capsicum annum* L. Several approaches have been made to manage the foot rot of pepper. A large number of enzymes have been associated with induced systemic resistance (ISR), including peroxidase, phenyalalanine ammonia-lyase, lipoxygenase, β-1, 3 glucanase and chitinase (Ye *et al*., 1990; Koch *et al*., 1992; Schneider and Ullrich, 1994; Van Loon, 1997; Silva *et al*., 2004).

This work aimed to verify the specific protection in Black Pepper provided by rhizobacteria and endophytes against *Phytophthora capsici* pathogen. However, no attempts have been made for the management of *P. capsici* disease by using rhizobacteria and endophytic strain. *Trichoderma viride* were used to detect the increasing activity of enzymes involved with ISR such as lipoxygenase, peroxidase, phenylalanine ammonia lyase (PAL) activity, polyphenol oxidase (PPO), β-1, 3-glucanase assay and estimation of phenol.

II. MATERIALS AND METHODS

2.1. Plant materials and pathogen

The black pepper vines were obtained from Pepper Research Center, Appangala, Madikeri, Karnataka. The pathogen was isolated from the black pepper foot showing typical symptoms of *P. capsici* by using oat meal agar (OMA) medium and the fungal cultures were identified as *P. capsici* in the Department of Studies in Biotechnology, University of Mysore, Mysuru, India.

2.2. Biocontrol agents

Endophytic fungal strains of *Trichoderma viride* and *Trichoderma asperellum* were isolated from medicinal plant *Azadiracta indica*, respectively. In addition, the rhizobacterial strain *Bacillus subtilis* and *Pseudomonas fluorescens* isolated from soil was reported to contain many plant diseases and pest. Pure culture of *T. asperellum* and *T. viride* strains were maintained on potato dextrose agar (PDA) slants and *B. subtilis* and *P. fluorescens* strain was maintained on nutrient agar (NB) agar slants and King's B medium at 4°C.

2.3. Efficacy of rhizobacterial and endophytic fungal strains biocontrol agents on radial growth of *P. capsici*

Rhizobacterial strains *P. fluorescens* and *B. subtilis* and endophytic strains were *T. asperellum* and *T. viride* were tested against *P. capsici* by dual culture technique (Webster, 1971). The mycelial disc (9 mm) from seven days old culture of *P. capsici* was placed in one side of the petriplate containing 15 ml of PDA medium. After three days of pathogen inoculation, 24 h old bacterial strains *P. fluorescens* and *B. subtilis* strains were streaked on the opposite of the petriplate by the help of sterilized inoculation needle. Endophytic fungal strains *T. asperellum* and *T. viride* were streaked simultaneously against *P. capsici*. Three replications were maintained for each treatment. The plates were incubated at room temperature (22 ± 2°C) for three days and seven days, respectively, and inhibition zone was measured. The radial growth of the pathogen and per cent reduction over control was calculated by using the formula as follows.

$$\text{Per cent reduction over control} = \frac{C\text{-}T}{C} \times 100$$

where, C – mycelial growth of the pathogen in control (mm) and T – mycelial growth of the pathogen in dual plate (mm).

2.4. Preparation of individual and mixtures of PGPR and PGPE bio-formulations

A loopful of *P. fluorescens* and *B. subtilis* were inoculated into the sterilized KB and NB and *T. asperellum* and *T. viride*, respectively, and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (26 ± 2°C) for bacteria and seven days of incubation for endophytic fungi. Bacterial suspensions (9×10^8 cfu/ml) and fungal endophytic suspension (5×10^5/g inoculum) was used for the preparation of talc-based formulation. To the 400 ml of suspension, 1 kg of the talc powder (sterilized at 105°C for 12 h), calcium carbonate 15 g (to adjust the pH to neutral) and carboxymethyl cellulose (CMC) 10 g (adhesive) were

mixed under sterile conditions, following the method described by Nandakumar *et al*. (2001). After shade drying for overnight, it was packed in polypropylene bag and sealed. At the time of application, the population of bacteria and fungal endophytes in talc formulation was 3×10^5/g of the inoculum. Finally, strains that are going to make up the mixture were added equally (v/v) and mixed with talc powder, $CaCO_3$ and CMC.

2.5. Greenhouse studies

2.5.1. Effects of bio-formulation mixtures on the incidence of foot rot disease

To study the ISR against *P. capsici* of pepper veins, a pot culture experiment was conducted with rhizosphere bacteria *B. subtilis*, *P. fluorescens* and endophytic fungi *T. viride* and *T. asperellum*. Six months veins were grown in earthen pots (size-0.35 m diameter, 0.50 m height, volume of soil: 0.04 m^3) filled with sterilized potting soil per vein per pot. In all the treatments, the talc-based bio-formulation mixture was applied for vine treatment and soil application. The fungicide (**RIDOMIL** GOLD® **MZ**) was used as a positive control. For treatment, the pepper vine **cv.** Subhakara was used. Six months pepper vines were sprayed with bacterial suspensions (9×10^8 cfu/ml) for one vine per pot (size-0.35 m diameter, 0.50 m height, volume of soil: 0.04 m^3) containing sterilized soil mixture (cow dung: sand: soil in 1:1:1 ratio) and inoculated with the spore suspension (2×10^5 spores/g of inoculum) of *P. capsici*. In another set, *Trichoderma* inoculum (5×10^5/g) per pot was sprayed for six months old vines (40 - 45 cm height). Vine treated with **RIDOMIL** GOLD® **MZ** (2 g/litre) was considered as positive control. The pepper vine inoculated with the pathogen alone served as inoculated control and without any pathogen inoculation served as healthy control. The observation on the development of *P. capsici* symptoms was recorded after 30th day of the inoculation. Each treatment was replicated thrice in completely randomized block design (CRD). The per cent disease index (PDI) was estimated using the formula suggested by Mckinney (1923).

2.5.2. Inoculation preparation of *P. capsici* for enzyme study

For inoculation purpose, *P. capsici* was cultured on a series of 9 cm diameter petriplates containing OMA at 22±2°C until mycelia cover the media. Mycelia suspension was prepared in sterilized distilled water. Using haemocytometer, inoculum load was adjusted to 2×10^5 spores/g.

2.5.3. Induction of defense-related protein and experimental design

Organisms from rhizosphere, *B. subtilis*, *P. fluorescens* and endophytic fungal strains *T. viride* and *T. asperellum* were used in the induction of defense reactions in pepper. The bio-formulations treated vine were grown at the rate of one vien per pot (size-0.35 m diameter, 0.50 m height, volume of soil: 0.04 m^3) filled with sterilized potting soil containing the spore suspension (2×10^5 spores/g of soil) of *P. capsici*. Cultures of rhizosphere bacteria and spores of endophytic fungi treated plants were challenge inoculated with *P. capsici* in the first set and in the second set of experiment, treated plants were not challenged with the pathogen. Plants without prior biocontrol treatment was inoculated with the pathogen and kept as control. The plants neither treated with bio-formulation nor challenged by the pathogen were kept as healthy control. Three replications were maintained in each treatment. Each replicate consisted of three pots and in each pot one vine were maintained. The experiments were conducted using completely randomized block design in a greenhouse. The humidity in the greenhouse was maintained at around RH 70%. The temperature was adjusted to 26°C (day)/20°C (night).

2.5.4. Sample collection and assay of defense-related proteins

Vine leaf tissues were collected at different time intervals (0, 4, 8, 16, 24, 48 and 72 h after pathogen inoculation). Three pepper vines were sampled from each replication of the treatment separately and were maintained for biochemical analysis. Leaf samples were homogenized with liquid nitrogen in a pre-chilled mortar and pestle. One gram of leaf sample was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 10,000 rpm. The supernatant was used as a crude enzyme extract for assaying PO (Hammerschmidt *et al*., 1982), polyphenol oxidase (PPO) (Mayer *et al*., 1965) and PAL (Dickerson *et al*., 1984). Enzyme extracted in 0.1 M sodium citrate buffer (pH 5.0) was used for the estimation of chitinase (Boller and Mauch, 1988) and β-1, 3-glucanase (Pan *et al*., 1991). The total phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993).

2.5.5. Chitinase activity

Chitinase assay was performed by grinding 1 g of leaf sample using a chilled pestle and mortar with 0.1 M sodium citrate buffer (pH 5.0) at 4°C. The homogenate was centrifuged at 8000 rpm for 20 min. The supernatant was used as a crude enzyme extract for assaying chitinase activity. The changes in the chitinase activity were determined by colorimetric assays described by Boller and Mauch (1988).

2.5.6. Assay for peroxidase

Fresh vine leaves (1 g) were homogenized in 3 ml of 0.1 M sodium phosphate buffer (pH 7.0) with pre chilled mortar and pestle. The homogenate was centrifuged at 18,000 rpm at 4°C for 15 min and used within 2 h. Supernatant was served as an enzyme source. To a spectrophotometric sample cuvette, 3 ml of buffer solution, 0.05 ml guaiacol solution, 0.1 ml enzyme extract and 0. 03 ml H_2O_2 solution were added and mixed well. Absorbance was recorded at 470 nm using spectrophotometer (Hitachi, 2000, Japan). The enzyme activity was expressed as changes in absorbance/min/g fresh weight (Hammerschmidt *et al*., 1982). Three replicates were maintained for each treatment.

2.5.7. Determination of phenylalanine ammonia lyase activity

Leaf tissues (300 mg) from each of three replicates for each treatment were homogenized in the ice-cold 0.25 M borate buffer (pH 8.7) in an ice bath. The homogenate was centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was then centrifuged at 15000 rpm for 15 min at 4°C. The resultant clear yellowish-green supernatant was used as crude enzyme extract. The reaction mixture contained 1 ml of enzyme extract, 0.5 ml of 0.2 M borate buffer (pH 8.7), 1.8 ml of distilled water and 0.2 ml of 1 M l-phenylalanine. Changes in absorbance at 290 nm were observed using spectrophotometer (Hitachi, Japan, 2000). Reaction mixture without substrate served as control. One unit of enzyme activity produced 3.37 nm of cinnamic acid/h (Singh and Prithiviraj, 1997). Three replicates were maintained for each treatment.

2.5.8. Assay of polyphenol oxidase

One gram fresh weight from biocontrol agents, endophytic fungal treated and control plants were ground to a fine powder in liquid nitrogen and extracted in 1 ml of extraction buffer containing 0.1 M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 15,000 g for 15 min at 4°C and the supernatant was used as enzyme source. The reaction mixture was started when 0.2 ml of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm at 30 sec intervals for 3 min. The mean change in absorbance was calculated for 1 min and the activity was expressed as changes in absorbance/min/mg of protein of the plant sample (Mayer *et al*., 1965).

2.5.9. **Assay for β-1, 3-glucanase**

The crude extracts was added to 62.5 µl of lamanarin and then incubated at 40°C for 10 min; the reaction was stopped by adding 375 µl of dinitrosalicylic acid and heated for 5 min on boiling water bath. The resulting solution was diluted with 4.5 ml distilled water and the absorbance was read at 500 nm. The crude extract preparation with lamanarin with zero time incubation served as blank. The activity was expressed as µg equivalent of glucose/min/mg of protein (Kavitha *et al*., 2005).

2.5.10. Estimation of phenol

One gram of fresh sample was homogenized with 10 ml of 80% methanol (Zieslin *et al*., 1993). One milliliter of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1 N) and the solution was kept at 25°C. The absorbance of the blue was measured using a spectrophotometer (Hitachi, 2000, Japan) at 725 nm. Catechol was used as the standard (Kagale *et al*., 2004).

2.5.11. Statistical analysis

The data on effect of the treatments on the growth of pathogens, severity of diseases and activity of enzymes in pepper vine were analyzed by analysis of variance (ANOVA), and treatment means were compared by Duncan's multiple range test (DMRT). The data on disease severity was arcsine transformed before undergoing statistical analysis (Gomez and Gomez, 1984).

III. RESULTS

3.1. Compatibility among bacterial strains

PGPR strain of *B. subtilis*, *P. fluorescens* and PGPE strains of *T. asperellum* and *T. viride* were tested for their compatibility *in vitro*. None of the antagonistic bacteria were inhibited by each other, so the absence of inhibition zone suggesting that these biocontrol agents and endophytic fungal strains were compatible with each other.

3.2. Effect of biocontrol agents on radial growth of *P. capsici*

PGPR strain of *B. subtilis*, *P. fluorescens* and endophytic fungal strains of *T. viride* and *T. asperellum* were tested individually to assess the radial growth of *P. capsici*. All the treatments were effective in reducing the mycelial growth of the pathogen. However, *B. subtilis* had resulted with mycelial growth of 50 mm and inhibition zone of 25 mm and P. *fluorescens* showed 63 mm of mycelial growth and 18 mm inhibition zone, respectively. *Trichoderma viride* recorded the maximum inhibition zone of 26 mm, and *T. asperellum* showed 19 mm inhibition zone and 60 mm mycelia growth, respectively. The control plates recorded the highest mycelial growth of 91 mm (Table 1).

Table.1: Effect of biocontrol agents on the mycelial growth of P. capsici

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Sl. No	Treatments	Mycelial growth (mm)	Inhibition zone (mm)
1	*Bacillus subtilis*	50±0.32^{de}	25±0.50^{f}
2	*Psuedomonas fluorescens*	63±0.22^{ef}	18±0.28^{g}
3	*Trichoderma asperellum*	59±0.5^{bc}	19±0.50^{h}
4	*Trichoderma viride*	49±0.32^{a}	26±0.50^{a}
5	**RIDOMIL** GOLD	56±0.32^{bcd}	17±0.50^{e}
6	Control	91±0.12^{h}	0.00

Values are mean of three replications. In a column, mean followed by a common letter (s) are not significantly different at the 5% level by DMRT

3.3. Efficacy of PGPR and PGPE strains on foot rot incidence under greenhouse conditions

Talc-based bio-formulation of PGPR strains *B. subtilis*, *P. fluorescens* and *T. asperellum*, *T. viride* strains individually were tested for their efficacy against *P. capsici* on pot method along with **RIDOMIL** GOLD as a chemical check. *Bacillus subtilis* significantly reduced the foot rot incidence by 20% and *P. fluorescens* showed 31% disease incidence compared to untreated plants upon control with 68% as shown in figure 1. Conspicuously, *T. viride* resulted in a significantly lower foot rot disease index (PDI) than any of the strains of 18%, and *T. asperellum* showed 33% disease incidence, respectively.

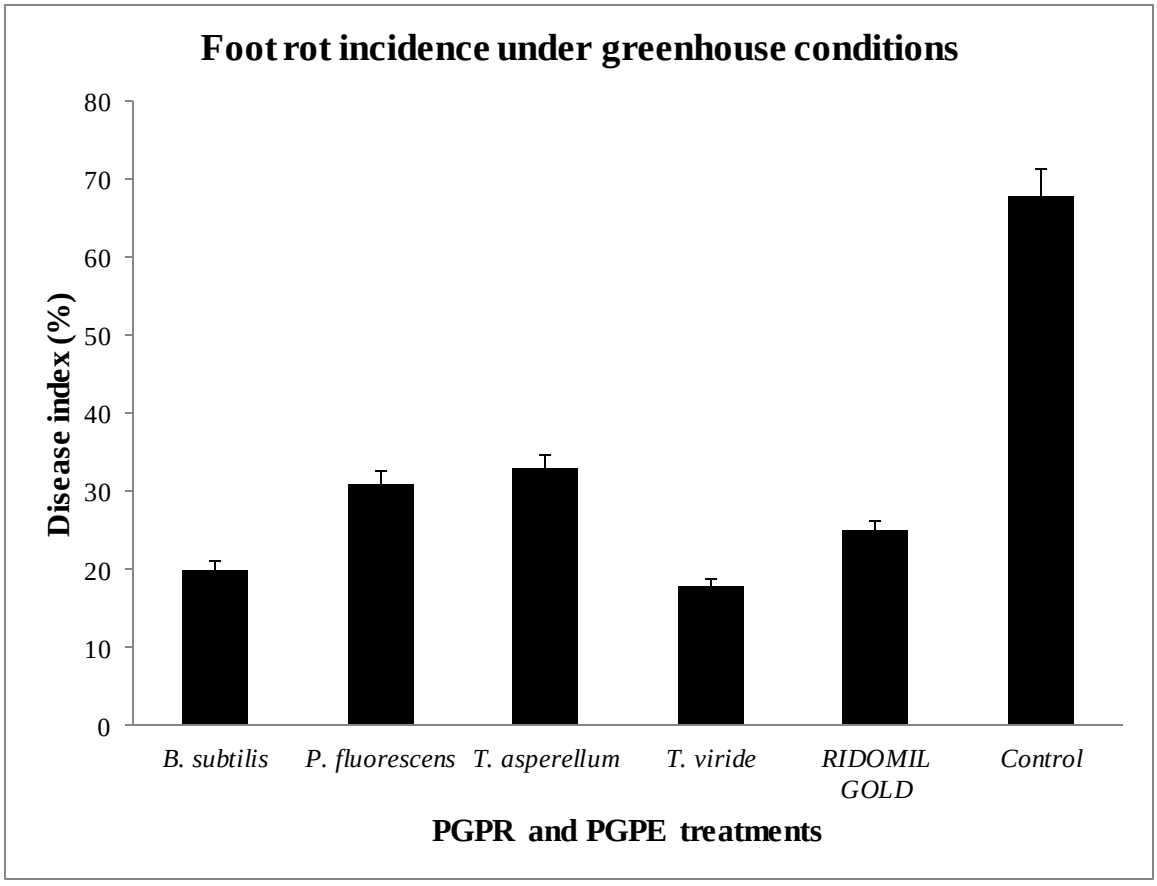

Fig.1: Efficacy of individual and mixture of biocontrol agents on foot rot incidence in black pepper under greenhouse conditions

Values are mean of three replications. The line on each bar represents ±SEM

and the post test analysis was carried out using DMRT. The results suggest that $p>0.05$.

3.4. Effect of PGFR and PGPE treatment on Chitinase activity

Higher chitinase activities were observed in endophytic fungal strain *T. viride* pre-treated black pepper

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challenge inoculated with pathogen (*P. capsici*) as seen in figure 2. Plants grown from different pre-treated vines presented the highest chitinase activity. *Trichoderma viride*, *T. asperellum* and *B. subtilis* were effective in yielding highest activity compared with other strains. The presence of pathogen (uninoculated control) alone not increased the chitinase activity compared to healthy control and inoculated, respectively.

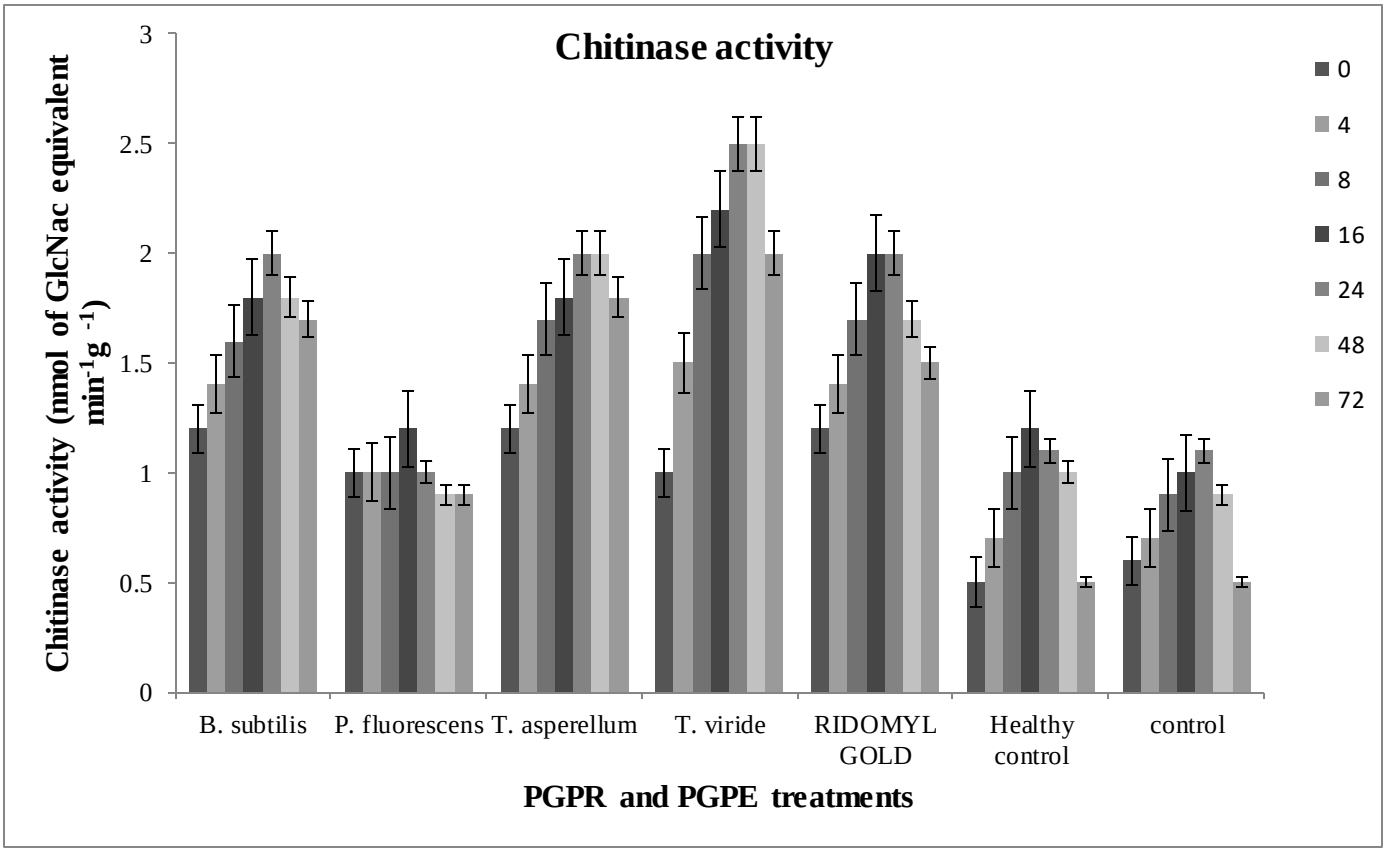

Fig. 2: Induction of chitinase in black pepper in response to PGPR and PGPE treatments against foot-rot disease

3.5. Effect of PGFR and PGPE treatment on peroxidase activity

Peroxidase assay indicated the increased peroxidase activity in the pepper vine inoculated with the target fungus *P. capsici*. After 48 h of challenge inoculation, and gradually decreases at 72 h this shows that peroxidase activity increased gradual persistence of resistance as shown in figure 3. *Trichoderma viride* were very effective in enhancement of peroxidase compared to *T. asperellum* and *B. subtilis* strains.

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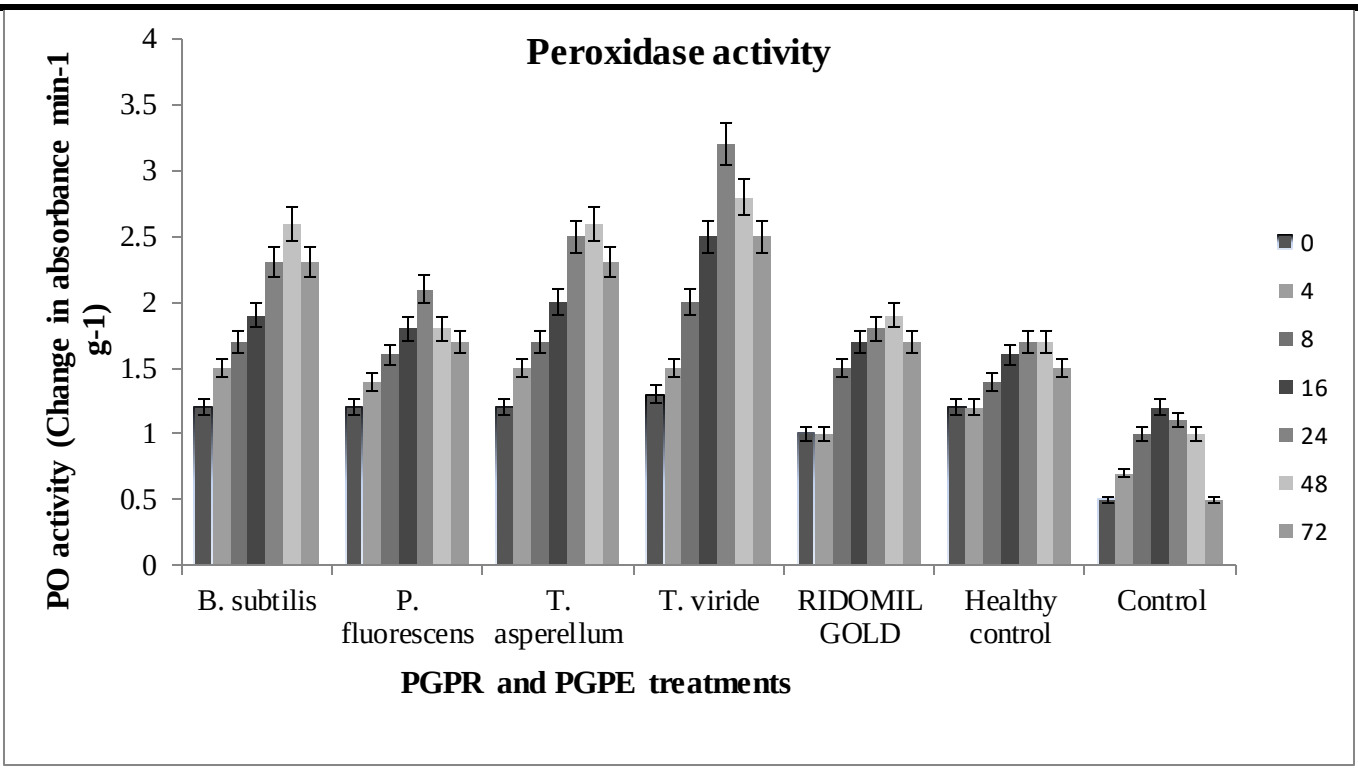

Fig.3: Induction of peroxidase activity in black pepper in response to PGPR and PGPE treatments against foot-rot disease

3.6. Effect of PGFR and PGPE treatment on polyphenols oxidase activity

Increase in polyphenol oxidase activities were observed in *T. viride*, *T. asperellum* and *B. subtilis* compared to *P. fluorescens* treated black pepper vines which showed a drastic increase from 24 to 48 h and decreases the activity at 72 h after inoculation as shown in figure 4.

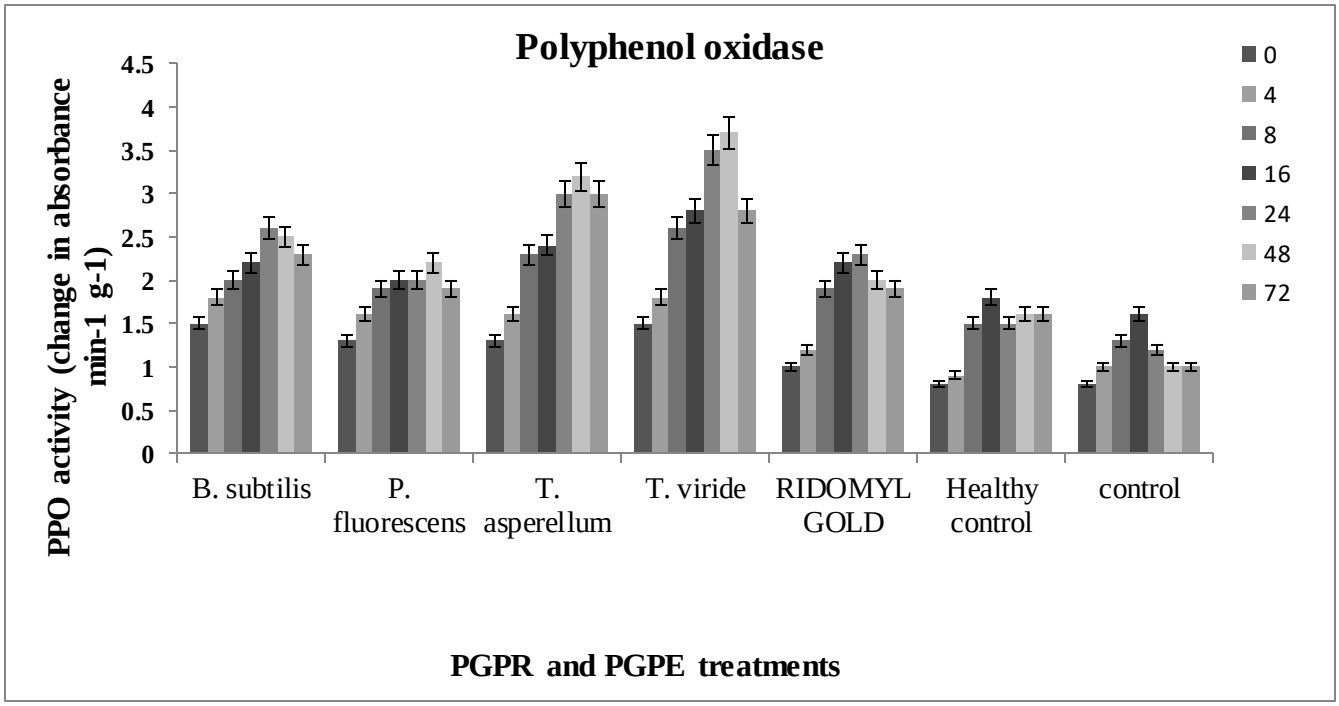

Fig.4: Induction of polyphenol oxidase in black pepper in response to PGPR and PGPE treatments against foot-rot disease

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3.7. Effect of PGFR and PGPE treatment on Phenylalanine ammonia lyase activity

Phenylalanine ammonia lyase (PAL) can be seen that microbiolization of vines with different PGPR and PGPE strains lead to increase in PAL activity. Also, changes in PAL activities were observed after challenge inoculation with the target pathogen *P. capsici* up to 48 h and drastic decrease at 72 h after challenge inoculation as shown in figure 5. *Trichoderma viride* was most effective in enhancing the PAL activity compared to other strains. *Trichoderma asperellum* and *B. subtilis* also showed higher activity when compared to *P. fluorescens*, healthy control and control.

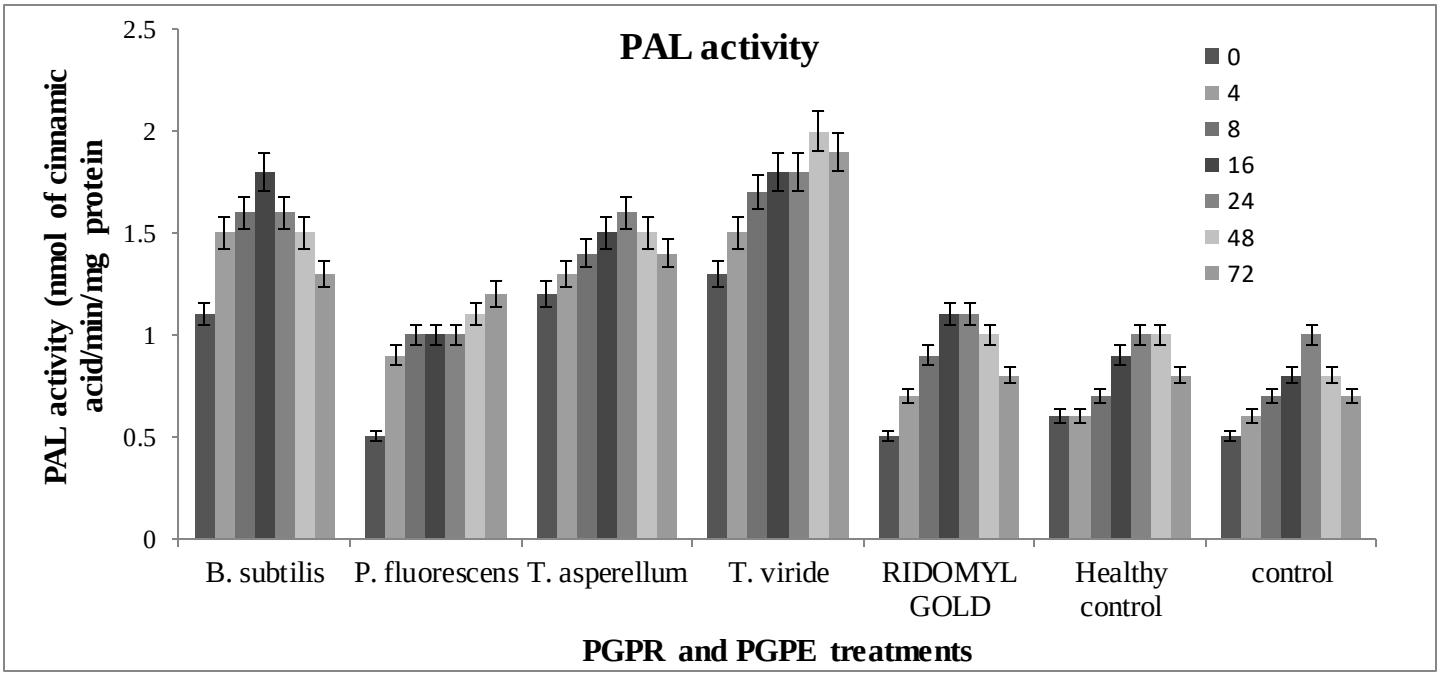

Fig. 5: Induction of phenylalanine ammonia lyase in black pepper in response to PGPR and PGPE treatments against foot-rot disease

3.8. Effect of PGFR and PGPE treatment on β 1,3-glucanase activity

The β 1, 3-glucanase activities were increased at 24 h up to 48 h after challenge inoculation. *Trichoderma viride*, *B. subtilis* and *T. asperellum* pretreated vines enhance their β1, 3-glucanase activity compared with other strain *P. fluorescens* as shown in figure 6.

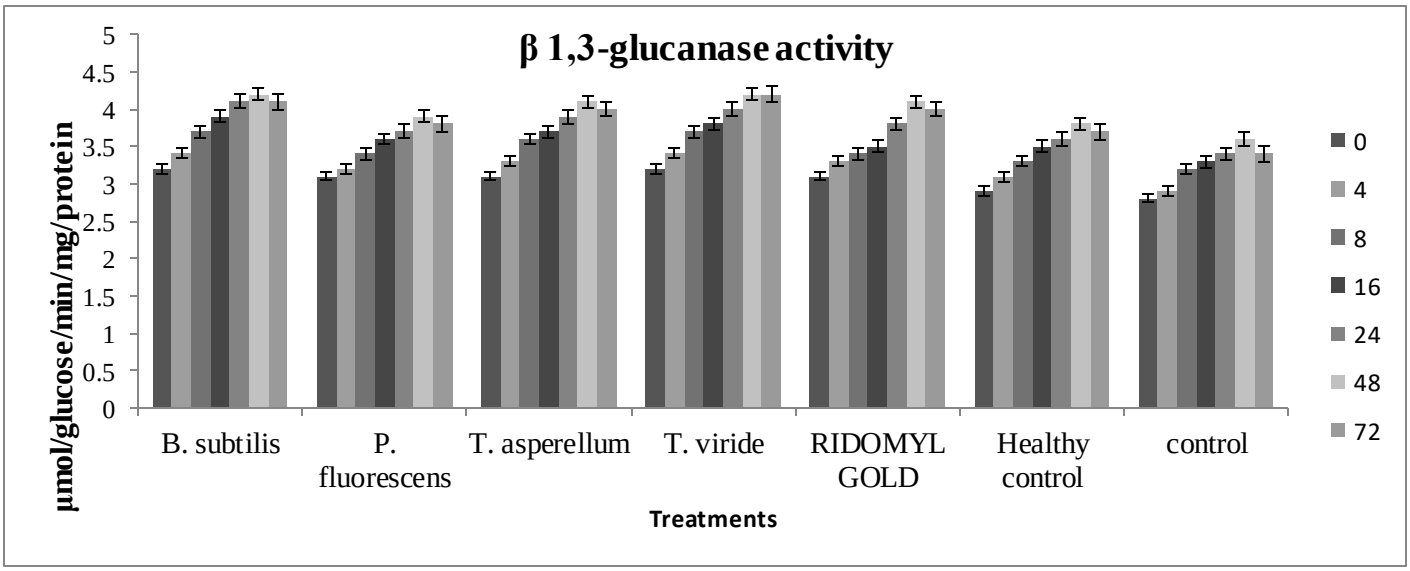

Fig. 6: Induction of β 1, 3-glucanase in black pepper in response to PGPR and PGPE treatments against foot-rot disease

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3.9. Effect of PGFR and PGPE treatment on phenol content

Phenolic compounds may be fungitoxic in nature and may increase the mechanical strength of the host cell wall. In the present study, vine treatments with *T. viride* and *B. subtilis* resulted in increased accumulation of phenolic substances in response to pathogen infection. Higher level of phenol activity was observed in *T. viride*, *B. subtilis* and *T. asperellum* treated vines when compared with *P. fluorescens*, healthy control and control treated vines as shown in figure 7.

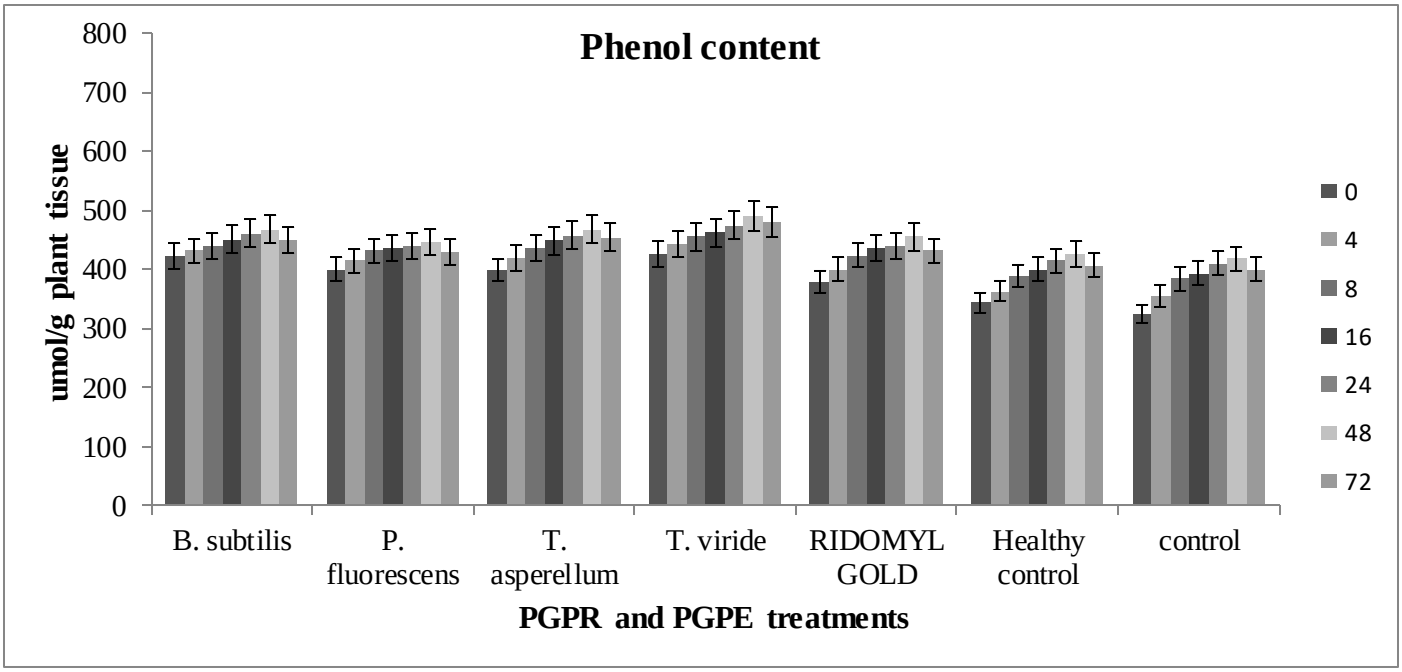

Fig. 7: Induction of phenol content in black pepper in response to PGPR and PGPE treatments against foot-rot disease

IV. DISCUSSION

Two strains of rhizobacterial isolates and two fungal endophytes were used for the efficacy study of foot rot disease. These isolates also induced the systemic resistance against *P. capsici* and in turn they enhanced the plant height. The massive accumulation of phytoalexins and phenolic compounds, the increased accumulation of PR proteins and peroxidase, increased the levels of mRNAs encoding phenylalanine ammonia-lyase (PAL), chalcone synthase and PR1a proteins and enhanced lignifications have been reported in plants following treatment with PGPR isolates (Hynes and Lazarovits, 1989; Van Peer *et al*., 1991; Zdor and Anderson, 1992; M'Piga *et al*., 1997). PGPR are free-living bacteria having a beneficial effect on plants as they enhance emergence, colonize roots and stimulate growth (Kloepper *et al*., 1988). In recent years, the concept of using PGPR and PGPE for the promotion of plant growth is gaining worldwide acceptance (Kloepper *et al*., 1991). PGPRs stimulate host plant growth through several possible mechanisms, including biological control (Pleban *et al*., 1995), induced systemic resistance to plant pathogens (Benhamou, 1996; Hallman *et al*. 1997), phytohormone production and the improvement of nutrient and water uptake (Pleban *et al*., 1995; Nowak and Lazarovits, 1997). They also improve plant performance in stress environments and consequently enhance yields (Frommel *et al*., 1991). The present findings are in agreement with these findings.

Increase in the chitinase activity was observed in PGPR treated pepper plants. Our results are in confirmation with the findings of Silva *et al*. (2004). Reports on the increase in chitinase activity in tissue expressing resistance in diverse pathosystems have been made (Croft *et al*., 1990). The mode of pathogen infection that excludes direct penetration in host cells (Romeiro, 1995), suggests the possibility that products of chitinase activity posses some antimicrobial activity (Sailaja *et al*., 1998).

The present study demonstrated that the isolates of rhizobacterial *B. subtilis*, endophytic fungi *T. viride* and *T. asperellum* consistently reduced the radial mycelial growth of *P. capsici* by producing various antibiotics and reduced the foot rot of pepper under green house and field conditions by inducing ISR compared to individual agents. Further, plant growth promoting rhizobacteria (PGPR),

especially *B. subtilis* and plant growth promoting rhizobacteria (PGPR), especially *P. fluorescens* (Pf1) strains have been developed commercially as a talc-based formulation and tested against several crop of plant diseases (Vivekananthan *et al*., 2004; Rajendran *et al*., 2007). Sundaramoorthy *et al*. (2012) reported that the combination of *P. fluorescens* strains and *B. subtilis* strain together resulted in significant growth promotion that was correlated with induced resistance in *Capsicum annum* L. Several approaches have been made to manage the foot rot of pepper. A large number of enzymes have been associated with ISR, including peroxidase, phenyalalanine ammonia-lyase, lipoxygenase, β-1, 3 glucanase and chitinase (Koch *et al*., 1992; Schneider and Ullrich, 1994; Van Loon, 1997; Ye *et al*., 1990; Silva *et al*., 2004). Furthermore, interactions among the bacterial strains may have synergistic effects that could induce ISR and promote the growth of the plants (Sundaramoorthy *et al*., 2012). Several literature have been documented that the use of biocontrol agents and endophytic fungal strains were more effective for management of plant diseases (Kavino *et al*., 2007; Harish *et al*., 2008; Sivakumar, 2012).

In the present study, *T. viride* and *B. subtilis* increased the peroxidase (PO) and polyphenol oxidase (PPO) activities, which increased the maximum activities on 48th h after challenge inoculation with *P. capsici*. The PO and PPO catalyse the last step in the biosynthesis of lignin and other oxidative phenols. Zdor and Anderson (1992) noticed that rhizosphere *B. subtilis* induced PO activity in bean. Chen *et al*. (2000) reported that the higher PO activity was observed in cucumber roots treated with *P. fluorescens* challenged with *P. aphadermatum*. Our results are in agreement with the results of Podile and Laxmi (1998) and Silva *et al*. (2004). The high peroxidase activities are usually associated with later stages of the infection process and are linked to generation of hydrogen peroxides that inhibit pathogens directly or generate other free radicles with antimicrobial effects (Hammerschmidt *et al*., 1982).

PAL activity was also increased at 48th h after challenge inoculation with target pathogen due to rhizobacterial and endophytic strains. In which, *B. subtilis* and *T. viride* and *T. asperellum* effectively enhanced the enzyme activity in the presence of pathogen when compared to other strains of *P. fluorescens*. Similar results were reported by Sundaramoorthy *et al*. (2012) in capsicum against *P. capsici*. The product of phenyl alanine trans-cinammic acid, is directly linked to cell lignifications processes and the highest levels of PAL activity usually about 24 h after initial infection (Podile and Laxmi, 1998). So, PAL generally shows increased activity during pathogen establishment in the host tissue (Bhattacharya and Ward, 1988). PAL plays an important role in the biosynthesis of phenolic phytoalexins (Daayf *et al*., 1997). The increase in PAL activity indicates the activation of phenyl propanoid pathway. In several host–pathogen interactions, increased levels have been shown to be correlated with incompatibility (Rathmell, 1973; Bhattacharya and Ward, 1988; Ralton *et al*., 1989). The product of PAL is trans-cinnamic acid which is an immediate precursor for the biosynthesis of SA, a signal molecule in systemic acquired resistance (SAR) (Klessig and Malamy, 1994).

β 1, 3-glucanase activity was increased in PGPR and PGPE treated black pepper after 48 h of challenge inoculation with target pathogen. Sundaramoorthy *et al*. (2012) have reported that seed treatment with PGPR and PGPE has produced hydrolytic enzymes such as chitinases and β1, 3-glucanases in capsicum. These host lytic enzymes accumulates at the site of penetration of the fungus *P. capsici* resulting in the degradation of the fungal cell wall (Benhamou *et al*., 1996). In a number of plant species, β1, 3-glucanase exists in multiple forms. These enzymes solubilize elicitor's active glucan molecules from the fungal cell wall (Mauch and Staechelin, 1989) and also induce defense enzymes. When the pathogen grows initially in the intercellular spaces, the fungus may come in contact with β1, 3-glucanase localized in the middle lamellae.

Increment of phenol was high in vine treated with *B. subtilis*, *T. viride*, *T. asperellum* and *P. fluorescens* resulted in increased accumulation of phenolic substances in response to infection by the pathogen among them *T. viride* and *B. subtilis* showed increased phenolic substance when compared to *T. asperellum* and *P. fluorescens*. M'Piga *et al*. (1997) reported that *B. subtilis* induced the accumulation of phenolic substances which exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. Ramamoorthy and Samiyappan (2001) have reported the accumulation of phenolic substances and PR proteins in response to infection by *F. oxysporum* f. sp. *lycopersici* in tomato. Our results substantiate the inhibition of various plant pathogen and disease management by using several biocontrol agents through the induction of ISR in plants reported by Van Peer *et al*. (1991), Kloepper (1993), Van Loon (1997), Chen *et al*. (2000). Thus, our findings provide evidence that the induction of defense enzymes and PR proteins by application of endophytic fungi and rhizosphere bacterial strains may strengthen the plants against various biotic stresses.

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V. CONCLUSION

The present study demonstrates that PGPR and PGPE are due to isolates of *T. viride* and *B. subtilis* and their role in enhancing growth on foot rot disease of pepper. Moreover, it is concluded that *T. viride* and *B. subtilis* increase the plant growth and resistance to *P. capsici*. It is easily imagined that the different isolates of rhizobacterial strains and endophytic fungal strains produces antimicrobial products and defense enzymes restrict the development of challenging phytopathogenic fungi.

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Minimizing the Impact of Yellowfin Tuna *Thunnus albacares* fishing in Banda Sea

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Abstract*—The declining trend of Yellowfin Tuna Thunnus albacares production in Fishery Management Territory of Indonesia 714 nowadays will affect the demand for tuna products in Indonesia and global. Regarding the management purposes, the impact of fishing needs to be minimized. Therefore, the estimation of population structure based on the length and age is very important to be studied. The data were collected from November 2015 to October 2016 from longline and handline catching activities operated in the Banda Sea. The average length of yellowfin tuna caught with longline and handline was 98.5 cm and the first length maturity was L_m 103.6 cm. The result showed that the first yellowfin tuna caught was Lc_opt 125.2 cm, where fishing mortality ratio toward natural mortality was at the level that endangered the sustainability of yellowfin tuna fisheries in the Banda Sea. To minimize the impact, there needs to be a minimum size regulation of yellowfin tuna that should be landed.*

Keywords*— *Yellowfin Tuna, First Caught Length, Yield, Biomass, Banda Sea.

I. INTRODUCTION

Yellowfin Tuna Thunnus albacares is one of the important commercial big pelagic fish and lives in tropical and subtropical waters (Wu et al., 2010; Collette and Nauen, 1983). Banda Sea is one of the potential tuna fishing areas in Indonesia, the types of fishing gear used consist of purseseine, pole and line, tuna longline, handline and trolling line. The production of tuna in Maluku during the period of 2012 - 2016 has decreased; the average decline was 24.3%. The decrease of the production was caused by the temporary suspension of licensing of catch fishery business in the fisheries management territory of the Republic of Indonesia, especially the purse seine fisheries fleet (Ministry of Fisheries and Marine Affairs Regulation No. 56, 2014), and banning the purseseine system of two vessels to catch big pelagic fish (Candy KP number 71 , 2016).

The status of yellowfin tuna stocks at Western and Central Pacific Ocean at the end of 2012 is slightly above maximum sustainable yield, and the highest impact of catching occurs in the tropics area (Davies et al, 2014; Brouwer et al, 2016; IOTC, 2018). The impact of high catching in the tropic area is due largely to the catch of yellowfin tuna with purseseine belonging to young fish (Widodo et al, 2015; Brouwer et al, 2016; Haruna et al, 2018).

Overfishing is one form of over-exploitation of fish populations to a dangerous level. The reduction of the fish catch impact of is discussed in international forums such as Rio + 20 summit of June 2012 (UN 2012), and meeting on fish catching that are responsible at international level (FAO 2012). According to Froese et al (2016), minimizing the impact of fishing on a population is close-to-natural figures of individuals should participate in important life history events. There are two important characteristics of the population in fisheries management, i.e. the average duration of the reproduction phase if the total mortality of Z = M + F is fairly constant after the age of the fish reaches maturity, and the average duration of the reproduction phase is the opposite of Z (Carnov, 1993). There are three options proposed by Froese et.al (2016) regarding the handling of fish populations, the first is fishing mortality is smaller than natural mortality, second is that catch should not reduce the population under half of natural abundance that is not exploited, and the third is the exploited individual population should be allowed to reproduce in order to realize its growth potential before it is captured. Fox (1970) or Schaefer (1954) production model shows that maximum sustainable yield can be obtained on stock sizes between 37% and 50% of unexploited biomass. Beverton and Holt (1957) say that the yield per recruit of catch mortality, catch and largest biomass can be obtained by increasing the length of the first time catch. The fact is minimizing the catch impact on biomass and age structure if the allowable catch is approximately the optimum size of the

individual (about 2/3 of the maximum length, L∞) where the biomass cohort is maximum (Froese et al., 2008).

In 7 regions at the Western and Central Pacific Ocean, the highest annual yellowfin tuna recruitment in region 7 (Indonesia, Philippines and Vietnam) are 0.42, the highest catch mortality due to the catch of yellowfin young age with purse seine was in region 3 and fisheries of Indonesia, Philippines, and Vietnam are in region 7 (Davies et al, 2014). According to Damora and Baihaqi (2013), the total length of yellowfin tuna that is first catch with the shelling line in the Banda Sea is 131.85 cm, and the natural mortality and catch mortality are 0.68 and 1.79. Fishing mortality of adult yellowfin tuna in Western and Central Pacific Ocean (WCPO) in 2000 ranged from 0.18 to 0.56, at Indian Ocean at the highest was 0.2 in 2010, and at WCPO 2010, it ranged from 0.25 to 0.45 (Hampton, 2004; Langley et al., 2011; Langley et al., 2012).

The estimation value of fishing mortality depends on the fishing gear, the method of catching and the distribution of the sampled fish size. The estimation of fishing mortality in Banda Sea by Damora and Baihaqi is only to the fishing gear and the samples which are limited to large fish, while in WCPO and Indian Ocean on purse seine and fishing gear. High catch mortality indicates high fishing pressure on fish stock in a waters. After purse seine of two vessel system is prohibited from the operation to catch tuna fish in all Indonesia Fishery Management Area including Banda Sea, tuna production depends only on long line and others fishery. Therefore, it is necessary to evaluate the condition of yellowfin tuna stock, in order to obtain the option to minimize the impact of the catch for its sustainability through limitation of the fish size that may be caught.

II. MATERIALS AND METHODS

Forked length size data of yellowfin tuna were obtained from tuna fishing activities (long line and hand line) operating in the Banda Sea from November 2015 to October 2016. The collected data were as much as 4,829 individuals of yellowfin tuna. The map of yellowfin tuna fishing operations in the Banda Sea is shown in Fig. 1.

The growth parameter of Von Bertalanffy was estimated by using *ELEFAN-I* developed completely by Gayanilo Jr. et al. (1996) by projecting some possible combinations of growth parameters of von Bertalanffy (L_∞ and *K)*. Growth parameter t_o is counted according to Pauly (1980) by the equation:

Log (t_0)= -0.3922 - 0.2752 (log L_∞) - 1.038 (log K) and natural mortality (M) is estimated by the equation: Log M = 0.0066 - 0.279 LogL_∞ + 0.6543 + 0.4634 LogT LogK .

In which M= the rate of natural mortality / (year),L_∞ = asymptotic length (cm), K = growth rate coefficient / (year), T = the average of water surface temperature (^{0}C) = 29 ° C.

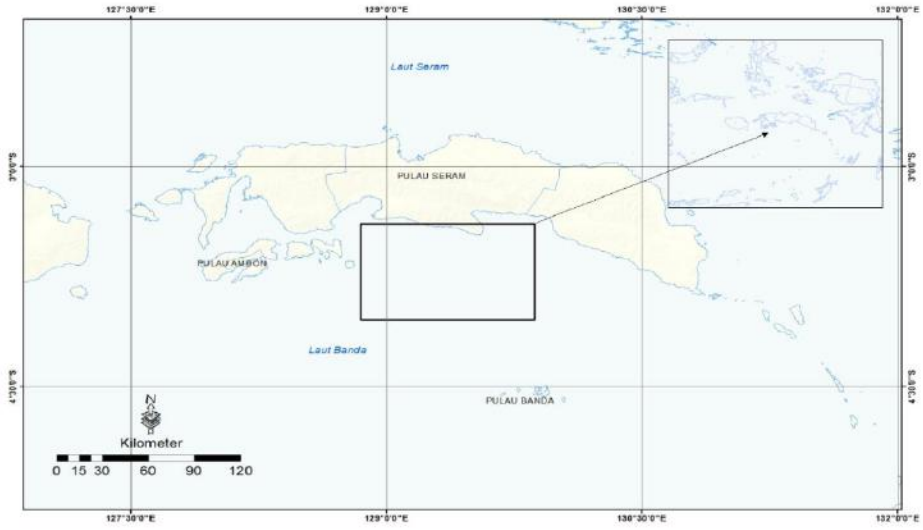

Fig. 1. Map of yellowfin tuna fishing operations in the Banda Sea.

The first size estimation of ripe gonads using the Sperman-Karber method (Udupa, 1986) used two criteria of gonad maturity which were immature group of gonads (Gonad Maturity Level I and II) and mature gonad group (Gonad Maturity Level III, IV and V). The estimation of the average size of gonads first mature fish usedsize group of gonad mature fish (Gonad Maturity Level III and IV) based on morphological criteria (Itano, 2001) and was analyzed by referring to Sparre and Venema (1989).The length of first catch $L_{(c_opt)}$is obtained by the equation (Froese et al., 2016):

$$L_{(c\_opt)} = \frac{L_\infty(2 + 3F/M)}{(1 + F/M)(3 + M/K)}$$

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In which M and F are obtained from L_∞ and K, L_∞ and K are the parameters of von Bertalanffy growth the equation (1938), and the other variables are determined based on various equations that have been published previously such as Froese (2006), Holt (1958), Beverton (1992), Charnov (1993), Beverton and Holt (1957) and Holt (1966).

Analysis of minimizing the fishing impact to meet the 3 options of yellowfin tuna fishery management in the Banda Sea was the curve of the relative first length capture (L_c/L_∞) as function of relative fishing mortality F to natural mortality M, and the yield curve per recruit relative to the theoretical and biomass maximum yield per recruit relative to unexploited biomass as a function of the F/M ratio for different lengths at first catch. L_{c_opt} was analyzed by using MS Excel worksheets according to Froese et al. (2016).

III. RESULTS AND DISCUSSION

3.1. Size Structure

The size of yellowfin tuna caught during the annual period was distributed in the range of 25-178 cm (**Fig. 2**). Fish catches were dominated at the frequency of 98.5 cm length mode.

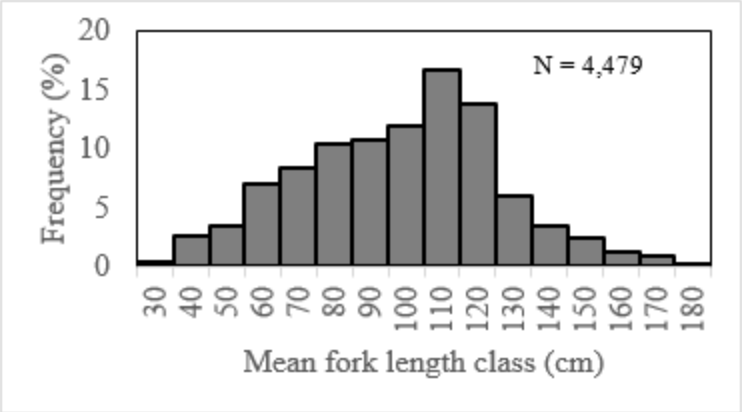

Fig. 2. Size Structure of Yellowfin Tuna Caught by Line Fishing in Banda Sea Until November 2015 to October 2016

3.2. Growth, Mortality and Maturity

Based on the equation of growth parameters by von Bertalanffy, it was obtained growth rate coefficient (K) = 0.31 per year, asymptotic length (L_∞) = 215 cm, theoretical age of yellowfin tuna when the length is zero (t_0) = - 0.311. Maximum estimated age of yellowfin tuna was 9.37 years. Total mortality (Z) was 1.47 per year and the rate of natural mortality (M) was 0.49 per year, and the fishing mortality (F) was 0.98 per year.

The observation toward 629 samples of female gonads including Gonad Maturity Level IV which was distributed in a size range of 69-178 cm was obtained the first mature gonad size (length at first maturity $L_{m)}$ was 103.6 cm, and the average size of a decent catch ($L_{50)}$ was 115.2 cm. The accumulation percentage of fish catches towards a decent size of yellowfin tuna fishing in the waters of the Banda Sea amounted to 23.09%.

3.3. First length Catch

Each fishing mortality has relationship with the first caught which will maximize the yield of stocks in a given period (Beverton and Holt, 1957, 1966). Fishing mortality (F) of yellowfin tuna in the Banda Sea from November 2015 until Oktober 2016 was 2.0 M. The length of first caught (L_{c_opt}) of yellowfin tuna for fishing mortality 2. 0 M was 125. 2 cm, whereas L_{c_opt} was 117.4 cm for F = M and 109.5 cm for F = 0. 5 M (Figure 2). In fishing mortality (F) level is equal to natural mortality (M), the first catch (L_{c_opt}) was 54,5% from L_∞. To meet option 1 proposed by Froese *et al* (2016), F should be smaller than M, and to meet option 3, F=M in which Lc_opt is bigger than the length where yellowfin tuna has reached gonad maturity (**Fig.3**).

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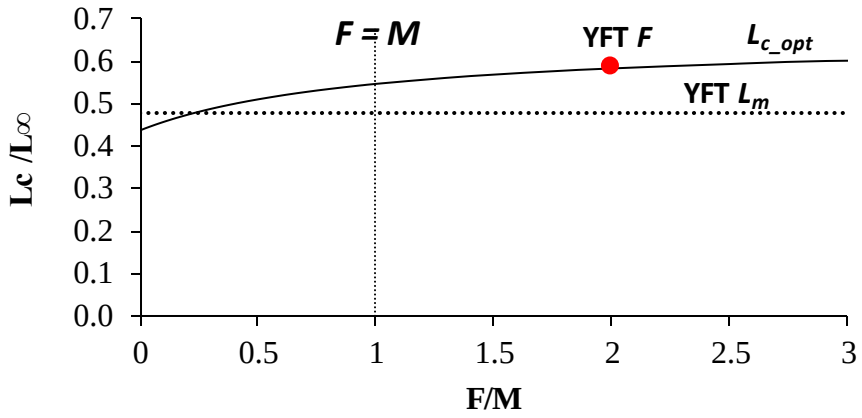

Fig. 3.The Relative First Caught Length (L_c /L_∞) of Relative Fising Mortality F to Natural Mortality M. the Doted Curve (Lc_Opt) was the Length of First Caught. Lm Indicates the Length in Whichthe Yellowfin Tuna Reaches Gonad Maturity, and Red Round Color Indicates the Actual Fishing Mortality During the Period November 2015 toOctober 2016.

3.4. Yield and Biomass per Recruit

Fig.4 is a relative yield per recruit toward theoretical yield per recruit as a function of the ratio F / M for the first time caught length (L_{c_opt}) which were different. It shows that theincreasing of fishing mortality will cause the relative yield per recruit to the theoretical yield per recruit increasing to $L_{c\_opt.}$ If the length size of caught yelowfin tuna is with no size limit (dashed line), relative yield per recruit toward the theoretical yield per recruit for L_{c_opt}reaches the maximum at F / M = 0.6, the increasing of F/M then the relative yield per recruit of the theoretical yield per recruit for L_{c_opt} is diminishing (**Fig. 4**).

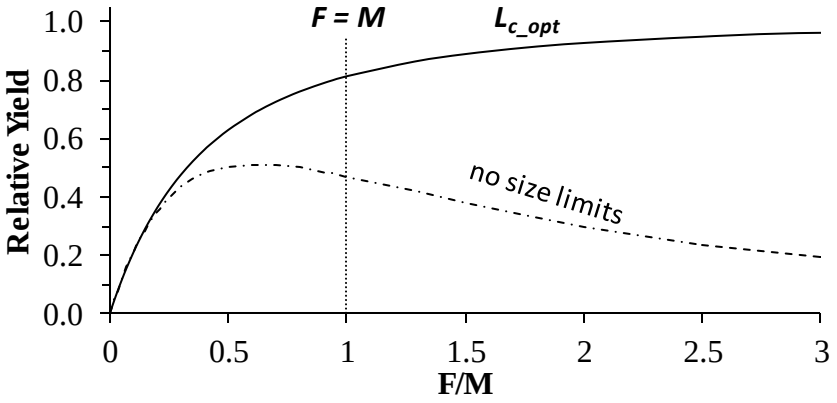

Fig. 4. The Relative Yield per Recruit to the Theoretical Maximum Yield as a Function of the F/M Ratio, for Different Lengths at first Catch of Yellowfin Tuna in Banda Sea. Lc_Opt (Solid Line) and No Size Limit (Dotted Line).

Sea (red round mark in **Fig. 5**) is below B = 0.5 $B_{0,}$ to meet the second option then the biomass per recruit relative to the unexploited biomass for L_{c_opt} is at F/M = 0. 8 (bold line indicated in **Fig. 5**). Thus, the catch pressure by 80% is equivalent to the natural death rate marking the highest theoretical fishing pressure that still meet the 3 proposed options in managing the yellowfin tuna fishery in the Banda Sea.

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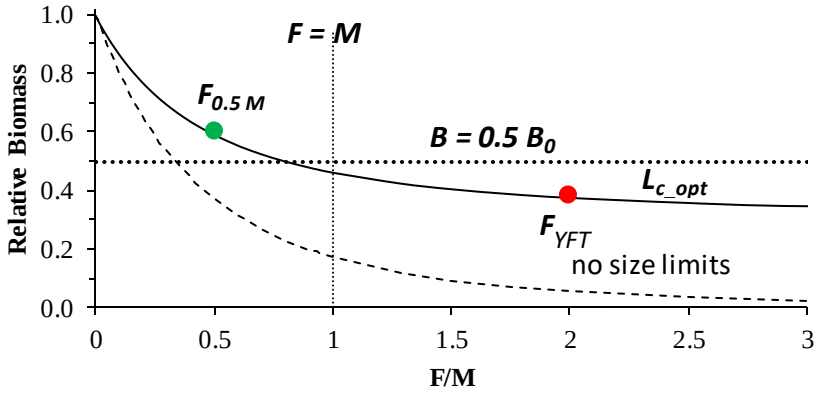

Fig. 5. Biomass per Recruit Relative to Unexploited Biomass, as a Function of the F/M Ratio, for Different Lengths at first Catch of Yellowfin Tuna in Banda Sea.

From this research, the average size of yellowfin tuna caught in the Banda Sea was 98.5 cm. This size was still below the size of gonad first mature. Therefore for the benefit of tuna fishery management in the Banda Sea, the minimum size that must be landed needs to get attention with a regulation from the government. This is needed to be done in order to meet the substance of minimizing the impact of tuna fishing in the Banda Sea.

IV. CONCLUSION

To minimize the catch impact of yellowfin tuna in the Banda Sea, the catching pressure with no feasible size requires attention by providing a regulation of the minimum size that can be landed. This is needed to be done so that fishing mortality becomes smaller than natural mortality.

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The Effect of Combination of Picloram and Bap on the Calculation of Clean Plants (*Postogemon cablin Benth*)

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***Abstract*—** *This study aims to determine the best Picloram and BAP concentrations in InVitro patchouli callus formation. This experiment was conducted in November 2018 to January 2019 at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University, Padang. This study used an experimental method with Completely Randomized Design (CRD) consisting of two factors. The first factor was the administration of Picloram concentration with 5 levels of treatment and the second factor was the administration of BAP concentrations with 5 levels of treatment. This experiment consisted of: This experiment consisted of Factor I: Picloram (A) Without Picloram (A1), Concentration of 1 mg / L (A2), Concentration of 2mg / L (A3), Concentration of 3 mg / L (A4), Concentration of 4mg / L (A5), Factor II: Concentration of BAP (S) Without BAP (S1), Concentration of 0.5 mg / L (S2), Concentration of 1 mg / L (S3), Concentration of 1.5 mg / L (S4) Concentration 2 mg / L (S5). The data obtained were analyzed using the F test at the level of 5%, if F count is greater than F table then the analysis is continued with the DMNRT test at the level of 5%. From the results of the study showed that the concentration of picloram had a significant effect on the growth of patchouli callus with an average callus appearance of 7 HST and percentage (%) of explants growing up to 100%. High picloram concentration did not significantly affect callus growth, 2 mg / l picloram + BAP concentration did not produce callus.*

***Keywords*— Patchouli (Pegestemon cablin Benth), Picloram, BAP, Tissue Culture.**

I. INTRODUCTION

Patchouli plant (*Pogostemon cablin Benth*.) Is one of the important export commodities in Indonesia because 90% of the world's needs for patchouli oil are supplied by Indonesia. Patchouli oil is widely used in the perfume, pharmaceutical, food and aroma therapy industries containing essential oils (Mangun, 2005 in Hatta et al., 2008). Patchouly oil is obtained from the distillation of patchouli leaves. Indonesian patchouli oil exports amounted to 800-1,500 tons worth US $ 18-53 million (Mustika and Nuryani, 2006). Patchouli oil exports reached 700 to 1,500 tons, with a foreign exchange value of US $ 14 to 30 million (Sulfiani, et al., 1998). Based on data from the National Export Development Agency, in Indonesia there are 14 production centers spread in four provinces. Patchouli oil is the largest product for essential oils and its use in the world shows an increasing trend. It can be said that until now there have not been any products, both natural and synthetic, that can replace patchouli oil in its position as fixative. BPS export data shows that the contribution of patchouli oil to the export income of essential oils is around 60%, vetiver oil (Vetiner oil) is around 12.47%, citronella oil (Citronella oil) is around 6.89%, and ginger oil (Ginger oil) around 2.74% (Krismawati, 2005). Until now 90% of the world's patchouli oil needs, equivalent to almost 2000 tons, are supplied from Indonesia. West Sumatra is the largest patchouli oil producer in Indonesia. In 2017 the area of West Sumatra patchouli plant reached 2,762 ha with a production of 200 tons (Directorate General of Plantation Indonesia, 2017).There are three types of patchouli in Indonesia, namely Pogostemon cablin Benth, P.hortensis Baker, and P. heyneanus Bent. The last two types of patchouli are rarely cultivated, because of the low yield and quality of oil, so that commercially is no longer profitable. Pogostemon cablin Benth is patchouli which naturally cannot flower in Indonesia. West Sumatra as one of the centers marked by the extent of patchouli cultivation by farming communities is 2765 ha with the main production center in West Pasaman with an area of

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1,496 ha, Mentawai islands 783 ha and the area of 237 ha (plantation statistics 2015). In addition to the three central areas of patchouli cultivation there are still several other areas in West Sumatra that can also develop patchouli plants and new areas of development of patchouli plants.

Patchouli potential in West Sumatra, especially in West Pasaman Regency, there are many patchouli plants that have been cultivated by the community since long ago. This is evidenced by the presence of local clones in each District. From 11 Subdistricts in West Pasaman there are 6 Subdistricts which have been found Nilam clones, including (1) Kinali Subdistrict (ai maruok clone), (2) Pasaman District (rimbo binuang and bukik nilam clones), (3) Talamau District (tombang clone), (4) Gunung Tuleh District (tanjung durian clone), (5) Lembah Melintang District (situ clone), and in (6) Koto Balinka District (lubuk godang clone) (Hidayat, 2017) Lately it has been known that there was a decrease in patchouli oil production which was partly due to low genetic quality, simple cultivation technology, the development of various diseases, and improper harvesting and post-harvest techniques (Nuryani 2006). According to Kadir (2011) the low genetic variability causes a narrow genetic diversity and difficulty in forming new clones. Until now the patchouli superior varieties that have been released are still very limited. One of the efforts to improve the quality of this oil can be done by using superior seeds that have the desired oil quality, but the availability of the number of superior seeds available is still limited (Directorate General of Plantation, 2007). One alternative for the rapid supply of seeds is by using vitro propagation techniques or tissue culture. Plant tissue culture is a technique to develop aseptic and accentic parts of plants in vitro on culture media containing complete nutrients and controlled conditions for specific purposes. Tissue culture is based on the theory of cell totipotence which states that every living plant cell has complete genetic information and physiological devices to grow and develop into intact plants if the conditions are appropriate. Plant propagation in tissue culture can produce large quantities of plant seeds in a short time so it is more economical, does not require a large area, can be carried out throughout the year without depending on the season, and the seeds produced are healthier (Yusnita, 2015). The success in in vitro propagation is determined by many factors including types of explants and growth regulators (Swamy et al. 2010; Hua et al. 2014; Norrizah et al. 2012).These growth regulating substances play a role in stimulating and increasing the growth and development of cells, tissues, and plant organs in the direction of certain differentiations. The use of growth regulating substances at the right concentration can stimulate explant growth, especially the formation of roots, shoots, and callus. Plant growth regulators play an important role in controlling biological processes in plant tissues (Davies, 1995; Gaba, 2005). Its role, among others, regulates the growth rate of each network and integrates these parts in order to produce the form we know as plants. The activity of growth regulators in growth depends on species, chemical structure, concentration, plant genotypes and plant physiology phases (Satyavathi et al., 2004; George, 1993; Dodds and Roberts, 1982). In the process of forming organs such as shoots or roots there is an interaction between exogenous growth regulators added to the media with endogenous growth regulators produced by plant tissue (Winata, 1987). Addition of auxin in a larger and more stable amount tends to cause callus growth from explants and inhibit the regeneration of plant shoots (Wetherell, 1982) while cytokinins and auxin combined with their main activity is to encourage cell division (Karjadi and Buchory, 2008). Picloram is one of the synthetic auxin which is widely used for callus induction (Aprisa, 2012). Picloram is more effective in increasing callus induction if compared to growth regulator 2.4 D (Chernova, et.al, 1975). Tu et al. (2001) added that picloram in low concentrations can stimulate RNA synthesis and DNA replication in controlling cell division and growth. while BAP is synthetic cytokinin which has a higher activity compared to natural cytokines (Santoso and Nursandi, 2003). Cytokinin (BAP) which is balanced with auxin (Picloram) can cause callus growth (Abidin, 1985). According to Litz and Gray, 1995 the use of a combination of cytokinin (BAP) and auxin (Picloram) will improve the induction process. Based on the exposure, the research on the use of various concentrations of picloram and cytokinin (BAP) for the best callus formation and can provide information about the announcement of the composition of picloram and cytokinin (BAP) which is most suitable for the formation of (*Pogostemon cablin Benth)*.

II. MATERIALS AND METHODS

This experiment was conducted in November 2018 until January 2019 at the Network Culture Laboratory, Faculty of Agriculture, Andalas University, Padang. The materials used were situ clone patchouli plants (Jorong Situak in Lembah Melintang District) (Appendix 2), Picloram, BAP (Benzyl Amino Purine), MS media (Murashige and Skoog), agar agar (7 g / L), fungicide (Dhitane M45) 2 g / L, bactericidal (Agrept20WP) 2 g / L, antibiotics (Streptomycin) 0.05 g / L, Tween 20, sterile distilled water, 70% alcohol and 96%, 3% sucrose, HCL 1 mol / L , 1 mol / L NaOH, pH meter, plastic, rubber band, plastic wrap, tissue, spritus, masking tape (clear tape), disinfectant (formalin), aluminum foil, micropipette tips,

pH paper, and label paper. The tools used in this experiment are Laminar Air Flow Cabinet (LAFC), autoclaves, analytical scales, hot plate magnetic stirers, ovens, scalpell blades, tweezers, erlenmeyer 1000 mL, 50 mL cup cups, culture bottles, bunsen, petridisk, measuring cups 10 mL, glass bottles, culture rack, micropipette, Color chart, handsprayer stationery, camera. This study used an experimental method with Completely Randomized Design (CRD) consisting of two factors. The first factor was the administration of Picloram concentration with 5 levels of treatment and the second factor was the administration of BAP concentrations with 5 levels of treatment. This experiment consisted of: This experiment consisted of Factor I: Picloram (A) Without Picloram (A1), Concentration of 1 mg / L (A2), Concentration of 2mg / L (A3), Concentration of 3 mg / L (A4), Concentration of 4mg / L (A5), Factor II: Concentration of BAP (S) Without BAP (S1), Concentration of 0.5 mg / L (S2), Concentration of 1 mg / L (S3), Concentration of 1.5 mg / L (S4) Concentration 2 mg / L (S5). Each treatment was repeated 3 times so that there were 75 bottles of explants. The data obtained were analyzed using the F test at the level of 5%, if F count is greater than F table then the analysis is continued with the DMNRT test at the level of 5%.

III. RESULTS AND DISCUSSIONS

1. Appearing Callus

This research was carried out by giving auxin group Picatinam ZPT and cytokinin group namely BAP with 25 combinations of treatments using shoot explants, with explants position after cutting, the position of the leaf bone facing upward and the remaining part of the leaf slightly removed to accelerate callus formation process. Callus is a cell that has not been differentiated formed from slices of explants (Hendaryono daan Wijayani, 1994). Table 1 shows the diversity of patchouli plant callus time can be seen from the average callus emergence on the combination treatment of picloram concentration without BAP concentration and the treatment of picloram concentration with a concentration of BAP 2 mg / l that is at 7 HST, followed by concentration treatment picloram + BAP 1 mg / l, treatment of picloram + BAP concentration of 1.5 mg / l while for treatment 2 mg / l Picloram + BAP concentration does not occur in callus formation. According to Gunawan (1987) the contrast of different ZPTs gave a different response to callus induction. Slow and fast formation or formation of callus is influenced by endogenous hormones, it is thought that the patchouli plant of endogenous BAP hormone can not meet the needs of the explants so that the callus formed only at the high BAP. Callus growth is influenced by several factors related to explants such as the availability of energy sources, the environment and Growth Regulatory Substances, especially the balance between cytokinin and auxin hormones in tissue culture (sumardi, 1996). In line with research by Wattimena et al. (1992) in the in vitro structure, morphogenesis of explants always depends on the interaction between auxin and cytokinin given and contained in explants strengthened by the study of Wulandari et al. (2004) that the concentration of auxin an cytokinin often controls the amount of growth and form of culture , both in callus growth and organogenesis. Callus growth in explants increases when leaf bones containing file / tissue transporter, this is due to the transport network can be more nutrient when compared to leaf leaves that do not have a transport network (Nurwahyuni) in Intias (2012). Callus growth is influenced by several factors related to explants such as the availability of energy sources, the environment and growth regulators, especially the balance between cytokinins and auxin in tissue culture (Sumardi, 1996). Wattimena et al. (1992) in in vitro culture, morphogenesis of explants always depends on the interactions between auxin and cytokinin given and those contained in explants. The concentrations of these two ZPTs often control the shape and amount of growth of a culture, both in callus growth and organogenesis (Wulandari et al., 2004). Callus can emerge from the former part of the explan at the time of cutting the segment, which is also a process of opening, as in the study of Marlin et al. (2012) that callus formation is caused by stimulation of injured explant tissue to cover existing wounds, resulting from the formation of callus causes the cell wall to change direction, where some protoplasts flow outward. In line with the research of George and Sherrington (1984), which states that cell division that leads to the formation of callus occurs from the response to injury and the supply of natural or artificial hormones from the outside into the explants. Can be seen in figure 2 below: After the explants experience changes by sensitizing the observations then the callus begins to appear, the callus does not appear throughout all parts of the plant. in line with the research conducted by Gunawan (1988), that cell division does not occur in all cells in the original tissue, but only cells that actively divide continuously. The initiation of cell division is only limited to the outer layer of tissue can be caused by higher oxygen availability, CO2 gas release, more nutrient availability, phenolic inhibitors that evaporate faster, and light.

2. Percentage (%) of life explants

Percentage (%) of living explants is a condition in which explants planted in the media grow. According to Fauzan et al. (2004) eksplan life is characterized by fresh

explants, brightly colored and not experiencing browning or decay. All treatment studies of the percentage (%) of life explants in patchouli plants can be seen in table 2 as follows: Table 2. Shows the percentage (%) of live explants that of the 25 combinations of picloram and BAP treatments in patchouli plants there are 22 live explant treatments. Treatment Concentration of 1 mg / l picloram + concentration of 0.5 mg / l and 1 mg / l BAP, and concentration of 0.5 mg / l picloram + 1 mg / l BAP produced explants growing 100% meaning that there was an effect between the treatment of picloram and BAP concentrations to the percentage (%) of explant growth. At a concentration of 2 mg / l picloram + BAP concentration there is no explant that grows, meaning that there is no effect on patchouli explant growth in line with the statement of Hendaryono and Wijayani (1994), namely at high levels, auxin is more inhibiting than stimulating growth. Picloram is not a growth regulator for explants but rather becomes a herbicide. This is in accordance with the opinion of Tu, et. Al. (2001) that picloram with high concentration can inhibit cell division and cell growth. From the table above, it can be seen that the combination of 2 mg / l picloram and BAP concentration has not been able to increase the growth power of patchouli explants.

3. Callus Texture

Callus texture is a marker used to assess the quality of a callus. Callus texture can be divided into three types, namely compact (non friable), intermediate and crumb (friable) (Andrayani, 2010). Callus is good for use as a secondary metabolite-producing material that has a compact (non friable) texture. Compact callus texture is considered good because it can accumulate more secondary metabolites (Indah and Dini, 2013). Callus which has a compact texture generally has small cell size with solid cytoplasm, large nucleus, and has a lot of wheat starch (carbohydrate) according to Dodd (1993) in Ariati (2012). Andri (2012) states that compact callus has a nodule-like structure. Nodules are proembryonic and can be used as an inoculum for induction as a somatic embryo. According to Sitorus, (2011) crumb callus is a callus that grows apart into parts that are small, easily separated, and contain lots of water. Callus formation is influenced by certain substances in the medium such as growth regulators. 2.4 D concentration and high yeast extract will produce friable textured callus (Rahayu et al., 2003). The results of observations about callus texture in this study can be seen in table 3 below: Can be seen from the table of observations of the callus texture of each treatment on patchouli explant growth has a compact texture and crumbs. In line with the study of Pieril (1987) stated that the texture of the callus, which is compact to weaker, depends on the type of plant used, the composition of nutrient media, regulating growth temperature and environmental conditions of the culture. In the study conducted by Lim et.al (2009), the combination treatment of growth regulator 3 mg / l picloram and various cytokinin concentrations on Ocimum sanctum leaves produced compact texture callus. This can occur because the administration of high picloram concentrations can produce crumb callus. In line with the statement of Chernova et al. (1975) picloram has almost the same properties as 2.4 D. Rahayu (2003) states that a high concentration of 2.4 D will produce friable textured callus. Below this is the callus texture image after being identified in the observation of 3MST.

IV. FIGURES AND TABLES

Table.1: When Appears Callus of Patchouli Plants at Various Treatments (HST)

Treatment		BAP concentration (mg/l)					
		0	0,5	1	1,5	2	Average
Picloram concentration (mg/l)	0	7	10	7	11	7	8.4
	0,5	7	9	9	7	7	7.8
	1	7	8	12	10	7	8.8
	1,5	7	10	7	7	7	7.6
	2	0	0	0	0	0	0

The numbers in the column above are not significantly different according to the F test at the level of 5%

Table.2: Percentage (%) of explants of patchouli plant life at various treatments

Treatment		BAP concentration (mg/l)				
		0	0,5	1	1,5	2
Picloram concentration (mg/l)	0	66.67	66.67	66.67	66.67	33.33
	0,5	33.33	33.33	100.00	33.33	33.33
	1	-	100.00	100.00	66.67	33.33
	1,5	-	66.67	66.67	33.33	100.00
	2	-	-	0	0	0

The numbers in the column above are not significantly different according to the F test at the level of 5%

Table.3: Results of Observation of Callus Texture at 4 MST at Various Treatments

Sample Code	Picloram and BAP combination	Forming Callus (%)
A_1S_1	0 mg/l PIC + 0 mg/l BAP	-

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A_1S_2	0 mg/l PIC + 0.5 mg/l BAP	-	A_3S_3	1 mg/l PIC + 1 mg/l BAP	-
A_1S_3	0 mg/l PIC + 1 mg/l BAP	Crumb	A_3S_4	1 mg/l PIC + 1.5 mg/l BAP	-
A_1S_4	0 mg/l PIC + 1.5 mg/l BAP	Crumb	A_3S_5	1 mg/l PIC + 2 mg/l BAP	Compact
A_1S_5	0 mg/l PIC + 2 mg/l BAP	Compact	A_4S_1	1.5 mg/l PIC + 0 mg/l BAP	Compact
A_2S_1	0.5 mg/l PIC + 0 mg/l BAP	Compact	A_4S_2	1.5 mg/l PIC + 0.5 mg/l BAP	Crumb
A_2S_2	0.5 mg/l PIC + 0.5 mg/l BAP	Crumb	A_4S_3	1.5 mg/l PIC + 1 mg/l BAP	Crumb
A_2S_3	0.5 mg/l PIC + 1 mg/l BAP	-	A_4S_4	1.5 mg/l PIC + 1.5 mg/l BAP	-
A_2S_4	0.5 mg/l PIC + 1.5 mg/l BAP	-	A_4S_5	1.5 mg/l PIC + 2 mg/l BAP	-
A_2S_5	0.5 mg/l PIC + 2 mg/l BAP	-	A_5S_1	2 mg/l PIC + 0 mg/l BAP	-
A_3S_1	1 mg/l PIC + 0 mg/l BAP	Compact	A_5S_2	2 mg/l PIC + 0.5 mg/l BAP	Compact
A_3S_2	1 mg/l PIC + 0.5 mg/l BAP	-			

Figure 1: Changes in explants into callus

a b

Figure 1. Changes in explants into callus (a), Callus Nilam with the treatment of 1 mg / L 2 mg/l PIC + 0.5 mg/l BAP; (b), Callus Nilam with the Treatment of A3S5 1 mg/l PIC + 2 mg/l BAP

V. CONCLUSION

Based on the results of research on it can be concluded that: The concentration of picloram has a significant effect on the growth of patchouli callus with an average callus appearance of 7 HST and percentage (%) of explants growing to 100%. High picloram concentration did not significantly affect callus growth, 2 mg / l picloram + BAP concentration did not produce callus. Suggestions that can be conveyed related to this research are that further research is needed and with a longer observation time, so that patchouli callus growth can be more clearly seen.

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Effect of Poultry Manure and N.P.K 15: 15: 15 Fertilizer on the Growth and Yield of Nsukka Yellow Pepper (*Capsicum annum*)

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***Abstract*—** *A field experiment to evaluate the effect of poultry manure and N.P.K 15:15:15 fertilizer on the growth and yield of Nsukka yellow pepper (Capsicum annum) was conducted in the faculty of Agriculture and Natural Resources Management Teaching and Research Farm Enugu State University of Science and Technology Enugu during the 2018 cropping season. The experiment was carried out using a randomized complete block design (RCBD) with four (4) treatments replicated five (5) times. The result of the experiments showed significant treatment effect (P = 0.05) on the following agronomic parameters measured; percentage (%) plant survival, leaf area index, plant height, number of leaves per plant, number of days to 50% flowering, number of fruits per plant and fruit yield (kg/ha). Poultry manure and N.P.K 15:15:15 fertilizer combination performed significantly (P = 0.05) better than poultry manure, N.P.K 15:15:15 fertilizer and no application of the treatments on the number of leaves per plant, number of fruits per plant and fruit yield (kg/ha). There was neither significant interaction effect (P = 0.05) of poultry manure + N.P.K 15:15:15 fertilizer nor significant main effect (P = 0.05) of poultry manure and N.P.K 15:15:15 fertilizer on leaf area index.*

Keywords*— *Nsukka yellow pepper (Capsicum annum), poultry manure, N.P.K 15:15:15: fertilizer, interaction effect, main effect.

I. INTRODUCTION

Capsicum species popularly known as pepper is the world's second most important vegetable crop after tomato (Yoon *et. al.* 1989). In contrast, (Uzo, 1983) stated that pepper occupies the third position of importance among cultivated vegetable after onions and tomatoes. Bosland (1994) stated that genus *Capsicum* belongs to the family solanaceae. Uguru and Obieri (2008) stated that Nsukka yellow pepper belongs to the family of solanaceae and the genus *Capsicum*. This crop is grown for its fruits. It is an indispensable commodity and an integral component of many cuisines in the world due to its appealing flavour, taste and pungency (Bosland and Votava 2000). Five species of pepper were domesticated; *Capsicum annum, Capsicum frutescens, Capsicum baccatum, Capsicum Chinese* and *Capsicum pubscens*. Among these species, *Capsicum annum* is the most widely spread and most important. The plant is a herbaceous annual growing from 45 cm - 65 cm tall. One to three (1-3) fruits do occur in the axils of one leaf. It is also characterized by its yellow colour at fruit ripening and a unique aroma which distinguish it from other pepper varieties.

Capsicum species is grown in most countries of the world such as China, Turkey, Mexico, Spain and U.S.A, among which Nigeria is ranked third producer of pepper in the world (Uguru and Obieri 2008). Pepper is an indigenous vegetable of Nigeria and as such, its production is an important component of both subsistence and commercial farming system generally practiced in Nigeria especially Enugu State (Tanko 1995).

Nigeria is one of the most important countries in the world for pepper genetic resources since it accounts for 50% out of million tones believed to be produced in Africa, following the world estimated area of 1.6 million hectares with China being the largest producer and 1 million tones believed to be produced in Africa (FAOSTAT 2013). Consumption of pepper accounts for about 20% of the average vegetable consumption per person per day in Nigeria (Alegbejo, 2002). Nsukka yellow pepper is a vegetable fruit consumed either fresh or dehydrated/dried. It is very nutritious and has medicinal value and is a recognized source of vitamin A, C and E. In addition, it is a source of antioxidant, nutrients, as well as bioactive compound such as flavonoids, phenolic acid, carotenoids and also rich in natural colour and aroma. The key bioactive compounds in peppers such as flavonoids, capsaicinoides and capsicinoids have been linked to biochemical and pharmacological effect including anti-oxidation and anti-inflammation activities. Capsaicinoids provide the

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pungent sensation in hot pepper, whereas capsicinoids are non pungent compounds present in sweet peppers.

Capsicinoids have been reported to have anti-inflammatory activities as well as to promote energy consumption, suppress fat accumulation and increase body temperature in humans. The activities of capsicinoids and their lack of pungency, make them attractive for potential application in food and pharmacology. Other major bio-active compounds of pepper includes ascorbic acid, carotenoids and other anti-oxidants. The culinary properties and biological effect of bioactive compounds make them extremely important not only for nutrition, but also as pharmacological substance that are used in prevention of cardiovascular disease, cancers and cataracts. In addition, it is used for preservation of cowpea and other grains against weevils attacks.

Generally, pepper production has not attracted the same research patronage like other crops such as cassava, cowpea, cocoa, and Rice especially in Nigeria (Awoke and Okorji 2004). As a result, a few or no research has been conducted to find out why Nsukka yellow pepper is not grown in many parts of Nigeria especially in areas with low soil fertility. As a result of this, I decided to initiate a research work that aimed at evaluating the effect of poultry manure and N.P.K 15:15:15 fertilizer on the growth and yield of Nsukka yellow pepper (*Capsicum annum*) in Enugu area of south eastern Nigeria.

II. MATERIALS AND METHODS

Field experiment to evaluate the effect of poultry manure and N.P.K 15:15:15 fertilizer on the growth and yield of Nsukka yellow pepper (*Capsicum annum*) was carried out during the 2018 cropping season at the Faculty of Agriculture and Natural Resources Management Teaching and Research Farm of Enugu State University of Science and Technology Enugu, Southeastern Nigeria. The University lies between latitude 06^0 $50^/$ N – 06^0 $57^/$ N and longitude 07^0 $15^/$ E – 07^0 $18^/$ E with a mean elevation of 450 m above sea level.

Experiment Design

The experiment was carried out using a randomized complete block design (RCBD) with four (4) treatments replicated five (5) times. The experimental area measured 14 m x 11 m (154 m^2). Each plot (experimental unit) measured 2 m x 2 m (4 m^2), separated by 1m pathway, with plant spacing of 50 cm x 50 cm. the pepper seedlings were raised in a nursery before they were transplanted into the field.

Treatments

Treatments were; (i) No poultry manure and no N.P.K 15:15:15 fertilizer. (ii) Poultry manure (6.4 tons/ha) (iii) N.P.K 15:15:15 fertilizer (200 kg/ha) (iv) Poultry manure (3.2 ton/ha) + N.P.K 15:15:15 fertilizer (100 kg/ha).

Data Collection

Data were collected on percentage (%) plant survival, plant height (cm), number of leaves per plant, leaf area index, number of days to 50% flowering, number of fruits per plant and fruit yield (kg/ha).

Data Analysis

The data collected were subjected to analysis of variance for randomized complete block design (RCBD) experiment as outlined by Obi 2002 using Genstat Release 10.3DE (PC. windows) 2012 software. Differences between treatment means were detected using fisher's least significant difference (F – LSD) as outlined by Steel and Torrie (1980).

III. RESULTS

Effect of poultry manure and N. P.K15:15:15 fertilizer on percentage (%) plant survival, plant height (cm) and leaf area index.

The result of the experiment showed significant effect (P = 0.05) of poultry manure and N.P.K 15:15:15 fertilizer on percentage (%) plant survival and plant height (cm), and non-significant effect (P = 0.05) on leaf area index. Poultry manure recorded the highest mean number of 92.50% survived plants, followed by poultry manure + N.P.K 15:15:15 fertilizer that had mean number of 90.00% survived plants and lastly, no poultry manure and no N.P.K 15:15:5 fertilizer that had mean number of 67.50% survived plants. On plant height plots treated with N.P.K 15:15:15 fertilizer recorded the highest mean plant height of 42.70 cm, followed by plots treated with poultry manure + N.P.K 15:15:15 fertilizer that recorded mean plant height of 38.13 cm and lastly plots treated with no poultry manure and no N.P.K 15:15:15 fertilizer that had mean plant height of 29.83 cm. Although there was non-significant effect of the treatments on leaf area index, poultry manure recorded the highest mean leaf area index of 3.66 followed by poultry manure + N.P.K 15:15:15 fertilizer that recorded mean leaf area index of 3.29 and lastly no poultry manure and no N.P.K 15:15:15 fertilizer treatment that had mean leaf area index of 1.73 (Table1).

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Table.1: Effect of poultry manure and N.P.K 15:15:15 fertilizer on plant survival (%), plant height (cm) and leaf area index.

Treatments	Plant survival (%)	Plant height (cm)	Leaf area index
None	67.50	29.83	1.73
Poultry manure	92.50	35.50	3.66
N.P.K 15:15:15 Fertilizer	76.30	42.70	1.73
Poultry manure + N.P.K 15:15:15 fertilizer	90.00	38.13	3.29
F-LSD$_{0.05}$	12.20	9.26	NS

Effect of poultry manure and N.P.K 15:15:15 fertilizer on the number of leaves per plant and number of days to 50% flowering.

Statistical analysis of the experiment showed a significant effect (P = 0.05) of poultry manure and N. P.K15:15 15 fertilizer on the number of leaves per plant and also number of days to 50% flowering.

Plots treated with poultry manure + N.P.K 15:15:15 fertilizer recorded the highest number of 296.0 leaves per plant followed by plots treated with poultry manure that recorded 183.4 leaves per plant and lastly plots treated with no poultry manure and no N.P.K 15:15:15 fertilizer that had a record of 38.00 leaves per plant.

The result also showed that plots treated with no poultry manure and no fertilizer recorded the highest number of 76. 50 days to 50% flowering, followed by plots treated with N.P.K 15:15:15 fertilizers that had a record of 61.60 days to 50% flowering and lastly plots treated with poultry manure that recorded 35.00 days to 50% flowering (Table 2).

Table.2: Effect of poultry manure and N.P.K 15:15:15 fertilizer on the number of leaves per plant and number of days to 50% flowering.

Treatments	Number of leaves per plant	Number of days to 50% flowering
None	38.00	76.50
Poultry manure	183.40	35.00
N.P.K 15:15:5 fertilizer	84.20	61.60
Poultry manure + N.P.K 15:15:15 fertilizer	296.00	35.20
F-LSD$_{0.05}$	85.40	13.12

Effect of poultry manure and N.P.K 15:15:15 fertilizer on the number of fruits per plant and fruit yield (kg/ha).

The result of the experiment showed significant effect (P = 0.05) of poultry manure and N.P.K 15.15.15 fertilizer on the number of fruits per plant and fruit yield

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(kg/ha) of Nsukka yellow pepper (*Capsicum annum*). Poultry manure + N.P.K. 15:15:15 fertilizer recorded the highest number of 26.0 fruits per plant followed by poultry manure that recorded 4.0 fruits per plant. Again poultry manure + N.P.K 15:15:15: fertilizer had the highest fruit yield of 4768.75 kg/ha, followed by poultry manure that recorded fruit yield of 2106.25 kg/ha and lastly, no poultry manure and no N.P.K 15:15:15 fertilizer that had fruit yield of 81.25 kg/ha (Table 3).

Table.3: Effect of poultry manure and N. P.K15:15 15 fertilizer on the number of fruits per plant and fruit yiel ded (kg/ha).

Treatments	Number of fruits per plant	Fruit yield (kg/ha)
None	4.0	81.25
Poultry manure	14.0	2106.25
N.P.K 15:15:15 fertilizer	11.0	581.25
Poultry manure + N.P.K.15:15:15 fertilizer	26.0	4768.75
F-LSD$_{0.05}$	9.8	79.25

IV. DISCUSSION AND RECOMMENDATION

The result of the experiment showed that plots treated with poultry manure recorded the highest plant survival which was not significantly different from that of poultry manure + N.P.K 15:15:15 fertilizer (Table 1). Again, on fruit yield (kg/ha), plots treated with poultry manure + N.P.K 15:15:15 fertilizer recorded the highest fruit yield of 4768.75 kg/ha which was significantly different from that of poultry manure that recorded fruit yield of 2106.25 kg/ha (Table 3). Since plots treated with poultry manure + N.P.K 15:15:15 fertilizer produced fruit yield that was significantly higher than that of poultry manure, and also produced percentage plant survival that was not significantly different from that of the poultry manure, Nsukka yellow pepper producer/farmers in Enugu area should combine poultry manure and N.P.K 15:15:15 fertilizer in order to maximize yield and also reduce crop loss in the farm.

On plant height, plots treated with N.P.K 15:15:15 fertilizer recorded the highest plant height of 42.70 cm, followed by plots treated with poultry manure + N.P.K 15:15:15 fertilizer that recorded plant height of 38.13 cm which was not significantly different from that of N.P.K 15:15:15 fertilizer. Therefore, Nsukka yellow pepper farmers within Enugu area, whose aim is to produce crop of high yielding and optimum plant height for easy harvest should apply a combination of poultry manure and N.P.K 15:15:15 fertilizer since the combination recorded a significant higher yield as earlier indicated (Table 3).

In areas where erosion of top soils by rain drops is a problem, Nsukka yellow pepper producers/farmers should apply a combination of poultry manure and N.P.K 15:15:15 fertilizer as this treatment recorded the highest leaf area index of 3.66 and also the highest fruit yield since leaf area index indicates the extent of land area coverage by plant leaves.

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Relationship between Fibre Characteristics and Physico-Mechanical Properties of *Aningeria robusta* (A.CHEV) Wood

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***Abstract*—** *Aningeria robusta wood is fast becoming popular in Nigeria, due to its excellent performance in structural applications especially in roofing, and in recent times, door frames and furniture. Thus, consideration must be given to its mechanical properties, and fibres in wood is known for providing mechanical support for the wood. Notwithstanding, mechanical failure of wood in service still occurs. To limit such failures assessment is needed to be done on some parameters associated to quality of wood. This study thus assess the relationship between fibre characteristics and physico-mechanical properties of Aningeria robusta wood. Three trees of Aningeria robusta were obtained wood samples of 20x20x300mm were collected to determine modulus of elasticity and modulus of rupture (mechanical property), 20X20X20mm for fibre characteristics, and 20x20x60mm for wood density and moisture content. The mean fibre length, fibre diameter, lumen, cell wall thickness (CWT), 1.55mm, 11.71µm, 8.78µm, 1.46µm, while the mean moisture content, density, modulus of rupture (MOR) and modulus of elasticity (MOE) were 55.54%, 429.34kg/m^3, 123.91N/mm^2, 5876.89N/mm^2 respectively. There existed a negatively significant correlation between fibre length and MOR, and well as CWT and moisture content. This existing relationship implies that the shorter the fibre length, the more the MOR. It can therefore be concluded that existing relationship between fibre characteristics and physico-mechanical properties taken cognisance of where mechanical failure of wood is to be limited.*

***Keywords*— A.robusta wood, fibre, mechanical, physical, relationship.**

I. INTRODUCTION

The wood of *Aningeria robusta* is fast becoming popular due to its excellent performance in structural applications especially in roofing, and in recent times, door frames and furniture. *Aningeria robusta* belongs to the family Sapotaceae, a hardwood. It is referred to as 'agengre' in Cote d'Ivoire, 'landosan' in Nigeria and 'osan' in Uganda (TRADA 1979 cited in Ajala and Ogunsanwo, 2011), 'mukali' in Angola, 'mukangu' in Kenya (Chudnoff 1980 cited in Ajala and Ogunsanwo, 2011) and 'asafonia' in Ghana (Okai 2003).

Wood fibre are usually a thread like cellulosic elements that are extracted from trees and used to make materials including paper, paperboard, tissue, cardboard etc. However, its main function in a tree is to provide mechanical support. Sizes of fibre cell wall range from thin-walled to thick-walled fibre depending on the wood species.

Physical properties are the quantitative characteristics of wood and its behavior to external influences other than applied forces. Density and moisture content are some of the relevant physical properties of wood that are relevant to wood structural performance. Familiarity with physical properties is important because they can significantly influence the performance and strength of wood used in structural applications (Winandy, 1994). Mechanical properties are the characteristics of a material in response to externally applied forces. They include elastic properties such as modulus of elasticity, which relate the resistance of a material to deformation under an applied stress to the ability of the material to regain its original dimensions when the stress is removed, and modulus of rupture, which measures wood strength before rupture when load is applied. Mechanical property values are given in terms of stress (force per unit area) and strain (deformation resulting from the applied stress.

Also, Samuel 2004 stated that mechanical properties of wood are its fitness and ability to resist applied or external forces. This means that mechanical properties

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determines to a large extent the use of wood for structural and building purposes.

There has been records of wood failure when in use, and these failures may be attributed to poor mechanical factors. Samuel 2004 reported that a buckling and bending failure will occur where the fibre walls of a wood show gradations of thickness i.e. transition from the thin-walled cells of the early wood to the thick-walled cells of the late wood is gradual. Also that moisture in wood decreases the stiffness of the fibre walls and enlarges the region of failure. It can therefore be stated that the manner of failure depends partly upon the anatomical structure and partly upon the degree of humidity of the wood, such that the fibres (tracheids in conifers) act as hollow tubes bound closely together, and can buckle or bend when force is applied.

Therefore, to limit the failure of wood in service, it is imperative to assess the wood quality parameters. This involves the consideration of anatomical such as fibre characteristics, physical and mechanical properties of wood. Thus, the objective of this study is to investigate the relationship between fibre characteristics and physico-mechanical properties of *Aningeria robusta* (A.chev) wood.

II. MATERIALS AND METHOD

Three trees of *Aningeria robusta* with at least 25cm DBH were obtained from Onigambari Forest Reserve. From each tree, bolts were collected at the base, middle and top portion, and the wood samples was processed using circular machine and planning machine to a dimension of 20x20x300mm for modulus of elasticity and modulus of rupture (mechanical property), 20X20X20mm for fibre characteristics, 20x20x60mm for wood density and moisture content according to ASTM, 1991.

2.1 Determination of Fibre Characteristics

For the fibre dimensions determination, small slivers were obtained each at the different sampling height. The slivers were placed in an equal volume (1:1) of 30% hydrogen peroxide and 10% glacial acetic acid, boiled until soft and bleached white (ASTM D 1413-61 2007). The slivers were then washed, placed in 30 mL-test tubes with 20 mL-distilled water and shaken vigorously to separate the fibre bundles into individual fibre. The macerated fibre suspension was carefully aligned on a slide using a rubber teat. The resulting image on Rheichert visopan microscope screen was measured for fibre length, diameter, lumen width and cell wall thickness was calculated with relevant formular.

2.2 Determination of Wood Density

The 20x20x60mm wood samples collected were oven dried to a constant weight at 103°C ± 2 for 24 hours and the weight afterward was recorded. The volume of samples at green weight was recorded and the following formula was adopted for the calculation of wood density.

$$D = \frac{m}{v}(kg/m^3) \qquad (1)$$

D = Density
m = oven-dried mass
v = green volume

2.3 Determination of Moisture Content

The samples were weighed when wet (original weight), it was then dried to a constant weight at 103°c ± 2 in an oven for 24 hours, after which it was re-weighed. The loss of weight of the wood samples on drying to a constant weight was noted. Calculation of the loss in weight as a percentage of the samples weight after drying was done by using the formula below

$$MC = \frac{ww - ow}{ow} \times 100 \qquad (2)$$

MC = Moisture Content
ww = wet weight
ow = oven dry weight

2.4 Determination of Modulus of Rupture (MOR) and Modulus of Elasticity (MOE)

This involved the use of standard test specimen (20 x 20 x 300mm), in a universal testing machine. The test sample was prepared in such a way that growth rings will be made parallel to one edge. Load was applied at the rate of 0.2mm/sec, with the growth rings parallel to the direction of loading, that is, specimen was loaded on the radial face. From the graph generated by the universal testing machine, the peak and breaking force were recorded; hence MOR and MOE was calculated as thus;

$$MOR = \frac{3PL}{2bd^2}(N/mm^2) \qquad (3)$$

$$MOE = \frac{PL^3}{4\Delta bd^3}(N/mm^2) \qquad (4)$$

Where:

P = load in Newton (N)

L = span / length (mm)

B = width (mm)

D = depth (mm)

Δ = the displacement at beam centre at proportional load

III. RESULTS AND DISCUSSION

Table 1 shows the axial variation in fibre characteristics of *A.robusta* wood. Mean for Fibre length, fibre diameter, lumen diameter and cell wall thickness were 1.55mm, 11.71µm, 8.78µm, 1.46µm respectively. Meanwhile, Table 2 shows the selected physical and mechanical properties tested for the wood sample, while Table 3 shows the correlation analysis between these properties.

Anon, (1984) stated that a mean fibre length of 1.6mm and above are classified as long fibre. Therefore, fibre length of *A.robusta* was considered short. As reported by Kpikpi (1992), some Nigerian hardwood species also had value within this value. Although the fibre length of A.robusta was short, yet it compared favourably with some Nigerian wood species. Meanwhile, the observed fibre diameter for *A.robusta* was lower to other selected wood species considered with it for comparism. Ogunleye *et al.,* (2017) recorded 41.5µm for *Ricinodendron heudelotii* wood, 30.67µm was recorded for *G. arborea* (Roger *et al.,* 2007) while 36.09 and 34.25µm for *R. racemosa* and *R. harrisonii*, respectively (Emerhi 2012), and 20.3µm for *T. scleroxylon* (Ogunsanwo 2000). Similarly, lumen diameter and cell wall thickness of *A.robusta* wood had values which compared lower to the selected wood species afore-mentioned.

Wood density obtained in this study was similar to Chudnoff (1980) (400–480kg/m^3), Ajala and Ogunsanwo (2011) (430 kg/m3) for *A. robusta* but slightly lower to Arowosoge *et al.,* (2008) (510kg/m^3), and Okai (2003) (500 kg/m^3) for the same species. Age and location could have contributed to these differences (Arowosoge *et al.,* 2008). Furthermore, the mean MOE value obtained in the course of this study is slightly lower (5876.89N/mm2) to what was obtained by Ajala and Ogunsanwo (2011); a reason for this may be an effect of wood maturity, and/or inconsideration of radial pattern.

Existing relationship between fibre characteristics and selected physico-mechanical properties revealed that fibre length had a negatively significant correlation with wood density and MOR alone. Similarly, cell wall thickness had a negatively significant correlation with moisture contents only. This implies that the shorter the fibre length of *A.robusta* wood, the more its wood density and MOR. Also that, a thinner cell wall will means more moisture content.

This relationship is tenable because shorter fibres could mean larger percentage of fibre, which in turn equate to higher MOR. Uetimane and Ali (2011) supports this tendency in their work which showed a significant positive relationship in fibre length and MOR for sapwood. One of the parameters that determines high density is the amount of mass of a substance. Then, higher fibre percentage may also be responsible for higher density. Thus, supporting the relationship observed in this study. However, Emmanuel (2014) recorded no significant correlation between any of the fibre characteristics and physico-mechanical properties of selected wood species but recorded a significant difference among wood species sample. The resulting no significant correlation could have been caused by a significant difference in wood species samples tested. Thus, this study suggests that determining correlation of wood properties of more than one species should be done independently, as a significant difference among species may alter correlation analysis.

Table.1: Axial variation in fibre characteristics of Aningeria robusta wood

Sampling Height	TREE	FL(mm)	FD(µm)	LD(µm)	CWT(µm)
TOP	1	1.73±0.06	11.83±0.22	10.13±0.29	0.85±0.07
	2	1.37±0.03	10.67±0.37	8.16±0.49	1.26±0.08
	3	1.51±0.03	11.48±0.24	10.45±0.25	0.52±0.05
MEAN		**1.54±0.10**	**11.33±0.34**	**9.58±0.71**	**0.88±0.21**
MIDDLE	1	2.04±0.49	12.21±0.41	8.43±0.52	1.89±0.12
	2	1.34±0.03	10.99±0.33	6.67±0.40	2.16±0.26
	3	1.52±0.05	12.43±0.58	9.60±0.61	1.42±0.28
MEAN		**1.63±0.21**	**11.88±0.45**	**8.23±0.85**	**1.82±0.22**

BASE		1	1.44±0.16	12.64±0.63	9.12±0.62	1.76±0.02
		2	1.44±0.09	11.41±0.46	8.00±0.31	1.71±0.17
		3	1.54±0.06	11.73±0.31	8.59±0.30	1.57±0.13
	MEAN		**1.47±0.03**	**11.93±0.37**	**8.57±0.32**	**1.68±0.06**
	P. MEAN		**1.55±0.07**	**11.71±0.21**	**8.78±0.38**	**1.46±0.17**

FL – Fibre Length FD – Fibre Diameter LD – Lumen Diameter
CWT – Cell Wall Thickness P. mean – Pooled mean

Table.2: Mean values of physico-mechanical properties determined for A. robusta

	TREE 1	**TREE 2**	**TREE 3**	**MEAN**
MC (%)				
TOP	58.02	62.65	65.12	**61.93±2.08**
MIDDLE	38.79	48.44	57.65	**48.29±5.44**
BASE	65.88	48.49	54.77	**56.38±5.08**
MEAN	**54.23±8.04**	**53.19±4.72**	**59.18±3.08**	**55.54±2.98**
WOOD DENSITY (kg/m^3)				
TOP	380.42	407.5	434.50	**407.47±15.61**
MIDDLE	361.67	517.67	452.92	**444.08±45.24**
BASE	412.25	515.25	381.92	**436.472±40.35**
MEAN	**384.77±14.76**	**480.14±36.32**	**423.11±21.2**	**429.34±18.91**
MOR (N/mm^2)				
TOP	122.1	129.51	129.56	**127.06±2.47**
MIDDLE	95.04	116.33	145	**118.79±14.47**
BASE	116.73	136.87	124.08	**125.893±5.88**
MEAN	**111.29±8.27**	**127.57±6.0**	**132.88±6.26**	**123.91±4.74**
MOE (N/mm^2)				
TOP	5807.8	6435.92	6609.44	**6284.39±243.50**
MIDDLE	5754.87	5576.14	5660.49	**5663.83±51.62**
BASE	6862.52	3176.22	7008.62	**5682.45±1253.82**
MEAN	**6141.73±360.71**	**5062.76±975.37**	**6426.18±399.81**	**5876.89±382.82**

*MOR = Modulus of rupture, **MOE = modulus of elasticity

Table.3: Pearson correlation analysis between fibre characteristics and physico-mechanical properties

	Wood density	Moisture content	MOR	MOE
Fibre length	-0.66*	-0.54	-0.63*	0.03
Fibre diameter	-0.36	0.01	-0.16	0.17
Lumen width	-0.50	0.55	0.29	0.36
CWT***	0.30	-0.62*	-0.48	-0.29

IV. CONCLUSION AND RECOMMENDATION

This study was successful in establishing the relationship that existed between fibre characteristics and physico-mechanical properties of *A.robusta* wood, and as such this existing relationship should be taken into consideration where mechanical failure of wood is intended to be limited.

Thus, recommending more studies into the relationship of other anatomical and physico-mechanical properties that wasn't considered in the scope of this study.

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Waste Management Practices in Selected Poultry Farms and its Effect on the Environment and Human Health in Makurdi, Nigeria

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***Abstract*—** *The poultry industry produces large amounts of waste that include solid waste and wastewater. These waste if not properly disposed can be risky to the environment and humans through the spread of diseases and pollution of soil and groundwater. The investigation to determine poultry waste management practices and its effect on the environment and human health was carried out in Makurdi. Benue State. The study was conducted from October to December 2017. A structured questionnaire was administered to 20 randomly selected poultry farmers and 20 neighbours in the study area by one on one interview and observation of the environment. Data collected from the questionnaires were analysed using descriptive statistics. The major poultry waste generated by the farms was faecal waste (50%) with about 50% of the respondents getting 25 bags or more of waste after clearance of poultry houses. There was no significant difference (P=0.056) between the flock size and the number of bags gotten from clearance of the litter. About (40%) of the farms sell their poultry waste immediately after clearing their farms while 30% applied theirs directly to nearby farm lands. The challenges in disposing of poultry waste were shortage of labour (45%) and lack of dumping sites (25%). The major complaint about poultry farms by neighbours living around the farms was bad odour (60%). It was observed that improper handling of poultry production waste from start to finish could lead to environmental and health hazards with 60% of the respondents reported being sick with signs of respiratory problems and 40% had digestive problems. Eighty-five (85%) percent of the farmers indicated that improper disposal of litter causes surface water contamination while all (100%) the farmers agreed that poultry waste causes air pollution. It was concluded that waste management by poultry farmers in the study area was inefficient. There is a need for further studies to assess the magnitude of the risk posed by poor poultry waste management practices to the environment, livestock and human health.*

Keywords* — *Disposal, poultry waste, litter, poultry farms, Makurdi.

I. INTRODUCTION

Worldwide, the poultry industry is growing rapidly and contributes towards addressing key national development goals, as well as improving the standard of living of people through poverty alleviation and creating employment opportunities(Agblevor*et al*., 2010). Poultry farming is one of the largest and fast growing agricultural businesses worldwide, this is due to its economic and health benefits(Adeoye*et al*., 2004). There is high demand for poultry products in form of meat and eggs which makes poultry business to be lucrative with high source of income(Akilu, 2008).Waste is defined as anything that is no longer useful and needs to be disposed of. The poultry industry produces large amounts of waste that include solid waste and wastewater. The solid waste consists of bedding material, excreta (manure), feed, feathers, hatchery waste, shells, sludge, abattoir waste (offals, blood, feathers and condemned carcasses) and mortality(Moreki and Chiripasi, 2011). The wastewater results from washing and disinfection of chicken houses and abattoirs(Moreki and Chiripasi, 2011). Reports from Dong and Tollner(2003) stated that poultry densities on farms continue to increase and have caused manure related problems which are water, air and land pollution. Livestock manure can be either a valuable resource or an environmental pollutant. Generally, manure refers to faeces and urine produced by animals, and it contains organic matter and nutrients, that has fertilizer value when applied on the land and used by crops. The proper handling and management of manure can augment or replace purchased commercial fertilizers(Tao and Mancl, 2008). On the other hand, poultry litter is a mixture of poultry droppings and bedding materials, such as wood shavings and rice or peanut hulls). In Nigeria, like any developing nation, there is a rapid expansion of small and medium scale poultry farms with the attendant effect of

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huge waste generation. The magnitude of this generated poultry waste has given rise to improper disposal which include over application to land, improper timing of application thereby creating pollution problem to soil water and air environment(Adewumi*et al*., 2011).There are several ways of disposing poultry waste which include burial, rendering, incineration, compositing, feed for livestock, fertilizer or source of energy.(Moreki and Kealkitse, 2013). Other waste disposal methods include conversion of poultry waste to energy and use of poultry waste for treatment of heavy metal contaminated water(Moreki and Chiripasi, 2011). Modern management methods for poultry waste like re-feeding to animals, green disposal, gasification and biogas production have not gained prominence in Nigeria probably due to level of awareness, lack of strict regulation from government in respect of poultry waste disposal and care-free attitude of the farm owners(Adeoye, *et al*., 2004). It is still a common site in Nigeria to see huge deposit of poultry waste around the farm, flushing of the waste into water courses through open canals from farms are also common sites (Ojolo*et al*., 2007). These methods are not only unsightly, it also creates a lot of environmental nuisance and surface and groundwater pollution(Akinbile, 2012). This work was conducted to identify the waste management methods used in poultry farms in Makurdi metropolis, Benue State, the problems encountered during waste disposal and its associated effects on the environment and human health.

II. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Makurdi Local Government Area of Benue State. Makurdi is the capital of Benue state, Nigeria. The city is located in North Central Nigeria along the Benue river and lies on latitude 07°43'50 North and Longitude 08°32'10 East. The climate of the state is characterized by an average annual minimum and maximum temperature of 22.43°C and 33.41°C respectively and the annual rainfall ranges from 1270-1397 mm(Olaniran and Sumner, 2006). The local government has 11 council wards with an estimated population of 500,797 people(NPC, 2010).

2.2. Methods of data collection

For this study, twenty (20) registered poultry farms and 20 neighbours living around the poultry houses were randomly selected.A structured questionnaire was designed to collect information from poultry farmers about their manure/litter management procedures and neighbours about the effect of poultry waste on the environment and human health. The questionnaire was designed to adequately capture relevant information such as type of management system, kind of disposal methods used to remove manure/litter, constraints or major challenges of waste disposal in poultry farms, symptoms of sickness experienced by neighbours etc. Some data were collected through one on one oral interview with the farm owners and household heads using the questionnaire. This study was carried out during the dry season from October to December 2017. Forty (40) questionnaires were administered, 20 to poultry farms and 20 to neighbours in North Bank 1, North Bank 2, Agan, Wadata, Modern Market, Fiidii and Waimalayo council wards of Makurdi, Benue State.

2.3. Data analysis

The data obtained from the questionnaires were analyzed using descriptive statistics using SPSS (version 20). The frequencies, percentages and chi square values where calculated. Results were presented in charts and tables. Value of $P \leq 0.05$ was considered significant.

III. RESULTS AND DISCUSSION

The results from this study showed that most poultry farmers preferred to rear broilers(50%) (Table 1). Majority of them had broilers in their farms at the time of sampling. This might be due to the fact that broiler production is more profitable than layer production in this part of the country (Laseinde*et al*., 2005). Also, due to the high temperature in the study area, most poultry farmers would prefer keeping birds for a short period of time before selling(Amos, 2006).Most (80%) of the poultry farms visited kept birds in deep litter system and all (100%) of the poultry houses had concrete floors. The use of the deep litter system by most poultry farmers in Makurdi may be attributed to its cost effectiveness in terms of construction and thereby making it easier to manage(Musa andSaidu, 2012). Concrete floors are damp proof thereby making it easier to manage litter(Moore *et al*., 2004).About 15% of the poultry farmers used saw dust as their bedding material, 40% used wood shavings while 5% do not use any bedding materials (Table 1).The type of bedding material used by farmers depends on locality and availability. The fact that wood shavings and saw dust was mostly used by the poultry farmers in the study area showed that it is relatively cheap and readily available(Ekenma,2015).Some other workers reported that saw dust was the most popular litter material used in most nations for poultry production(Charles, 2005). Sixty-five (65%) of the farms sampled change their litter once a week, 10% change theirs every day and every month respectively with 15% changing theirs every 6 months (Table 1). Most (90%) of the respondents clear out all the litter and sweep the floor of the poultry house before replacing with new litter material while 10% remove only the topmost caked parts of the litter and replace with new one without

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sweeping the floors (Table 1).The type of bird also affects the frequency with which the litter materials are changed because daily production of litter by layers has been estimated to be higher than that of broilers(Tao andMancl, 2008). Also, the frequency in the change of poultry litter may also be due to poor quality structures, poor management practices and frequent weather variation(Sa'idu, *et al*., 2008). Most of the farms clear out all the litter and sweep the floor of the poultry houses i.e. total clean out before replacing with new litter, while others remove only the topmost caked parts of the litter without sweeping the floors. Only 10% of the farmers said they have heard of litter treatment before disposal while 90% have not heard of treatment of manure prior to disposal. This indicates that poultry waste treatment is not a familiar practice in Makurdi. Ninety (90%) percent of the farmers reported they got no visits from the state sanitation bodies while 10% acknowledged visitation from sanitation bodies which was sometimes once a year.The method of litter disposal by poultry farmers in the study area includes selling it off immediately to farmers (40%), storing and selling later (30%) and application as manure to nearby farm lands (30%). This is similar to the work done by Embury(2004) in Port Harcourt Nigeria where most of the poultry farmers sell off their bags of litter immediately after collection from the poultry houses with other farmers storing and applying to nearby farmlands. The majority of the farmers from these results have good market for their poultry waste hence a good disposal system because a good number of them sell off their poultry waste immediately. Whether poultry waste is sold off, stored or applied to farm land immediately, a greater portion of poultry waste end up in farm lands(Shah *et al*., 2009). All (100%) the farmers agreed that improper poultry waste disposal could be hazardous to human health with 60% of the respondents indicating respiratory problems as the major effect on human health while 40% stated digestive problems (Table 1).Symptoms of conjunctivitis and sneezing were reported by farmers and neighbours living around the poultry farms. This could be related to the ammonia produced from the poultry waste(Akanni and Benson, 2014).

Table.1: The frequency of the different variables used in the study.

Variables	Number of farms	Percentage (%)
Type of birds		
Layers	1	5.0
Broilers	10	50.0
Layers and broilers	9	45
Total	20	100.0
Management system		
Battery cage	1	5.0
Deep litter	16	80.0
Battery cage and deep litter	3	15.0
Total	20	100.0
Type of floor		
Concrete floor	20	100
Un cemented floor	0	0
Total	20	100
Type of litter		
Saw dust	3	15.0
Wood shaving	8	40.0
Both	8	40.0
Others	1	5.0
Total	20	100.0
Method of litter removal		
Clear all and sweep floor before replacing	18	90.0
Remove only topmost litter	2	10.0
Total	20	100.0
Frequency of litter change		
Every day	2	10.0

Once a week	13	65.0
Every month	2	10.0
Every 6 months	3	15.0
Total	20	100.0
Poultry waste disposal method		
Sell off immediately	8	40.0
Store and sell later	6	30.0
Apply to farmland	6	30.0
Total	20	100.0
Effects of poultry waste on human health		
Respiratory problems	12	60.0
Digestive problems	8	40.0
Total	**20**	**100.0**

The flock size of the farms ranged from 100 – 5000 birds (Table 2). Result showed that most of the poultry waste generated by these farms was faecal waste, others include staled eggs, dead birds and feathers (Table 3).

Table.2: Flock size of selected poultry farms in Makurdi metropolis, Benue State.

Flock size	Number of farms	Percentage (%)
100-500	4	20.0
500-1000	10	50.0
1000-1500	1	5.0
1500-2000	2	10.0
2000-5000	3	15.0
Total	**20**	**100.0**

Table.3: Type of poultry waste generated by farms in Makurdi metropolis, Benue State

Poultry waste	Number of farms	Percentage (%)
Faecal waste	10	50.0
Staled egg	3	15.0
Dead birds	3	15.0
Feathers	2	10.0
Others	2	10.0
Total	**20**	**100.0**

The quantity of waste generated by the farms showed that 90% of the farmers generate more than 400kg of poultry waste each time they cleared out their poultry farms, 5% get 200-400kg and another 5% get less than 200kg. About 50% of the respondents get 25 or more bags of waste after clearance, 20% get between 11-15 bags (Table 4). There was no association between the flock size and the number of bags gotten from clearance of the litter (P=0.056).The number of bags of litter gotten by individual farmers in this study depended on several factors such as type of feed, quantity of feeds consumed by birds, type of birds, feed conversion rate, stocking density and other managerial factors(Ekenma,2015).Also, the frequency at which the poultry houses are cleaned can affect the numbers of bags gotten from each clearance (Agblevor*et al*., 2010). The longer the clearance interval, the higher the quantity of poultry wastes gotten. According to FAO(2012),it is necessary a poultry farmer knows the quantity of waste produced by his birds because it helps in planning or designing an effective waste management program.

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Table.4: Number of bags after clearance of poultry houses in Makurdi, Benue State

Number of bag	Number of farms	Percentage (%)
1-5	2	10.0
6-10	3	15.0
11-15	4	20.0
16-20	1	5.0
21 and above	10	50.0
Total	**20**	**100.0**

The major complaint by the neighbours living around the poultry farms was the odour (60%) that emanates from the poultry houses while 25% complained about the noise from the birds (Table 5). Most of the respondents (45%) reported shortage of labour as their major constraint of waste disposal with 25% complaining of no dumping site, 20% reported no market to sell and 10% complained about transportation cost (table 6). There was a significant level of association between waste disposal methods and the constraints of waste disposal in the farms sampled (P=0.000).

Table.5: Complaints of neighbours about poultry farms in Makurdi, Beune State

Complaints	Number of farms	Percentage (%)
Foul odour	12	60
Noise from birds	5	25
Pollution of nearby waters	3	15
Total	**20**	**100.0**

Table.6: Constraints of poultry waste disposal by poultry farms in Makurdi, Benue State

Constraints	Number of farms	Percentage (%)
No dumping site	5	25.0
No market to sell manure	4	20.0
Transportation cost	2	10.0
Shortage of labour	9	45.0
Total	**20**	**100.0**

Poultry facilities are a source of odour and attract flies, rodents and other pests that create local nuisances and carry diseases(Moore,*et al*., 2004). Odour emissions from poultry farms adversely affect the life of people living in the vicinity and from this study, residents complained of the bad odour coming from the farms. This has led to most of the residents complaining and confronting the poultry farmers. Odour associated with poultry operations comes from fresh and decomposing waste products such as feaces, carcasses, feathers and bedding/ litter(Ferke*et al*., 2002; Kolominskas*et al*., 2002).

Results showed that most of the farmers were aware of the effect of improper poultry waste disposal on human health and the environment. Reports from other researchers (Akanni and Benson, 2014, Moore *et al*., 2004) also revealed that activities in livestock production facilities cause environmental problems such as odour nuisance and land pollution resulting from improperly discharged manure. Forty percent of the respondents indicated that creation of proper dumping sites was the best strategy for waste management while 35% were of the opinion to encourage farmers to use the waste material on farmland (Table 7). Eighty-five (85%) percent of the farmers also indicated that improper disposal of litter causes surface water contamination while all (100%) the farmers agreed that poultry waste causes air pollution.Also, 45% of the respondents knew that improper disposal of litter material had an effect on the soil (Table 8).

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Table.7: Strategies for proper waste management in Makurdi, Benue State

Strategies	Number of farms	Percentage (%)
Encourage famers to use poultry waste on farm land	7	35.0
Create proper dumping site	8	40.0
Educate farmers on other ways to utilize poultry waste	5	25.0
Total	**20**	**100.0**

Table.8: Knowledge and awareness of poultry farmers on the effect of poultry waste on the environment in Makurdi, Benue State (no. of farms =20)

Awareness		**Number of farms**	**Percentage (%)**
Contamination of ground water	Yes	11	55
	No	9	45
Contamination of surface water	Yes	17	85
	No	3	15
Air pollution	Yes	20	100
	No	0	0
Contamination of soil	Yes	9	45
	No	11	55

These results showed that excessive application of poultry manure on farm lands may lead to undesirable effects on forage crops and animals consuming the forage. This could also result to leaching of nitrate into ground water thereby causing water pollution(McGinley *et al*., 2003).

IV. CONCLUSION AND RECOMMENDATIONS

This study showed that there is poor management of poultry wastes in Markurdi metropolis, Nigeria. The disposal methods in practice may bear grave implications for the environment, livestock and public health. There should be strict enforcement by supervisory agencies of existing policies aimed at environmental sanitation and the protection of water quality. Alternative methods for the utilization of poultry wastes such as biogas production and conversion to inorganic manure using modern recycling facilities should be considered by stakeholders in the study area.

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In vitro Tissue Culture Technique as Tool for micro propagation of *Lepidium sativum* L. Plant under Abiotic Stress Conditions

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***Abstract*—** *This study was carried out at biology Lab, Biology Department, University College in ALKhafji, University of HAFR ALBATIN, to study the effect of MS salts strength, and sucrose concentration on micro propagation and phytochemical composition of Lepidium sativum Plant, using MS medium at five salt strength (1.0,1.5,2.0,2.5 and 3.0 mg\L) MS salt strength and sucrose at five concentration (30.0 g, 45.0 g, 60.0 g, 75.0 g and 90.0 g) \L. Ethanolic extracts and DPPH reagent were used to test the antioxidant activity.*

Lepidium sativum Plant used in folk medicine in Sudai Arabia, known as Garden cress belongs to Brassicaceae family. It is very famous in folk medicine. The tissue culture technique is a rapid method to propagate plants in vitro, especially medicinal plants for producing medicinal component. Through in vitro culture we can control the environmental conditions of plants; it enables us to study the effect of some factors like sucrose concentration and salt strength that determine growth of plants and chemical constituent. Impact of these stresses the plant micro propagation and the content of plants from active substances which plays a role as antioxidant was varied. For micro propagation, the effect of MS salt strength showed that, the use of MS media salt at double MS salt concentration (2.0 MS salt) obtained highest leaf number (8.11±1.3278), highest node number was (1.933±0.62098) and highest shoot length (4.608±0.5828cm) both were induced by basal MS (1.0 MS salt), while highest root length (3.787±0.3958) obtained at triple MS salt strength (3.0 MS salt (. Effect of sucrose concentration, MS media supplemented by 30 g sucrose obtained highest leaf number, highest shoot and root length (5.655±1.1076; 4.608 ±0.5828 and 2.985±0.3952) respectively. For antioxidant activity data represented that antioxidant activity increase with increasing salt strength and sucrose concentration. Our results suggested that increasing sucrose concentration decrease leaf number, shoot and root length, on the other hand increase antioxidant activity. While increasing MS salt strength decrease node number and shoots length, while root length and antioxidant activity was increase. The results contained in this research are the first study to Effect of some environmental stress on in vitro propagation and antioxidant activity of Lepidium sativum at Saudia Arabia..

Keywords*— *Antioxidant Activity, Phenolic contents, In vitro, Lepidium sativum, Micro propagation, MS media, Stress.

I. INTRODUCTION

Medicinal plants have been used from ancient time for their medicinal values. Nowadays, the crude extracts samples from medicinal plants have been shown interest for the development and preparation of alternative traditional medicine [1]. Plants are the best sources for chemical ingredients or phytochemical agents for cure of different diseases. Medicinal plants are an inexhaustible source of molecules with very different biological and pharmacological activities. *Lepidium sativum* is an edible plant. Seeds, leaves and roots of these plants have economic importance. It is widely used in folk medicine, known as garden cress, it is annual herb belonging to the Brassicaceae family, and it is widely cultivated in temperate climates throughout the world for different medicinal uses [2]. The plant is called “Hab el Rashaad” or “Thufa” in Saudi Arabia and is a popular herbal plant grown in many regions of Saudi Arabia, such as Hijaz, AL-Qaseem, and the Eastern Province [3 & 4]. Plant stress is a controversial environmental impediment affecting the plant growth and leading to an extensive loss in agricultural productivity. The environmental stress on plants can be biotic or abiotic. Biotic stress includes the wastage caused by various living organisms. Abiotic stress involves various environment factors including

aridity, salinity, heavy metal, high and low temperature stress that affect the plant growth and development, leading to decrease crop yield. *Lepidium sativum* Plant being grown in arid and semiarid regions is severely affected by both biotic stresses and abiotic stresses. Different agronomic practices, traditional breeding methods and biotechnological method were used for the management of different stresses and development of stress tolerance in several crops. However, all these methods were found to be unfavorable and less effective. Recently, tissue culture technique has proved to be more appropriate and cost- effective technique for the development of stress tolerance in plants. The technique carrying out under controlled environmental conditions with minimal time and space has very high potential for the development of various stress-tolerant crop plants. Therefore, in vitro selection technique provides new vista for improving stress tolerance in *Lepidium sativum* Plant for environmental sustainability.

Drought stress caused by water deficit, is probably the most impacting adverse condition and the most widely encountered by plants, not only in crop fields but also in wild environments. According to published statistics, the percentage of drought affected land area in the world in 2000 was double that of 1970 [5].

Another major environmental factor that limits crop productivity, mainly in arid and semiarid regions is high salinity. Approximately 19.5% of the irrigated soils in the world have elevated concentrations of salts either in the soil or in the irrigation water [6], damaging both the economy and the environment [7 & 8]. The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress), or a combination of these factors [9]. Abiotic stress leads to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity [10]. The resulting of these stresses induces production of reactive oxygen species, and accruement of hormones [11]. Secondary metabolites play a major role in the adaptation of plants to the environment and in overcoming stress conditions [12].

The similarities of the effects induced by the stress in the plant cultured in vitro and in vivo conditions suggest that the in vitro system can be used as an alternative to field evaluations for studying the general effect of water-stress on plant growth and development. The tissue culture technique is a rapid method to propagate plants in vitro, especially, medicinal plants. The present study is carried out at biology Lab, Biology Department, University College in ALKhafji, University of HAFR ALBATIN, to study the effect of MS salts strength, sucrose concentration on micro propagation and anti-oxidant activity of *Lepidium sativum* plant, Using MS medium at (1.0,1.5, 2.0,2.5 and 3.0) mg\L salt strength and (30.0,45.0,60.0,75.0 and 90.0)\g\L sucrose concentrations.

II. MATERIAL & METHOD

2.1. Plant Tissue Culture

2.1.1. Plant materials:

Lepidium sativum seeds were purchased from local market of khafji city, Saudi Arabia.

2.1.2. Surface sterilization of Explant:

Lepidium sativum seeds were surface sterilized for 30 sec with 70% ethanol followed by washing with sterilized distilled water, followed by sterilization by 5% chlorex supplemented by few drop of liquid soap for ten minute and washed thoroughly with autoclaved distilled water five times The sterilized seeds were dried with sterilized filter paper [13].

2.1.3. Establishment of Aseptic Seedlings

Media preparation: Murashige and Skoog [14] medium was used in the present study, sucrose (3%), and pH of the medium was adjusted to 5.8, then solidifying with 0.7% agar. The media was autoclaved at 121 ^{0}C and 15 lbs. pressure for 15 min

2.1.4. Inoculation of explants:

The seed inoculation was done with help of sterile forceps. After inoculation glass culture bottles were wrapped and incubate in growth room. Culture development and maintenance: Explants were maintained at temperature 25±2^{0}C, with 8 hours light and 16 hours dark condition Photoperiods, light intensity at 1000 to 2000 Lux. Data were reported after four week.

2.1.5. Effect of salt strength on Culture Medium on the plant micro propagation

Lepidium sativum sterilized seeds were inoculated on the MS basal medium with different salt strength (1.0, 1.5, 2.0, 2.5 and 3.0) salt strength per mg\L

2.1.6. Effect of Sucrose concentration on Culture Medium on the plant micro propagation

Lepidium sativum sterilized seeds were inoculated on the MS basal medium with different sucrose concentration, (30.0, 45.0, 60.0, 75.0 and 90.0) g\L.

2.2. Phytochemical Analysis

Explants were washed with distilled water to remove the trace of medium, used for extract preparation.

2.2.1. Extracts:

Whole plant Samples from each treatment were soaked separately with ethyl alcohol 70% in conical flasks for 24 h at 40^{o}C on water bath. After 24 hours it was filtered with Whatman No.1 filter paper. The filtrates were evaporated with rotary evaporator apparatus to obtained extraction for each sample. The extracts were

stored in sample bottles at 4ºC prior to use for further analyses.

2.2.2. Determination of Total Phenolic Content (TPC):

The total phenolic content for all plant samples was determined by using Folin- Ciocalteu method [15].

2.2.3. Antioxidant Scavenging Activity (DPPH) Assay:

The effect of methanolic extracts on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals was estimated according to [16].

2.3. Statistical analysis of the data

All experiments were replicated ten times having 10 explants per culture bottle and the experiment was repeated twice. Statistical analysis was carried using a statistical software program. Data were reported as means ± standard error

III. RESULTS AND DISCUSSIONS

Table (1) indicated that the different MS salt strength used effect on *Lepidium sativum* plant micro propagation, the effect of MS salt strength showed that, the use of MS media salt at double MS salt concentration (2.0 MS salt) obtained highest leaf number (8.11±1.3278), highest node number was (1.933±0.62098) and highest shoot length (4.608±0.5828cm) both were induced by basal MS (1.0 MS salt), while highest root length (3.787±0.3958) obtained at triple MS salt strength (3.0 MS salt) . MS supplemented by different sucrose concentration effect on *Lepidium sativum* plant micro propagation, MS media supplemented by 30 g sucrose obtained highest leaf number, highest shoot and root length (5.655±1.1076; 4.608 ±0.5828 and 2.985±0.3952) respectively, this result supported by [17] who reported sucrose at 30g/l and 45g/l concentration gave significantly higher mean number of Shoots and root of banana plant, while highest node number (1.949) was
Induced by 75g\L sucrose.
Polyphenol synthesis and accumulation in plants is generally stimulated in response of abiotic or biotic strsess [18].
Methanol extracts of studied samples were examined for their total phenolic content and antioxidant activities using a DPPH assay and expressed as IC_{50} value.

Table.1: Effect of MS Medium Salt Strength and Sucrose Concentration on Micro Propagation of Lepidium sativum plant after four weeks of culture.

Salt strength on Culture Medium (mg\l)					
MS salt Strength (mg\l)	MS Basal	MS1.5	MS2	MS2.5	MS3
TPC %	12.42	12.44	12.84	13.08	13.74
IC_{50} µgm/ml	181.33	212.27	245.51	257.46	276.12
Sucrose concentration on Culture Medium (g\l)					
MS sucrose concentration per (g\l)	MS Basal	45g	60g	75g	90g
TPC %	12.42	12.56	12.85	12.97	13.23
IC_{50} µgm/ml	181.33	203.94	232.11	249.90	253.16

Data in Table (2) showed that, total phenolic compounded (TPC) was increase with salt and sugar concentration increasing, which highest values were in MS 3.0 salt strength and 90 g\l sucrose and the lowest one was in MS as basal. The highest *total phenolic content values (13.74) and* (13.23*), also* antiradical assets as IC_{50} highest values (276.12) µgm/ml and (253.16) µgm/ml were determined for the plants culture on MS media contained 3.0 MS salt strength (mg\l) strength and sucrose at concentration 90 g\l (S3) respectively. Higher anti-oxidant activity of the extracts of the samples parallels to a high content of phenol in them, these result in agreement with [19]. Salinity causes hyperosmotic and hypertonic stress in plants, for the protection against oxidative stress, caused by salinity, antioxidants are produced. Some researchers have also reported a high positive correlation between free radical-scavenging activity and the total concentration of phenolic compounds in plant extracts [20]. The polyphenolic compounds include flavonoids and tannins and other active materials are reported to scavenge free radicals, superoxide and hydroxyl radical by single electron [21].

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Table.2: Effect of MS medium salt strength and Sucrose concentration on The Total Phenolic Content and Antioxidant Activities of Lepidium sativum plant grown in vitro.

MS salt strength mg\L	*Average*	*SE*	*Sucrose concentratio*	*Average*	*SE*
Leave					
MS	5.655	1.107653	S 30	5.655	1.107653
1.5 MS	6.991	0.530828	S45	3.423	0.242606
2 MS	8.11	1.327791	S60	3.4085	0.19038
2.5MS	3.623	0.407423	S75	4.223	0.564856
3MS	4.101	0.125516	S90	2.245	0.103099
Node					
MS	1.933	0.620989	S 30	1.933	0.620989
1.5 MS	1.141	0.102268	S45	1.67	0.126588
2 MS	1.672	0.34587	S60	1.3152	0.133498
2.5MS	1.768	0.345057	S75	1.949	0.238702
3MS	1.025	0.016816	S90	1.017	0.017
Stem					
MS	4.608	0.582826	S 30	4.608	0.582826
1.5 MS	4.383	0.441852	S45	3.761	0.263166
2 MS	3.893	0.408085	S60	2.457	0.303813
2.5MS	2.216	0.303107	S75	3.536	0.596462
3MS	3.65	0.109067	S90	0.674	0.073684
Root					
1.0 MS	2.985	0.395239	S 30	2.985	0.395239
1.5 MS	2.547	0.206393	S45	1.977	0.149064
2.0 MS	2.172	0.283176	S60	1.7815	0.167886
2.5 MS	0.979	0.05986	S75	2.253	0.200793
3.0 MS	3.787	0.395865	S90	0.9	0.238607

IC_{50}= Median Inhibition Concentration (concentration that reduces the effect by 50%)

IV. CONCLUSION

Increase salt strength and sucrose concentration resulted in osmotic stress and induces the production of antioxidant metabolites. The results showed that MS salt strength played a significant role of micro propagation, phenols accumulation and antioxidant activity of *Lepidium sativum plant* the current study show that the strength of MS salts and sucrose concentration can be used to induce antioxidant metabolites of *Lepidium sativum plant*. The data contribute for furthermore extensive studies of the biologically active compounds of in vitro cultures of this valuable plant depending on the nourishing elements

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Stress Response at different Ages of Weaning in Cattle

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***Abstract*—** *Weaning is an inherent husbandry practice in cow-calf beef production systems that impose physical, psychological, and nutritional stressors on calves. In order to characterize the main stressors and corresponding stress responses of calves, two weaning schemes were studied. One of conventional weaning (6 month calves) and another of early weaning (1 month calves). Blood samples were analyzed in an hematology analyzer, the plasma was separated and an analysis of total protein and of cortisol levels was carried. Finally, the response to a vaccine was assessed by analyzing antibody response titers. A significant decrease in the number of lymphocytes and increase in the number of granulocytes was observed in the conventionally weaned group leading to an elevated GR:LY ratio. On day one post-weaning, plasmatic proteins showed a significant increase of similar range in both groups. With regard to cortisol levels, there was a strong increase at day one corresponding to a clear stress response in the conventional weaning group, wheras in the group of early weaning animals only a marginal increase which difficult returned to baseline levels could be observed. As to the antibody response to vaccination, early weaning animals did not show a satisfactory response as was observed in calves subjected to conventional weaning. In conclusion, leukocyte subsets, plasmatic proteins, cortisol levels, and immune status were altered after weaning challenge in both studied age groups of calves. Results indicate weaning as a stressor, regardless of age.*

Keywords*— *early weaning, cattle, cortisol, immune response, stress response.

I. INTRODUCTION

Weaning is an inherent husbandry practice in cow-calf beef production systems that imposes physical, psychological, and nutritional stressors on calves. Weaning involves separation of the calf from its dam, resulting in the split of the maternal-offspring bond and removal of milk from its diet. Thus, the weaning procedure can be an acute stressful event in young calves (O'Loughlin, McGee, Waters, Doyle, & Earley, 2011). In beef herds, calves remain with their cows until they are weaned, which traditionally occurs at six to seven month of age. Time of weaning has an impact on cow and calf performance as well as health and productivity of the native range or pasture. In an intense production system, the negative pressure exerted on animal comfort is potentially higher. To apply the concept of animal welfare, these interventions involve situations that are associated with different stress levels that possibly include different negative effects. With the emergence of early weaning (EW) and hyper-early weaning (HEW) systems, it is important to evaluate how much stress is experienced by calves and their mothers by these production systems. Both practices are used in a large commercial scale in Argentina and other Mercosur countries since the early 90's (Vittone, MORANO, SILVEIRA, & Romera, 2012). In Argentina, two systems have been implemented for weaning, EW involves the

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removal of the calf from the cow at 60 to 70 days of age while in HEW the calf is weaned at 30 to 45 days of age (Gonzalez et al., 2013). Early weaning is a management practice that reduces grazing pressure on pastures by decreasing the nutrient requirements of cows. In addition, it can result in improved conception rates, provided the calves are weaned during the breeding season. Calves that are weaned early are relatively efficient at converting feed to gain, and can weigh as much, or more, than calves allowed to remain with their dams until a conventional weaning age is reached (Rasby, 2007). The practice of EW tends to bring animal production to one of its most clear objectives, which is to produce a calf per cow per year.

The goal of low-stress weaning techniques is to minimize negative stress caused by nutritional, environmental, physical, and social changes experienced by calves through weaning (WSU, 2013)., Calves respond to abrupt weaning with increased vocalizations, agitation, walking activity, and reduced feed intake and rumination (Haley, Bailey, & Stookey, 2005; Veissier, Le Neindre, & Trillat, 1989). Furthermore, they show alterations in hormonal mediators of stress (Blanco, Casasús, & Palacio, 2009) and immune function (Arthington, Eichert, Kunkle, & Martin, 2003; Hickey, Drennan, & Earley, 2003) resulting in a suppressed immune system and an enhanced disease susceptibility (Lambertz, Farke-Röver, Moors, & Gauly, 2015). Accordingly, EW will be more successful and less stressful when adequate attention to nutrition, health, management, and facilities are considered.

Stress alters the internal animal homeostasis inducing changes in the hypothalamic-pituitary adrenocortical (HPA) axis and the sympathoadreno-medullary system. The endocrine activation promotes the release of cortisol as the principal stress marker hormone and several other hormones. Although different authors have used several blood constituents to determine stress, cortisol, despite its variability and short life, is still one the most used indicators of stress (Romero Peñuela, Uribe-Velásquez, & Sánchez Valencia, 2011). The interaction between the immune system and stress is complex; it is an important subject of investigation since it is vitally important for both understanding the biology of animals as well as for the study of many diseases and imbalances. Young animals without a developed adaptive immune system rely on the HPA axis, which serves as the primary regulator and modulator of immunity.

Previous studies have evaluated biochemical, immunological, and behavioral stress markers in the weaning period (Enríquez, Hötzel, & Ungerfeld, 2011; Hulbert & Moisá, 2016; Lambertz et al., 2015; O'Loughlin et al., 2011). There are also studies that evaluate the stress response in early weaning designs (Coppo, 2007; Rasby, 2007). Our objective was to analyze and compare biochemical and immunological markers in a conventional and an early weaning system that are both used in animal husbandry in order to characterize the main stressors and stress responses. The identification of biomarkers of stress may allow to improve management in order to maximize production, meat quality, and animal welfare.

II. MATERIALS AND METHODS

Experiments were conducted in Concepción del Uruguay INTA's Experimental Station, Province of Entre Rios, Argentina. Hereford and Polled Hereford cows and their respective calves raised at the Experimental Station were used. Cows were vaccinated 30 days antepartum and calves at weaning and 21 days post-weaning using a commercial vaccine (Biopoligen HS, Biogénesis-Bagó) containing inactivated IBR (BoHV1 and BoHV5), Bovine Viral Diarrhea Virus (BVDV1 and BVDV2), Para-Influenza Virus (PI3), *Haemophilus somnus*, *Pasteurella multocida*, and *Mannheimia haemolytica* (*Pasteurella haemolytica*).

Experimental Design

At weaning, calves were housed in four corrals, each covering a 50 m^2 area equipped with waterers and feed bunks. Calves were allotted to two experimental groups according to age and weight. One groups representing Early Weaning calves (EW: 56.2±6.84 days old; weight: 64.2±9.93 kg) and another group was composed of Conventional Weaning calves (CW: 218.2±11.4 days old; weight: 135.1±25.44). Each group was placed in two pens (5 animals/corral). One corral from each group was immunized with 2 dosis (at weaning and 21 days post-weaning) of a commercial vaccine. The animals from the other corral of each group remained unvaccinated.

Calves were weaned following the management requirements for EW and CW according to the Manual of early weaning in beef herds (Galli, Monje, Vittone, Sampedro, & Busto, 2005). All calves were offered ad libitum a calf ruminal starter (Ruter®, Asociación de Cooperativas Argentinas) and water.

Sample Collection

Peripheral blood samples were taken at different time points with respect to weaning at -7, 0 (weaning), 1, 7, 14, 21, 35, and 42 days via jugular venipuncture using sodium citrate 3.8 % as anticoagulant. Samples collected were analyzed in the hematology analyzer Nihon Kohden Celltac alpha MEK-6450 equipped with software for bovine blood. Then,

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after centrifugation at 1000 g for 30 minutes, the plasma was collected and stored at -20°C until further use.

Cortisol levels

Plasmatic cortisol concentration was determined by high performance liquid chromatography (HPLC). Cortisol was extracted from plasma by adding diethyl ether:dicloromethane (60:40) and the organic phase was evaporated. As an internal standard, Dexamethasone was added to each tube. Samples were resuspended with methanol, vortexed, and injected into the HPLC system. The guard column Venusil XBP C18 (particle size 3 µm, 150 x 4.6 mm) was equilibrated using HPLC-grade acetonitrile-water (40:60 v/v). The mobile phase was a mixture of distilled water:methanol (45:55) and the flow rate was set to 1 ml/min.

A series of standards (rat normal plasma with cortisol, SIGMA 31719) covering the range of 5-500 ng/ml were used. The concentration was determined from a calibration curve, made on the basis of the ratio of cortisol vs. dexamethasone peak-height.

Plasmatic total proteins

The total protein concentration was measured using the method of Lowry (Lowry, Rosebrough, Farr, & Randall, 1951). A standard curve was estimated based on a series of dilutions of bovine serum albumin (BSA) (0, 0.10, 0.25, 0.50, 1.00, 2.00 mg/ml) that were in duplicate. Samples were diluted so that they would fall within the BSA standard range. Biuret reagent was added to each tube and mixed thoroughly. The mixture was then allowed to incubate at room temperature for 15 minutes prior to the addition of Folin & Ciocalteu's reagent. The absorbance was measured at 650 nm and blanked on water as a control.

Detection of Antibodies (Ab) against BoHV1

Total Ab in bovine serum were determined using an indirect ELISA as described previously (S. Romera et al., 2014; S. A. Romera et al., 2000). Briefly, polystyrene microtiter ELISA plates (Immulon 1B, Dynatech Laboratories) were coated with 50 µl of positive (concentrated and semi-purified BoHV1 reference strain Los Angeles containing 109 DICT50mL-1) or negative antigen (non-infected MDBK cells) in carbonate buffer of pH 9.6 and incubated for 12 h at 4°C. Samples were tested at a dilution of 1:40 in three serial four-fold dilutions. A peroxidase-labeled affinity purified goat anti-bovine IgG (Kirkegaard and Perry Laboratories, KPL) diluted 1:2000 was used as conjugate. After each incubation, plates were washed four times with PBS-T. The reaction was developed using hydrogen peroxide/2,2B-azino-bis 3-ethylbenzthiazoline- 6 Sulfonic Acid (ABTS) in citrate buffer,(pH 5) as the substrate/chromogen system. The Ab titer of each sample was expressed as the $\log_{10}$ of the reciprocal of the serum dilution. A serum was considered positive when after correction for optical densitiy its value was above the cut off value of the assay.

Statistical Analysis

All data are expressed as mean ± standard error of the mean (S.E.M.). Differences among groups were evaluated by two-way ANOVA under a model of repeated measures throughout time. All data were checked for adherence to normal distributions. The effects of treatment, sampling time and the possible interactions were listed in the model statement. When the interaction was significant, simple effects were performed. When there was no interaction, the post hoc Tukey test was performed.

The Infostat statistical program was used (InfoStat Group, National University of Córdoba; http://www.infostat.com.ar/). Values of $p < 0.05$ = *, $p < 0.01$ = **, and $p < 0.001$ = *** were considered significant.

III. RESULTS

Cortisol

Weaning as a stressor can be identified by the observation of a strong stress response as indicated by the large increase of plasmatic cortisol on day one. It can be seen in Fig. 1 that the level of cortisol (ng/ml) is largely increased on day one both in conventional ($p<0.001$) and early weaning ($p<0.001$), but with differences in the type of response. In early-weaned calves, a peak characteristic for an acute stress response is not seen, but a downward curve most characteristic for chronic stress is observed. The acute stress response in the conventional weaning group is clearly visible, whereas in early-weaning animals it is hardly seen.

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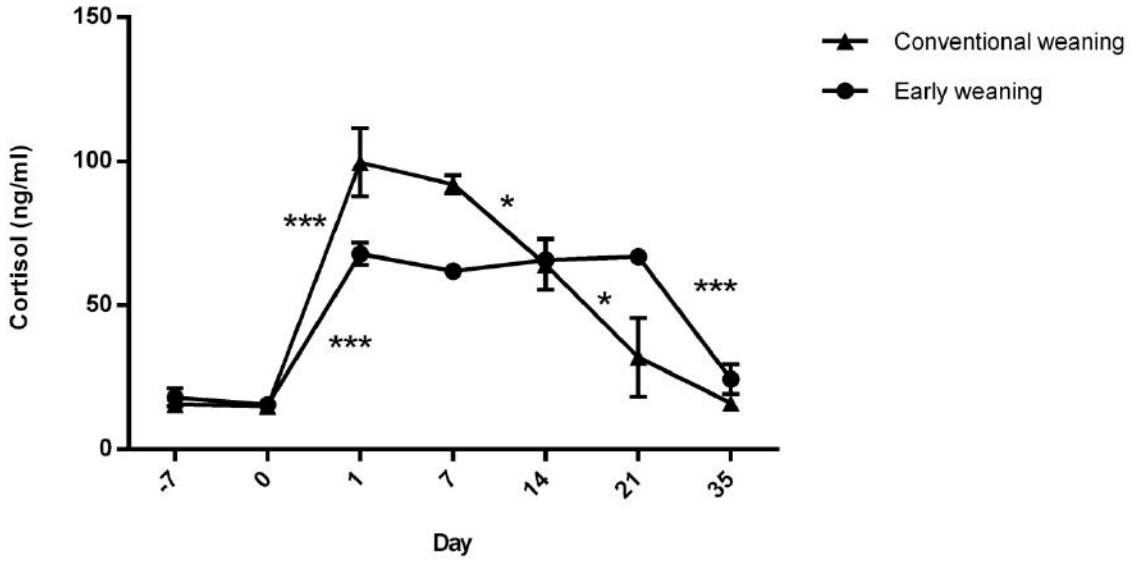

*Fig.1: Determination of plasmatic levels of cortisol by HPLC (ng/ml) (n=6). All data are expressed as mean ± standard error of the mean (S.E.M.); two-way ANOVA under a model of repeated measures; * p<0,05; ** p<0,01; *** p<0,001*

Total proteins

We noted in conventional and early weaning generated a response with a significant increase of plasmatic protein levels at day 1 ($p<0.01$) followed by a decrease at day 7 ($p<0.01$) and a return to the baseline at day 21 ($p<0.01$) (Fig. 2). The baseline ranges of protein levels observed comply with reference values (Rivas, Rossini, & Salvador, 2006).

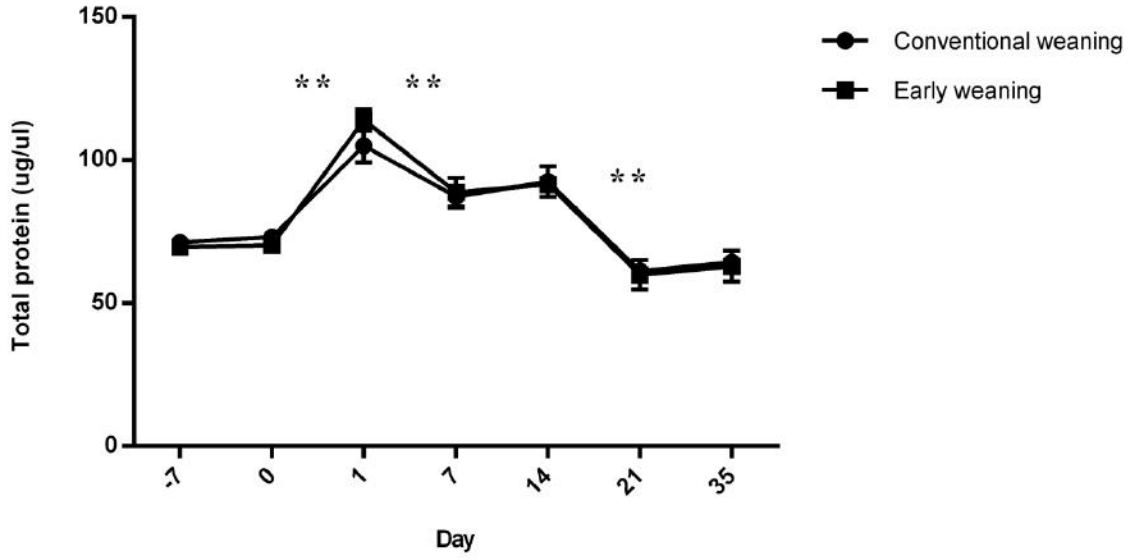

*Fig.2: Determination of total plasmatic proteins (µg/µl) by Lowry method (n=10). All data are expressed as mean ± standard error of the mean (S.E.M.); two-way ANOVA under a model of repeated measures; * p<0,05; ** p<0,01; *** p<0,001.*

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Hematologic parameters

Table.1: Effect of weaning on total lymphocyte and granulocyte number

	Granulocyte number (cel/ul)		Lymphocyte number (cel/ul)	
	CW	**EW**	**CW**	**EW**
Day				
-7	2,2 [a]	2,3 [a]	4,3 [a]	3,4 [a]
0	2,6 [a]	2,6 [a]	4,2 [a]	4,2 [a]
1	6,4*[,b]	3 [a]	1,7 *[,b]	3,7 [a]
7	6*[,b]	2,4 [a]	3,6 [a]	4,1 [a]
14	5,8*[,b]	3 [a]	3,25 [a]	3 [a]
21	6,1*[,b]	1,9 [a]	4,5 [a]	3,8 [a]

Two-way ANOVA under a model of repeated measures throughout time
*p≤0.05 group factor. a, b: time factor

Following weaning, in conventional weaned calves the lymphocytes number decreased from the baseline level at day one ($p < 0.05$, Table 1), whereas the granulocytes increased ($p < 0.05$, Table 1). Importantly, granulocytes remained elevated throughout the study while lymphocytes returned to the baseline by day 7. Thus, the post-weaning GR:LY ratio increased ($p < 0.01$, Fig. 3) and did not return to the pre-weaning ratio level during the evaluation period. Early weaning had no effect ($p > 0.05$) on any measured parameter and accordingly, the number of cells corresponding to reference baseline values were observed (Table 1).

No effect ($p > 0.05$) of weaning on the number of red blood cells, hemoglobin concentration, hematocrit percentage, mean corpuscular hemoglobin concentration, and platelet number was seen as all parameters stayed within reference values (data not shown).

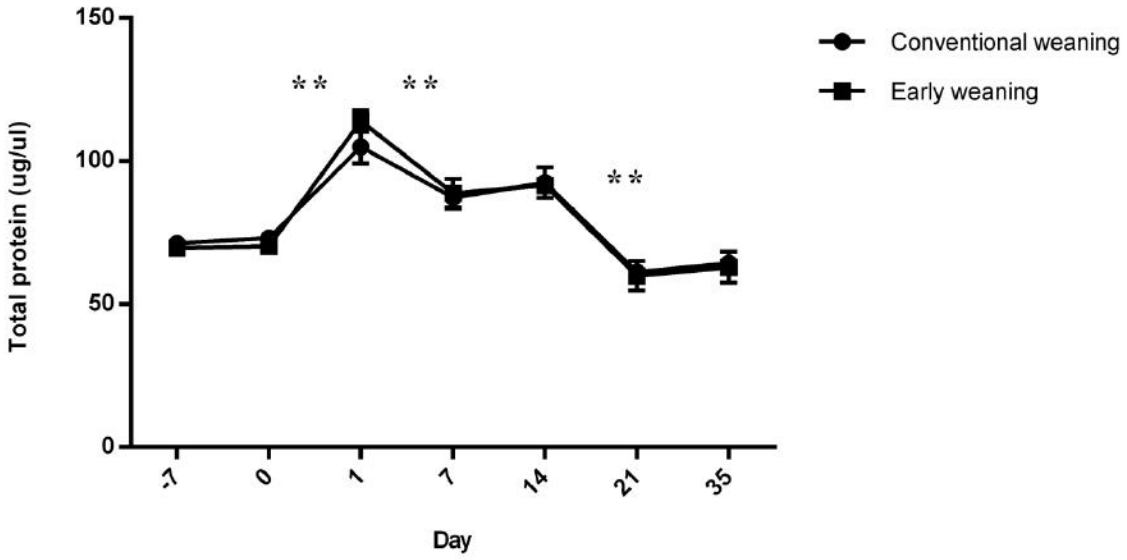

*Fig.3: Effect of weaning on granulocyte: lymphocyte (GR:LY) ratio (n=10); two-way ANOVA under a model of repeated measures; * p<0,05; ** p<0,01*

Total immune response to anti-BoHV1 vaccine

All animals of the conventional weaning group responded successfully to vaccination (5 of 5). In contrast, of the early weaning group only two animals showed a response. In conventional weaned animals antibody titers ranged between 1.6 to 2.8. The maximum titer was seen on day 35

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and lasted until the end of the evaluation period. In the early weaning group, only two vaccinated animals showed a humoral response with lower antibody titers (2 of 5) though the observed range of titers between the two animals was almost the same as in the group subjected to conventional weaning (Fig. 4).

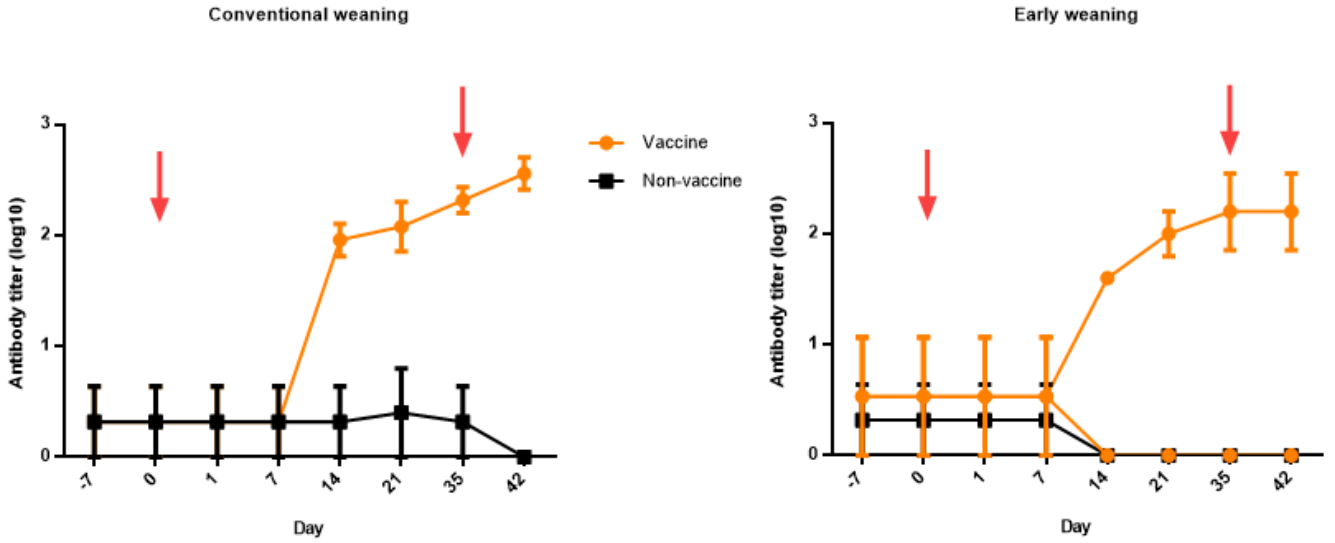

Fig. 4: Total antibody titers (log10) against Bovine Herpes Virus (BoHV) in weaned calves, both early (A) and conventional weaning (B). Vaccines administrations are indicated with arrows (from the total of 10 calves, 5 were vaccinated; orange line).

IV. DISCUSSION

The impact of early calf weaning on the reproductive performance of cows has been demonstrated under research and trade conditions in Argentina and other countries in the last thirty years (Galli et al., 2008). EW and HEW implementation has shown to increase the reproductive efficiency by accelerating postpartum rebreeding of the dam leading to increased pregnancy rates. As shown by our results, weaning generates a very marked stress response, with a cortisol peak and an increase of total plasmatic proteins at day one in both groups. As weaning practices are probably widely applied, a critical issue for the success of the practice is a correct nutritional and sanitary management of weaned calves (Diego Daniel Gonzalez et al., 2013).

The temporal course of total plasma protein levels did not vary between the two groups and in both a clear peak response was observed at day one post-weaning. This parameter seems to be a reliable weaning-stress marker and a clear response is seen.

With respect to plasmatic cortisol leves, early-weaned animals did not generate a typical acute stress response. Although, these animals showed a cortisol peak at day one values did not return to baseline and were still elevated at day 35 post-weaning. This result is important from the endocrinological point of view since cortisol impacts on many physiological functions of these animals. In particular, for the agricultural sector, its role in the immune system is very important since it directly impacts the health status of the animals. It is well established that incubation of cattle immune cells with physiologically relevant high concentrations of cortisol decreases lymphocyte proliferative responses. The immune response is one of the mechanisms through which animals defend themselves against environmental challenges. A common theory is that stress responses suppress immune activity, thus increasing susceptibility to disease. The underlying mechanisms of immune responses to stress remains unclear because of the complexity of the interrelation of the immune system and the stress responses, involving other physiological factors such as age, nutrition, genetics, and gender (Salak-Johnson, McGlone, & Norman, 1996). Extreme environmental conditions and stressful management practices influence the health and well-being of domestic farm animals. Converging evidence suggests that these stressors directly alter host immune function. These alterations have been linked to disturbances in the HPA axis and the hypothalamic autonomic nervous system (Moberg, 2012).

Regarding to the general parameters of the immune system we have measured the % of LY, GR, and the GR:LY ratio. We found that weaning influenced leukocyte populations in conventionally weaned calves but did not find any alterations in animals subjected to EW. Correspondingly, conventional weaned calves showed significantly elevated GR:LY ratios due to a significant reduction in the %LY accompanied by an elevation of

%GR. Previously it has been demonstrated that dexamethasone induces an elevation of GR:LY ratios in cattle (Anderson, Watson, & Colditz, 1999). Furthermore, it has been shown that the stress of weaning influences serum levels of acute-phase proteins, iron-binding proteins, inflammatory cytokines, cortisol, and leukocyte subsets in calves (Kim et al., 2011) suggesting that glucocorticoids are a contributing factor to the alteration of the GR:LY ratio. Our findings corroborate this previous studies that suggest that weaning affects leukocyte levels and that the GR:LY ratio may be an effective biomarker of the stress response in calves of six months of age. However, our findings also showes that in animals subjected to early weaning at an age of 60 days this effect is not seen. This difference may be attributed to developmental age differences of the cortisol response or the HPA and/or the immune system. Kampen et al., (2006) demonstrated that the percentages of CD4+, CD8+ and TCR+ lymphocytes stabilized during the first 10 to 12 weeks of life. The non-response of the GR:LY ratio of younger animals of 60 days of age may be due to the presence of still immature the leukocyte subsets or, alternatively, to an immature HPA system. At the moment, we can not identify a single factor responsible for this effect and/or draw any conclusions, but our results indicate that when using the GR:LY ratio as a stress indicator the animal age needs to be taken into account.

The HPA axis regulates the immune system mainly by the release of glucocorticoids. These exert a variety of effects on the immune system through signaling mechanisms mediated by steroid hormones. In rodents it has been demonstrated that increased glucocorticoids produce an increase in the release of mature neutrophils from bone marrow the magnitude of which decreases over time (Alvarez, 2002; Tadich, Kruze, Locher, & Green, 2003). In addition, it has been shown that in chronic stress neutrophilia-mediated leukocytosis occurs, whereas the number of lymphocytes and eosinophils decreases as long as glucocorticoid concentrations remain high (Alvarez, 2002; Yagi et al., 2004). Our results reproduce these observation in the group subjected to conventional weaning, but we emphasize the difference in the response according to age. It would be interesting to elucidate in the two animal age groups, on one hand the difference in the mechanism of interaction between the HPA axis and the immune system and, on the other hand the capacity of the HPA axis to generate immune responses and adaptations .

The goal of low-stress weaning techniques is to minimize negative stress caused by nutritional, environmental, physical and social changes experienced by calves at weaning time. Stress increases cortisol levels, which reduces immune system performance and can predispose calves to illness from a variety of disease pathogens, particularly organisms that cause the bovine respiratory disease (BRD) complex (WSU, 2013). Much of the failure of vaccination may lie in the timing of administration, failure of stressed calves to respond properly to vaccination, and the increased susceptibility of stressed calves to all pathogens. Our results show that the health status of early-weaned animals is potentially worse than that of conventional weaned animals at the day of weaning, since lesser animals show a lower response to vaccination. Recently, has been observed that EW and HEW calves showed no seroconversion after vaccination against BoHV1 (Diego Daniel Gonzalez et al., 2013). This may be due to the quality of the vaccine, the age of the animals, the state of weaning stress or a combination of these factors.

Previous studies have shown that there are no differences between early and conventionally weaning calves in their behavioral response to the stress caused by weaning as assessed by food consumption, displacement, and vocalizations (Vittone et al., 2012). Furthermore they also showed that the re-breeding and fattening pens provided greater efficiency and speed to the meat production process and that early weaning improved breeding rates.

Weaning is an inherently stressful process because it is usually connected with major changes that include nutritional, physical, and psychological elements as stressors in modern cattle production systems (O'Loughlin et al., 2011). More specifically these multifaceted stressors involve numerous husbandry practices, including the abrupt separation of the calf from its dam, a nutritional adjustment to a non-milk diet, social reorganization, and often housing (Enríquez et al., 2011; Lynch, Earley, McGee, & Doyle, 2010). Reducing the stress of weaning or finding the most adequate weaning age will help to reduce the risk of calfhood BRD and keep calves eating, gaining, and growing. Careful monitoring of weaned calves is essential to allow intervention when stress-related conditions arise and to ensure the welfare of animals. This is not only an ethical issue and social responsibility but improves also the quality of products derived from these animals. Trying to favor the quality of life and adaptation of livestock to their environment in a system that increases productivity, such as early weaning, we can say that both groups are generating a stress response to weaning, regardless of age. However, there are differences in mechanisms, particularly in the immune system, which is why it is recommended to the sector to verify and reinforce vaccination schedules.

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It is necessary to have solid indicators of the welfare state of the animals. As we show in this study, the ration of GR:LY, widely used as an indicator of stress, depends on calf age and is for this reason not a representative biomarker of stress across different calf ages. Therefore, we discourage its use to measure and compare stress associated events like weaning at different calf ages. Since Coppo, (2007) found a malnutrition status of early weaning calves resulting in lower weight gains, and low levels of nutritional indicators and we observed a deficient response to vaccination in EW animals. we believe it necessary to incorporate some additional immunological and nutritional indicators to those mentioned above in order to ensure a thorough surveillance of the sanitary or welfare state of animals and to act in consequence whenever necessary.

V. CONCLUSION

In this study, leukocyte subsets, plasmatic proteins, cortisol levels, and immune status were found to be altered after weaning challenge. Results indicated that weaning challenge represents an important stressor, regardless of age. Management strategies should be complemented with the screening of calf immunity to ensure the efficacy of implemented vaccination programs. We found that the conventional indicator (% GR: LY) is not very representative for the stress response at a younger calf age, and we recommend the use of cortisol plasma levels and total proteins as indicators of stress as more reliable biomarkers of stress across different calf ages.

It is necessary to have a robust group of biomarkers of stress that allows the assessment of calf welfare at different weaning ages associated with various production systems. This will facilitate to achieve two important goals, on one hand, the understanding of the relationship between stress and the immune system and, on the other hand, to analyze solidly the welfare state of weaning calves at different ages in diverse production systems.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Effect of pH on Hydroponically Grown Bush Beans (*phaseolus vulgaris*)

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Abstract**—** *Numerous environmental factors can influence the viability of plants. Several studies have shown that acidic and basic pHs have a noticeable effect on different types of plants when grown directly in soils. However not too many studies have been done on hydroponically grown plants with respect to pH. This study was conducted at the Botany laboratory and Green house at Alabama A & M University with bush beans.*

Five nutrient solutions with pH values ranging from 5 - 9 were used. Observations on plant height and number of leaves were done for six weeks after nutrient solutions with the different pH values were introduced to the plants 7 days after the seed germinated.

Plants grown in nutrient solutions with high alkalinity levels (8 & 9) were significantly shorter compared to those grown in nutrient solutions with pH 5, 6 and 7. Leaf number and size strongly correlated in a manner expected with plant height, plants in high alkalinity nutrient solutions had fewer and smaller leaves. Plants grown in neutral or near neutral nutrient solutions appear healthier with green leaves and strong stems.

Keywords— Effect, hydroponically, pH, bush beans.

I. INTRODUCTION

Hydroponics was derived from two Greek terms, Hydro (water) and Ponos (labor). In 1929, a plant physiologist by the name of William Gericke used nutrient solution formulae to grow crops and called the process "aquaculture". However, that term was used for another process, so Gericke instead coined the word "hydroponics" (Welch, 1990). Hydroponic systems have been utilized as one of the standard methods for plant biology research and also used in commercial production for several crops (Nguyen et al.). Hydroponics is the practices of growing plants using only water as a substrate with the addition of essential nutrients and is one of many methods used in nutrient- delivery systems (Kane et al, 2006). Most modern hydroponic solutions are based on the work of Hoagland and Arnon and have been adapted to numerous crops (Kane et al, 2006, Wheeler et al., 2008). Since the 1980s, hydroponic units have been commercialize for vegetable and flower production, and today more than 60,000 Ha of vegetables are grown hydroponically in green houses worldwide (Kane et al, 2006). Since hydroponic plants have access to unlimited nutrients and water, this specific environment helps to improve crop growth and yield (Paradiso et al,.2017) and they are able to grow up to ten times faster and healthier than soil grown plants (Ficks and Mitchell, 1993). Root zone pH is most importantly considered in hydroponic culture (Hyun-Ju Kim et al). The pH of a hydroponic solution influences the solubility and availability of nutrients for root uptake, especially iron and other micronutrients such as manganese and boron. A high pH decreases iron solubility and uptake by roots, which can result in deficiency symptoms. For most crops, keeping pH within a target range of 5.6 and 6.2 ensures adequate nutrient solubility and availability for plant uptake
(Dickson and Fisher, 2017).Plants grown in a hydroponic system have the perfect balance of nutrients and water freely available to their roots. Because plants are not forced to expand their limited energy searching for water and food, they are able to grow faster and healthier.

Plants must have certain nutrients in order to survive and function properly. Plants get their nutrients from air, water, or soil in which they grow. Of these three sources, the soil is often the greatest source for plant nutrients, but whether or not these nutrients are actually available for plant uptake, depends largely on the acidity or basicity of the soil (Huett, 1994). When growing plants without soil, or hydroponically, the pH of the nutrient solution is critical for proper development. If the pH of the nutrient solution is within the appropriate range for a particular plant, that plant will be able to utilize all the proper nutrients and thrive. If the pH of the solution is not within the appropriate range for a particular

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plant, then that plant will not be able to utilize an adequate amount of nutrients and will begin to show signs of poor health such as fragile stems, yellowish, and slow stunted growth. If the plant environment in which it is grown is too acidic then some nutrients can't be absorbed; likewise if too basic, then other nutrients cannot be absorbed. Ideally its environment should have a good balance, such that it is neither very acidic nor very basic. The pH number lets you know how soluble the nutrients in the water mixture are. It determines the mobility of these nutrients, and hence the plant uptake ability of these nutrients Green and Vibrant, 2017}Scientists have found that the ideal overall pH for optimum absorption of most nutrients is 6.8, slightly acidic. Any soil that has a pH range within 6.3 to 7.3 is considered good (Jett, 1999).

A simple hydroponic system comprising of plastic planting containers, planting medium, a reservoir, a wick, air space and a light source was used. The objective of this study is to show how altering the pH of a nutrient solution will affect the growth and health of bush bean plants.

II. MATERIALS AND METHODS

The study was conducted at the Botany Laboratory and Green House at Alabama Agricultural and Mechanical University. Bush beans grown in hydroponic nutrient solutions (contents: N 7.0%, P_2 O_5 4.0%, K_2 O10.%, Ca 4.0%, Mg 1.5%, S 2.0% and Fe 0.1%) with adjusted pH values of five, six, seven, eight and nine used in this experiment. Each treatment was replicated three times.

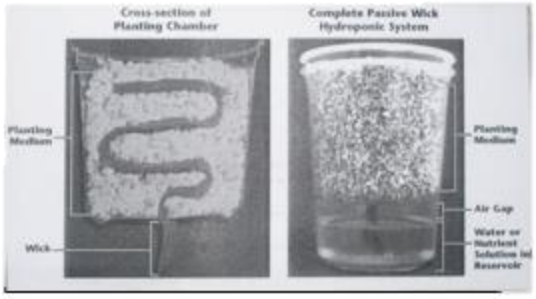

Figure 1: Hydroponic Set-Up System

The hydroponic system was assembled using two different sizes transparent plastic containers. One small sized container was used for the inert planting medium [perlite {70%} and vermiculite {30%}] and absorbent felt wick. The other size was used as a reservoir for the nutrient solution. The felt wick was weaved in an "S" shape through the container with the planting medium with at least 2 inches protruding through a slit at the bottom. The container was then placed in the other container with the nutrient solution, making sure an air gap was seen and the wick was immersed in the solution.

Figure 2: Bush Bean Plants seven (7) days after germination (grown with only water: no nutrient solution applies

Initially approximately 200 ml of water was placed in the reservoir container to allow the bush bean seeds to germinate, the seedlings were grown for one week and their heights measured before they were introduced to the different adjusted pH nutrient solutions. Plants were measured and number of leaves counted weekly. Other observations made were based on the general appearance of the plants (e.g. color, size and lost of leaves and color, strength of stem).

Using a pH strip and the pH up and pH down solutions, the nutrient solutions were maintained at their respective PHs. Reservoir containers were emptied, cleaned and refilled with newly mixed adjusted pH nutrient solutions every two weeks. At the end of the six weeks observational period the data for plant height and number of leaves per plant were compiled and graphical charts used to indicate correlations and trends. Plants were exposed to eighteen hours of light and six hours of darkness per 24- hour period. During the germination period light was kept on for 24-hours per day.

III. RESULTS AND DISCUSSION

Figure 3: Differences in growth/development of Bush Bean Plants Thirty-five (35) days after being fed with nutrient solutions. A= Nutrient solution at pH 5, B= Nutrient solution at pH 7, C= Nutrient solution at pH 6, D= Nutrient solution at pH 8, E= Nutrient solution at pH 9.

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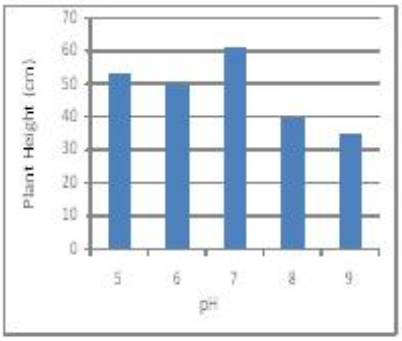

Figure 4: Effect of nutrient solutions at different pH levels on plant (*Phaseolus vulgaris*) height.

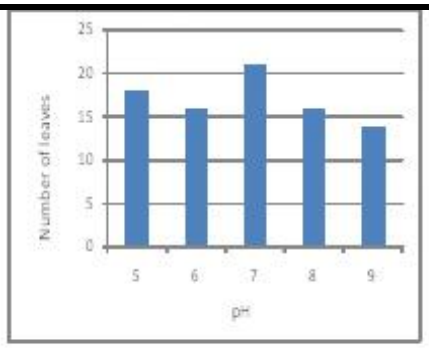

Figure 5: Effect of nutrient solutions at different pH levels on number of leaves/plant (*Phaseolus vulgaris*)

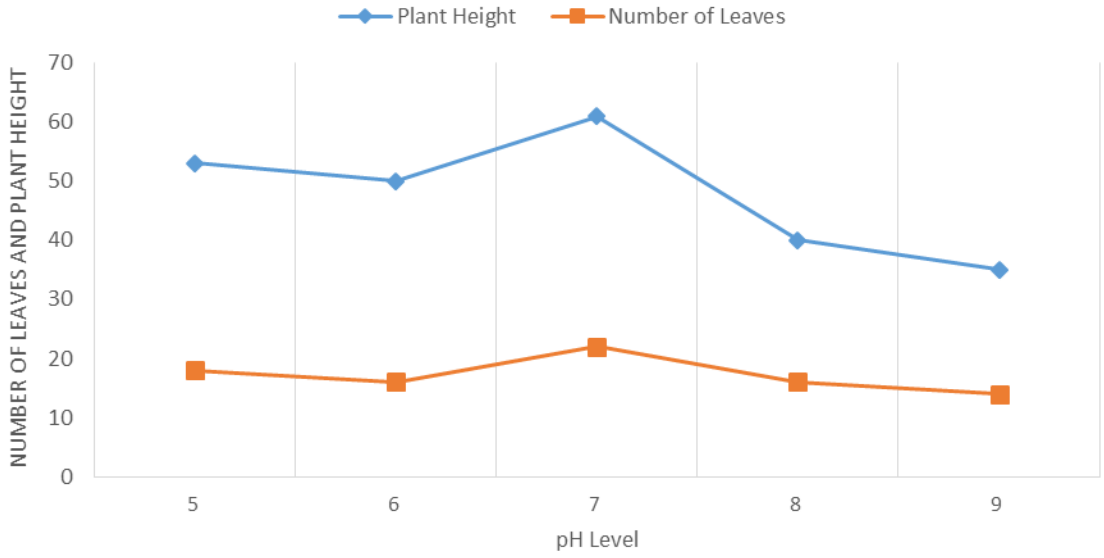

Figure 6: Correlation between Plant Height and Number of Leaves

The data of six week of observation on the effect of pH on hydroponically Bush Bean (*phaseolus vulgaris*) are illustrated in figures 4 and 5. Nutrient solutions with Very basic pH (8and 9) contributed to the stunted growth of bush bean, the data (Fig. 4) indicated that heights decreased when plants were grown in nutrient solutions at pH levels 8 (40cm) and 9 (35cm) when compared to nutrient solution with pH levels of 5 (53 cm), 6(50cm) and 7(61cm). This variation in growth was due to an observed decrease in internode length at both alkalinic nutrients solution levels (pH 8 & 9). This finding supports that of Green and Vibrant (2017) who indicated that each element has a specific tolerance to a respective pH level and main macronutrients (NPK) are hard to move in too high pH environment. Which makes it difficult for plants to absorb them, which results in deficiencies (Sedimentation occurred in nutrient solutions with pH levels of 8 and 9, indicating nutrients did not dissolve completely at those pH levels. **Ruano (1987)** and Heutt (1994) said pH values below 6 and above 7.5 cause the inhibition of phosphoric, calcium, magnesium, iron, manganese, copper, zinc and boron **ions** to be less available to plants. According to him extremes in pH, especially above 7 can result in precipitation of certain nutrients, for plant roots to be able to absorb nutrients, the nutrient must be dissolved in solution. The process of precipitation (the reverse of dissolving) results in the formation of solids in the nutrient solution, making nutrients unavailable to plants. These factors must have contributed to the deficiency in internode length, hence the height of the plant.

Data in Figure 5 indicated that less leaves were produced when plants were grown in nutrient solutions with pH levels of 8 & 9. Leaves were also observed to be smaller on plants grown in nutrient solution with pH levels of 5, 8 and 9. Lack of vital nutrients could be the contributing factor for

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these deficiencies. These results seem to support Huett (1994) finding that at extreme pH (very acid/very basic) essential elements become less available due some form of precipitation.

Based on general observations after the conclusion of the experimental period (Fig. 3), plants grown with nutrient solutions of pH 5, 8 and 9 show signs and symptoms that would eventually lead to their mortality. Plants grown with nutrient solutions of pH 5and 6, especially those with neutral (pH7) looked healthier. Excessive drying and falling of leaves, discoloration and fragile stems were indications that these plants would not survive. Similar trends in Figure 4 and 5 show a strong correlation between plant height and number of leaves per plant (Fig. 6) in this investigation.

IV. CONCLUSION

Plants grown in neutral or near neutral nutrient solutions (pH 6-7) appear healthier with green leaves and strong stems. This finding agrees with Kane et al. (2006)that biomass was greatest for plants grown at pH 6.5. According to Azad et al. (2009), plant growth is related to media pH and nutrient components.

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The Influence of Proximate Composition of Cow Dung on the Rate and Volume of Biogas Generation in Maiduguri, North Eastern Nigeria

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***Abstract*—** *This research investigates the Cow dung, Cow pats or Cow pies as a waste product of Cattle which constitute undigested residue of plant material and excreted by the animal as a waste. One dung cake made by hand of an average size gives 2100 kj of energy. The organic waste can be a source of renewable energy for rural areas especially in developing countries like Nigeria provided the material is subjected to anaerobic digestion to produce biogas as a means of waste disposal and alternative source of energy. Before this initiative of digesting Cow dung, the waste material is used as a direct fuel in clay stoves or three stone stoves and traditional fertilizer among peasant farmers in Nigeria and Asian countries. However, when discovered as a cheap, reliable and safe source of domestic fuel, energy experts and consumers thought of commercializing and patenting the gas.*

The research was carried out in the Laboratory of the Department of Biological Sciences, University of Maiduguri, Nigeria, using the batch system digester with a capacity of0.612 m^3 and 0.24 m^3as gasholder. About 0.2773 m^3 of gas was released daily to control the excess pressure imposed on the gas holder (size 24, tractor tube). Laboratory and field analysis of the cow dung were carried out, followed by daily records of the volume of gas generated using ruler to measure and estimate the volume of the gas in cubic meter using the standard formula for measuring a cylinder ($3.14 \times r^2 \times h$). The result of this research shows that the gas generated was methane due to combustion in combination with Carbon dioxide (CO_2) due to non-combustion, Hydrogen Sulphide (H_2S) due to rotten egg smell and water vapor. The daily volume of biogas generated was 0.27915 m^3 on the average, with a maximum daily record of 0.5165 m^3before declining after 73 days of the experiment and a cumulative volume of 77.62 m^3in 52 days of methane production. The sustainability of cooking trial utilized an overall volume of 1.17 $m^3$of the methane gas in 1hr to cook 1.5 kg of rice with ingredients and 0.553 m^3in 1:35 hrs.to boil 20 liters of water. The digestion process was done in an airtight drum of 0.612 m^3capacity as the digester.

***Keywords*— Biogas, methane, cow dung, bio-fertilizer, inoculum, carbondioxide, stove and sustainability.**

I. INTRODUCTION

The potential value of Cow dung and it's by products produced by the meat industry, theMaiduguri abattoir as a waste material or source of renewable energy, bio fertilizer and animal feed has been neglected by the urban community and researchers. This is indicated by the heaps of cow dung at the Maiduguri abattoir, diversion of the material to farmlands before treatment, dumping of the waste material in the nearest river and lack of scientific journals, reports or research carried out on the waste material by local scientists in Maiduguri, the Borno State capital. A related activity is the collection and dumping of the raw waste material by farmers to their farmlands as a manure to supplement the traditional inorganic fertilizer, which is expensive and polluting to the environment (Smith and Slater, 2010). This is because in most developing countries like Nigeria recycling of waste or agricultural wastes are rarely practiced leading to pollution and environmental degradation (Yahaya and Ibrahim, 2012).In Nigeria this scenario is sometimes promoted by ignorance of the value and accessibility to the organic material , thereby making fuel wood as the only source of fuel for domestic purpose for majority of the people living in the

region thereby creating unnecessary pressure on the already degraded land.It is noteworthy that, due to lack of provision for alternative source of energy like solar, wind and nuclear energy, fuel wood or biomass as the only traditional fuel remains inaccessible and expensive in Maiduguri, primarily due to insecurity, in addition to demand by the increasing human number, loss of vegetation, aridity or deforestation, high cost of fossil fuel, decreased supply of fuel wood, reduced crop residues, inefficient cooking devices or stoves and poor construction materials.This has created a lot of pressure on the already degraded arid environment. This phenomenon informs us that cow dung must be seriously looked into as a valuable alternative source of domestic energy for the rural poor community.

The main energy requirement in the ecological zone include domestic cooking, heating, warming during the cold harmatan period, small scale food processing industries and lighting in rare cases. Domestic homes in the urban areas have been using firewood, kerosene or electricity for their fuel requirement in homes and industries, while in the rural areas, particularly in the extreme northern arid zone of the region, these fuel materials are not accessible or completely not available due to acute aridity, loss of vegetation and where available they are expensive. Due to domestic pressure and necessity, the alternative fuel has always been the slashed twigs and branches of the sparsely populated trees, shrubs of the poisonous plant Calotropisprocera, cake of dung or expensive fuelwood imported from the city. It was reported that by 2050 the rural energy needs of Africa would be the traditional source of rural energy like forest biomass, wood and agricultural residues(Revelle, 1979).

This research, therefore intends to use cow dung as an alternative source of energy to produce biogas (methane) as a source of fuel for use in the rural areas of Nigeria particularly in the dry land zones in the northern part of the country. The gas has a number of advantages to the economy of a nation like Nigeria, which include reduction of dependence on imported or using petroleum gas, protecting the environment by minimizing greenhouse gas emissions(Torguati, et al.,2014). It will also empower the rural community by savingabout 144 minutes of their time from wood collection (Amare, 2015), the economy of a nation, the security of women and children during fuel collection in the bush and also children will have enough time to go to school. Biogas or methane production reduces the effect of deforestation caused by fuel wood extraction and climate change in Nigeria (Maiwada,et al.,2014),increased biomass yield from the use of biogas slurry as bio-fertilizer(Kasap, et al, 2012) and it also gives more time to vegetation to sprout and regrow. The nutrient rich sludge after digestion is recycled back to the land to maintain the fertility of the field growing feed for the cows at Mason-Dixon Dairy Farm located at Gettysburg, Pennsylvania (www.manuremanagement.cornell,2017). This suggest that the structure and components of biogas generation put in a community must be monitored for use and effectiveness in the community (Nagamani and Ramasamy,1990).

In Vietnam the biogas potential is due to livestock of more than 30 million, mostly pigs, cattle, and water buffalo. Although most of the livestock dung is used in feeding fish and fertilizing fields and gardens (Zafar, 2012). At the Mason-Dixon Dairy Farm located near Gettysburg, Pennsylvania manure from 2000 cows produced fuel engines that drive generators supplying not only all the electrical power for the Dairy, but the excess is sold to the utility company where the waste heat from the engine is used to heat the digester and the building (www.management.cornell,2017). Besides the benefits derived from biogas, it has some negative consequences on human health when used indoors. The gas is a major cause of respiratory morbidity for women and children (Dohoo, et al.2012)

Considering the above background this research intends to determine the influence of the proximate composition of cow dung and the rate of methane production during the season in the zone as its aim and objective and to specifically determine the volume of gas generated per unit time, the cumulative volume of gas per sludge of cow dung, the amount of gas required to sustain a cooking time and number of people in a family. Biogas or methane gas (CH_4) is referred to as a biofuel, because it is generated from degradable biological material. The gas is primarily made up of methane (CH_4) 55 – 70%, Carbon dioxide (CO_2) 30 – 45%, with some amount of other gases like hydrogen sulphide (H_2 S) 1 – 2% and traces of hydrogen (H_2) 0 – 1%, Nitrogen (N_2) 0 – 1%, carbon monoxide (CO), saturated or halogenated carbohydrates in traces and oxygen (O_2) traces which are occasionally present in the gas. This was confirmed byOnwuliri(2013) who reported that, the composition of the gas methane gas is (CH_4) 50 – 70%, CO_2 30 – 40% and other gases. It wasfurther reported that the composition of biogas depends on the type of decomposedmaterial, which may be as follows 50 – 85% methane(CH_4), 20 – 35% Carbon dioxide (CO_2), H_2, N_2 and H_2S form the rest of the composition(Pastorek et al.,2004) (Bharathiraja,2018). Usually the mixture is saturated with water vapor and may contain dust, some impurities and siloxane. According to Lawbury(2001) approximately 60%

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methane, 40% CO_2 with traces of other gases like hydrogen, nitrogen and hydrogen Sulphate are present.

Depending on the appliance used, the quantity of biogas has to be improved, due to interference by aeration and moisture. The Biogas Project(BP) (2015) reported that in order to upgrade the system and the quality of the gas some parameters like hydrogen Sulphate, water vapor, carbon dioxide, and halogenated compounds need to be removed. This will allow the gas to burn with blue flame, stable, non-toxic, tasteless and odorless. The presence of hydrogen Sulphate is noticed by a percentage of rotten egg smellwhen burned. When the gas burn in the presence of oxygen it produces a blue flame and large amount of heat energy and due to the presence of CO_2, this makes the fuel safe for use in rural homes as domestic fuel for cooking, heating and generation of electricity because it is not explosive like petroleum gas.

Cow dung is obtained from a cow, where about 50 liters of methane can be generated from a single cow after chewing the cud. The benefit of biogas generation is not only the biofuel and environmental sanitation but an organic fertilizer called bio-fertilizer or slurry is generated for use by farmer, especially when inorganic fertilizer is beyond the farmer's reach. The application of the slurry to soil is equal to bioremediation process of disposing excess nitrogen from animal farm and injecting it into crop land (Lopez-Ridaura, et al,2009). Further to this, the bio-fertilizer slurry improves the nutrient status of the soil, where it influenced the production of 5 t/ha of crop compared to inorganic fertilizer (Shaheb et al.,2015). Likewise, the physical properties of soil such as structure, texture, water holding capacity, cation exchange capacity and less erosion could be corrected or influenced by bio-fertilizer(Gurung, 1997)

II. MATERIALS AND METHOD

The experiment was carried out in the Faculty of Science Complex(UsienUdom Court) of University of Maiduguri, Nigeria, after collecting the required amount of cow dung from the Maiduguri abattoir on the 02/08/18 in a polythene bag and kept airtight to prevent loss of moisture and contamination.

Generation of Biogas(Methane)

Twenty four hours after collection of cow dung from the Maiduguri abattoir,400 kg of the cow dung was weighed using a weighing balance(Saltare model) and 400 liters of inoculum measured in a measuring cylinder(1000cm^3) was fed in to a batch digester(bioreactor) with a capacity of 0.612 m^3, after it wasanalyzed for proximate composition such as moisture content using moisture analyser(METTLER TOLEDO LJ16 and LP16 MODEL), Ph. using Ph.Meter(PHS-25 MODEL) and the temperature of the digester using a thermometer (0-100^0 C capacity) and the ash content was determined using an oven (Hot Air Oven). The 400 kg of Cow dung was homogenized to have fine and well separated particles to improve on the rate of digestion by the methanogen bacteria, 4000 liters of water were added to the quantity of cow dung to give a ratio of 10:1 water: cow dungAnjos et al. (2017) used batch digesters with and without solid separation in the substrates, Ezekoye et al.(2014) used cow dung to water in a ratio of 6:1. After feeding the digesterit was allowed to ferment, hydrolyze and methenation at a mesophyll temperature range of 38^0 C for a period of 14 days before a non-combustible gas was generated.

Carbon dioxide Scrubbing

The non-combustible gas generated was collected in a tractor tube of size 24 as an alternative gasholder for convenience of measurement and movement of gas from place to place by the end user in laboratories and rural community.From the 15th day to the 35thof the experiment an average of 0.1657$m^3$of thegas wasgenerated but notcombustible, so it was suspected to be carbon dioxide together with hydrogen Sulphate due to the rotten egg smell perceived, and it was expelled from time to time to time to reduce the pressure on the gas collector and the scrubbed carbon dioxide was injected in to water as waste for the growth of algae by another research.

The Gas Burning Flame

The nature of the burning flame was observed for colour and the existence of soot deposits and possible moisture from the burner.

The Combustible Gas (Methane)

The combustible gas, flame type and smoke observed and notedon the 36thday of the experiment and this was measured and recorded as methane gas. Daily records of the gas was taken and recorded for the period of 87 days of the experiment. The volume of gas generatedwas measured using a metric rule and substituted in the standard formula of measuring a cylinder(3.14 x r^2 x h) and recorded with daily room temperature as indicated in Table 1, while the volume of gaswas plotted in a graph (fig.1).

The values in cubic centimeter were divided by one million to give a volume measurement in cubic meter.

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Statistical Analysis

A graph showing the varying height of the gas was shown in figure 1, with a histogram at the background.

III. RESULT AND DISCUSSION

The result of this research indicates that gas production started14 days after feeding the digester, but it was not combustible and it was assumed to be carbon dioxide (CO_2) with other gases like hydrogen sulphate(H_2S) due to the rotten egg smell and water vapor during scrubbing. A similar trend was observed by Ezekoyeet al. (2014) where about 156.21 liters of the gas from waste material was produced after a period of 75 days with the methane constituting only 52.3% and combustible on the 45th day of the experiment. In this research the carbon dioxide exceeded the methane production on the 36th of the experiment when methane gas increased above carbon dioxide and reached its peak with a volume of 0.5165 m^3 of gas on the 73rd day before declining, when room temperature was 31^{0}c, although it has no influence on the rate of gas production, as indicated in the table below(table 1). In another research gas production started after 7th day with a steady increase and attend its peak on the 18th day before declining(Ugochukwu et al. (2018). From the above scenario it can be deduced that the time and volumeof initial methane production is determined by the type material and other secondary factors used as a substrate for the production of the gas.

Table.1: Showing the Daily Volume of Gas Generated and Room Temperature Recorded

Serial no.	Date/Days	Volume of Biogas generated/day (M^3)	Daily Temperature (^{0}C)
1	03/08/18	-	26
2	04/08/18	-	27
3	05/08/18	-	29
4	06/08/18	-	28
5	07/08/18	-	28
6	08/08/18	-	29
7	09/08/18	-	30.5
8	10/08/18	-	29
9	11/08/18	-	26
10	12/08/18	-	30
11	13/08/18	-	34
12	14/08/18	-	31
13	15/08/18	0.1657	31
14	16/08/18	0.1657	31
15	17/08/18	0.1657	29.9
16	18/08/18	0.1657	27
17	19/08/18	0.1657	39
18	20/08/18	0.1657	33
19	21/08/18	0.1657	30
20	22/08/18	0.1657	30
21	23/08/18	0.1657	31
22	24/08/18	0.1657	29.9
23	25/08/18	0.1657	25.5
24	26/08/18	0.1657	39.5
25	27/08/18	0.1657	32.5
26	28/08/18	0.1657	29.5
27	31 /08/2018	0.0.1657	_
28	01/09/2018	0.1657	_
29	02/09/2018	0.1657	_
30	03/09/2018	0.1657	_
31	04/09/2018	0.1657	_
32	05/09/2018	0.1657	_
33	06/09/2018	0.1657	_
34	07/09/2018	0.2862	_
35	08/09/2018	0.2566	_
36	09/09/2018	0.3020	_
37	10/09/2018	0.3155	27
38	11/09/2018	0.2722	29
39	12/09/2018	0.2739	30
40	13/09/2018	0.3212	28
41	14/09/2018	0.3588	28
42	15/09/2018	0.2579	30
43	16/09/2018	0.2821	31.5
44	17/09/2018	0.3125	29
45	18/09/2018	0.2475	26
46	19/09/2018	0.2678	30
47	20/09/2018	0.2929	34
48	21/09/2018	0.3337	31
49	22/09/2018	0.3237	32
50	23/09/2018	0.3128	32
51	24/09/2018	0.4166	29.9
53	25/09/2018	0.3432	25.5
54	26/09/2018	0.2843	30.5
55	27/09/2018	0.3343	33.5
56	28/09/2018	0.2926	29.5
57	29/09/2018	0.3368	31.5
58	30/09/2018	0.2533	32.5
59	01/10/2018	0.2934	34
60	02/10/2018	0.3127	30
61	03/10/2018	0.2777	28
62	04/10/2018	0.3013	29.9
63	05/10/2018	0.2978	31.0

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64	06/10/2018	0.3135	31.5
65	07/10/2018	0.3369	29.0
66	08/10/2018	0.3439	31.0
67	09/10/2018	0.3408	33.0
68	10/10/2018	0.3593	_
69	11/10/2018	0.3711	29.0
70	12/10/2018	0.3904	32.0
71	13/10/2018	0.3484	32.0
72	14/10/2018	0.3691	36.0
73	15/10/2018	0.5165	31.0
74	16/10/2018	0.1550	33.0
75	17/10/2018	0.1631	37.0
76	18/10/2018	0.1709	_
77	19/10/2018	0.1843	_
78	20/10/2018	0.1918	_
79	21/10/2018	0.2099	_
80	22/10/2018	0.2125	_
81	23/10/2018	0.2284	_
82	24/10/2018	0.2345	_
83	25/10/2018	0.0219	37.0
84	26/10/2018	0.1223	_
85	27/10/2018	0.0323	38.0
86	28/10/2018	0.0384	35.0
87	29/10/2018	0.0353	_
88	30/10/2018	0.0355	_

Throughout the period of methane generation there was a continuous daily fluctuation as indicated in figure 1. The fluctuation in gas production particularly the syncline indicated in figure 1 was attributed to a number of factors operating in the digester such as the high acidic condition of the digester with a Ph. value of 3.8, high moisture content raw material, incomplete digestion of materials and lower digester temperature. These factors were naturally controlled intermittently and more remittent on 72ndday, 24 hours before the peak. This volume of gas generated was not influenced by the daily measurement of temperature as indicated in table 1 above.Therefore, controlling the factors responsible for the intermittent gas production could give a steady and high gas production.

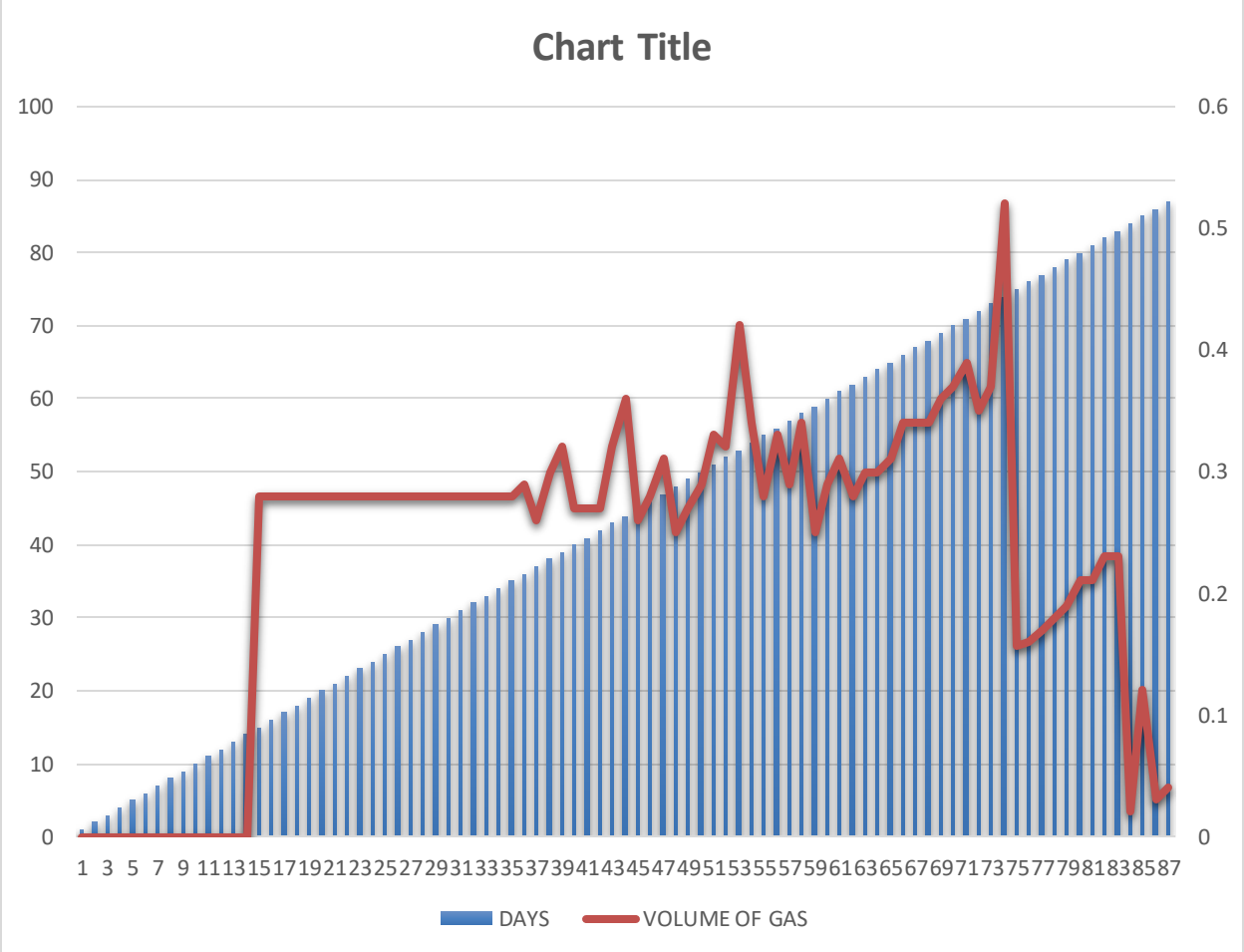

Fig.1: Showing the daily rate of Biogas production

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In this research the percentage composition of the gas was not measured, but the cow dung was well composted in order to produce the expected proportion of gas as obtained by other researchers on biogas. The composition of biogas depends mostly on the type of decomposed material, unless otherwise, the composition of biogas is 50-85% methane(CH_4),20-30% Carbon dioxide (CO_2), Nitrogen (N_2), and Hydrogen Sulphate(H_2S)(Pastorek et al., 2004), 60% methane 40% Carbon dioxide with other gases like hydrogen, Nitrogen and Hydrogen Sulphate(Lawbury, 2001).

The combustible methane was connected to a single stove burner and ignited for a cooking trial and sustainability based on an average daily production of 0.2773 m^3 of gas. Besides the triode peaks there was a number of fluctuations throughout the period of the 87 days of observation and record of value. A drastic decline in production of 0.5165m^3 to 0.1550 m^3 was recorded on the 74th day of the experiment. A similar trend was observed by(Ugochukwu et al,2018). The series of fluctuation in the daily production throughout the experiment could be ascribed to the high acidic condition (PH 3.8) of the digester as indicated in Table 2

Table.2: Showing the Composition of the Digester

Quantity of waste (kg)	Quantity of water (L)	Quantity of Innoculum (L)	Moisture content (%)	Ash content (%)	Ph value of slurry	Ph value of raw cow dung	Cumulative Volume of gas generated (m^3)
400	4000	400	48	48.7	3.8	6.8	77.62

Hyper acidic condition in a digester caused by factors like overload, low nutrient content of cow dung (Friedmann,2015),fermentation process and acidification during methenation was found to reduce biogas production (Chibueze et al,2017). The second factor that was associated with the fluctuation in production was high moisture content, which was high in this research(48%) Table 2.Lungkhimba et al. (2010) reported that use of high moisture content material, incomplete digestion and low temperature was responsible for lower gas yield. Also finer particle size can lead to acidification and ultimately to process failure at highest organic load rate. The cow dung collected from the field had a PH value of 6.8 which was near neutral, however when it was fed into the digester the process of hydrolysis was releasing more hydrogen ions which was not taken up by the carbon from the organic matter or cellulose from the cow dung. The carbon released was combining with the available oxygen to produce the excess carbon dioxide that was experienced(0.1657 m^3) for a period of 21 days. Methane was later generated when oxidation was replaced by reduction and hydrogen ions combining with carbon to form methane gas which it lasted for the whole period of the experiment(Table 1and Figure1).

Performance on Water Boiling Test

The cumulative period of gas generation was subjected to Kitchen trialby boiling 20 liters of water and the result obtained are shown in the table, table 3 below. The total cumulative volume of methane gas generated was 77.62m^3, out these the trial utilized 0.553 $m^3$of the gas in 1:35 hours to boil 20 liters of water (table 3). This means during the cold season the demand for hot water for bath and other purposes can be attain to using biogas to save the rural people from cold and diseases associated with it.

Table.3: Showing Sustainability trial of boiling water

Quantity of water (L)	Volume of Biogas utilized (m^3)	Time taken (hrs)
20	0.553	1:35

Performance on the Cooking Trial

The cooking trial was done with rice and the necessary ingredients for a family of thirteen as part of sustainability of the gas generated. The total cumulative volume of methane generated was 77.62m^3, out of these the cooking trial utilized1.17 m^3 of the gas in 1hour to cook the rice with ingredients and 10 liters of water to serve 13 plates of rice as lunch to 13 men and women with satisfaction (table 4). This indicate that using the biogas for a family of sixteen has saved the environment from loss of vegetation through fuel wood extraction, which has been recognized as means of cooling houses. This is because rural areas of developing countries are dependent on biomass fuels like fuel wood and dried dung for their energy need. For example, in Kaduna State of Nigeria Fuel wood accounts for about 1,722 ,904 t/year/person (Zaku et al, 2013). Therefore, if biogas can be introduced in Kaduna as an alternative the state could be saved from deforestation due fuel wood extraction, raping of women and danger of going to the bush to search for fuel, children going to search for fuel during school hours,

excessive carbon dioxide due to poor type of fuel wood and inefficient and poorly constructed cooking devices.

It was further reported that 83% of renewable is consumed in Nigeria and the greater part of it is fuel wood (UNDP, 2002), with a daily fuel wood consumption in Nigeria estimated at 27.5 million kg/day(Ogunsawa,2002). From the volume of biogas generated in this research Nigeria can institute a biogas use scheme in the country to reduce the rate of environmental degradation.

Table.4: Sustainability trail of cooking rice with ingredients

Quantity of Rice (kg)	Quantity of water utilized (L)	Volume of Gas utilized (m^3)	Time taken (hrs.)
1.7	10	1.17	1

IV. CONCLUSION

Therefore, from this research it can be concluded that cow dung as a waste material can be a source of wealth to a nation; especially in developing countries where the traditional source of domestic energy has been fuel wood without alternative which was contributing to deforestation, climate change, desertification and loss of soil fertility. However, this research observed that the most appropriate cooking stove must be employed to attain a sustainable level of benefit. Also in order to attain high and steady yield of gas production a number of factors like high moisture content of raw material, highacidity of digester, incomplete digestion and lower temperature must be avoided. Other benefits of biogas production like the sludge or slurry obtained could serve as bio-fertilizer to amend soil fertility of a degraded land. Also with the appropriate device, a sustainable electricity power can be generated as energy for the animal house and the excess can be sold to neighboring energy demand. It can be concluded that rearing of cows in a community can be source of income and poverty alleviation not only for milk and meat but energy and fertilizer. Finally, it can be concluded and advised that due to high ash content of 48.7% in raw cow dung it is not economical to use the raw cake as fuel.

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Variation in Species Richness and other Vegetational Parameters in Pine and Mixed Broadleaf Forest of Central Himalaya

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Abstract**—** *In the present study total two forest stand on different canopy cover (open canopy, moderate canopy and close canopy) were studied to assess the importance of these two parameters in supporting species regeneration and biodiversity in a P. roxburghii forest and mixed broadleaf forest between 1300 and 2000 m elevation in the Utrarakhand Himalaya. Tree species richness was high in mixed broadleaf forest compared to P. roxburghii forest. While, shrub and herb species richness was high in P. roxburghii forest. The tree and herb density was significantly high in mixed broadleaf forest. Tree shrub and herb diversity was high in mixed broadleaf forest. These forests are exploited variously for different resources and consequently the vegetation cover is decreasing. The disturbances are changing the species richness and diversity, which influences the soil and environmental conditions. Thus, the conservation and management of these forests will be important for the sustainability of human and land. Disturbances and climate change will alter the species composition of these forests, which may shift socio-economic and ecological condition of the region.*

Keywords**— Forest, canopy cover, richness and diversity.**

I. INTRODUCTION

Himalayan vegetation is subjected to various types of disturbances and most of them are either geological or anthropogenic or both. The geological disturbances are natural and include landslides, soil erosion and earth quakes where as the anthropogenic disturbances include deforestation, grazing, lopping of tree branches for fodder and fuel wood, removal of leaf and wood litter from the forest floor and frequent fire. Both types of disturbances affect ecosystem stability and retard the successional process (Kumar and Ram 2005). Disturbances favour plant invasion because they provide a pulse of resources for seedling establishment and growth (Pausas et al. 2006 and Arya et al. 2012). Tree of *P. roxburghii*, the dominant species from low to mid elevation, were harvested on a large-scale in 1960s and 1970s for timber and other industrial raw material and thereafter the continued disturbances either geological or anthropogenic is severely threatening the biological diversity (Singh 1998). Forest biodiversity is the main source of livelihood of the people living in Uttarakhand, Central Himalaya. The forests of this region are mainly dominated by *Pinus roxburghii* Sarg. (Chir Pine) and *Quercus leucotrichophora* A. Camus. (Banj oak). Chir pine often forms a pure stand in this area, but sometime it also mixes with certain broadleaved species like *Q. leucotrichophora*, *Q. glauca* Thumb, *Pyrus pashia* Ham., *Myrica esculanta* Linn. and *Rhododentron arboreum* Sm.

Himalayan biodiversity is severely threatened by natural and anthropogenic disturbances. Anthropogenic disturbances play an important role to change, loss or maintenance of plant biodiversity and more recent phenomenon of climate change is also responsible for the change in species composition and other ecosystem activities (Ram et al. 2005). The forest vegetation of Uttarakhand Himalaya has been described by different workers. These are Tewari and Singh (1981), Saxena and Singh (1982), Ralhan et al. (1982), Kalakoti et al. (1986), Sinhg and Singh (1987), Sundriyal and Bisht (1988), Tewari et al. (1989), Singh and Singh (1992), Pathak et al. (1993), Dhaulkandi (1996). Recently, Kumar (2000), Khera et al. (2001), Kharkwal (2002), Ram et al. (2004), Mishra et al. (2004), Kumar and Ram (2005), Sagar and Singh (2005), Samant and Joshi (2005), Semwal (2006), Lal (2007), Singh et al. (2008), Giri et al. (2008), Arya and Ram (2013), Singh et al. (2014) annd Pandey et al. (2014 4, b) have described various vegetational parameters of the Himalayan forest in relation to biodiversity. The present study deals with vegetational analysis and species diversity across the canopy cover and comparison of diversity between pine dominated and mixed broadleaf forests.

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II. MATERIAL AND METHODS

The study area is located between $29^0$20'and $29^0$30' N latitude and $79^0$23'and $79^0$42' E longitude at elevation 1350-2000m in Uttarakhand Himalaya. Altitudinally, Nainital is located in temperate zone of the Himalaya. Chir-pine (*Pinus roxburghii*) and chir-pine mixed broadleaf forest were selected for the detailed study of plant biodiversity and other vegetational parameters.

The monsoon climate is prevalent in the region. The minimum temperature varied from 5.0^0C (January) to 17.4^0C (June) and maximum temperature from 12.5^0C (January) to 17.4^0C (June). The total annual rainfall varied 1486.8 to 2213.4mm. The mean monthly rainfall (average of three years) was 2.25mm (November) and 498.5mm (July). The rocks of the study area are mainly sandstone, conglomeration, limestone, quartzite, schist's and granites (Valdiya, 1980).

A total of two forest types were selected with three different canopy treatment (open canopy <30%, moderate canopy 30-60% and close canopy >60%) were identified for the detailed study of vegetation parameters. In each forest, almost all species present (>90%) were collected, preserved, brought to the laboratory and identified with the help of plant taxonomist and flora. Species richness were determined as the number of species per unite area (Whittaker 1972, 1975). Vegetation analysis was made for all the three layers of forest, i.e. trees, shrubs and herbs. The size and number of samples were determined following Saxena and Singh (1982). The vegetation analysis was done by sampling 20, 10x10m quadrats on each site for tree. The shrub layer was analysed by sampling 20 quadrats of 5x5m and herb layer by placing 20 quadrats of 1x1m randomly at each site. The vegetation data were calculated for density, frequency and abundance (Curtis and McIntosh, 1950). The CBH (circumference at breast height, 1.37m) was used to calculate the basal area. The covers of shrub were measured by taking line transect of 5m. Herb cover were determined by placing a transect of 1m on the ground and percent ground cover occupied by each herb species was noted avoiding overlapping (Mishra 1968).The diversity index for all the three layers at each study site was calculated by using Shannon-Wiener diversity index (Shannon and weaver, 1963) using by density data, concentration of dominance by using Simpson's index (Simpson, 1949) and Evenness (Pielou, 1966) were also computed.

The index of the similarity (S) was calculated to compare the two forests (Sorensen, 1948) as:

$$S = 2C/A+B \times 100$$

Where, A and B represent the number of species in forest A and B, respectively and C is the number of species common to both the stands.

III. RESULT

Mixed broadleaf forest

A total of 192 species were recorded in close canopy, moderate canopy and open canopy of mixed broadleaf forest, among which 30 were trees, 45 were shrubs and 117 were herbs. The highest tree richness (26 species) was found in moderate canopy forest while close and open canopy forest recorded 25 and 22 tree species, respectively. In case of shrub, richness was highest in close canopy (38 species) compared to moderate and open canopy forest. Herbaceous species were also highest in moderate canopy (84 species) forest followed by close canopy (82 species) forest and lowest in open canopy (76 species) forest. The highest tree density (398 trees/ha) was found in close canopy forest and which decreased with increased disturbance level, 356 trees/ha in moderate canopy forest and 321.5 trees/ha in open canopy forest. In case of shrub species, *Eupatorium cannabinum* was dominant in open canopy forest and close canopy forest where as *Maesa indica* was dominating in moderate canopy forest. The tree density was significantly varied from moderate-close canopy and close-open canopy ($p<0.01$). Shrub density was highest (25683.4 shrub/ha) in close canopy forest whereas the lowest (23042.9shrub/ha) density was recorded in moderate canopy forest. It was significantly varied from open-moderate, moderate-close and close-open canopy cover ($p<0.01$). Among herbs, *Carex nubigena* and *Justicea simplex* was dominating in open canopy forest. *Polygonum fulganes* was dominating in moderate canopy forest and *Oplismenus compositus* was dominating in close canopy forest, close canopy forest was most dense with 7921183.4 herbs/ha compared to moderate and open canopy forest. Herb density was significantly varied from open-moderate, moderate-close and close-open canopy cover ($p<0.01$) (Table 1).

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Table.1: Various vegetational parameters along disturbance gradients in P. roxburghii and mixed broadleaf forest.

	***Pinus roxburghii* Forest**			**Mixed broadleaf Forest**		
	Close Canopy	**Moderate Canopy**	**Open Canopy**	**Close Canopy**	**Moderate Canopy**	**Open Canopy**
Tree						
Species Richness	8	14	12	25	26	22
Density (trees/ha)	355.0	299.0	294.0	398.0	356.0	321.5
Shannon Diversity Index (H)	0.69	0.80	0.89	2.11	1.91	1.71
Simpson Index (CD)	0.74	0.72	0.68	0.31	0.36	0.37
Evenness	0.12	0.21	0.16	0.43	0.41	0.29
Shrubs						
Species Richness	35	37	33	38	33	32
Density (shrubs/ha)	27773.4	29239.9	30887.1	25683.4	23042.9	25513.3
Shannon Diversity Index (H)	3.22	3.28	2.89	3.21	3.12	3.16
Simpson Index (CD)	0.137	0.162	0.194	0.177	0.181	0.174
Evenness	0.049	0.042	0.035	0.052	0.055	0.054
Herbs						
Species Richness	104	105	116	82	84	76
Density (herbs/ha)	3524080.8	3485394.9	4140229.3	7921183.4	6170537.9	7212821.8
Shannon Diversity Index (H)	4.06	4.09	4.11	5.53	5.40	5.63
Simpson Index (CD)	0.083	0.077	0.077	0.336	0.413	0.903
Evenness	0.073	0.071	0.071	0.174	0.157	0.172
Cover (%)	82.2	56.6	27.6	81.9	56.3	27.9

Shannon diversity index for tree species varied between 1.71 and 2.11 and the minimum and maximum value were observe for open canopy and close canopy forest, respectively. It was opposite for Simpson dominance index. Simpson index was recorded highest (0.37) for tree species in open canopy forest followed by close canopy (0.31) and moderate canopy (0.36) forest. The tree diversity was significantly varied from open-moderate canopy and close-open canopy ($p<0.01$). Whereas in case of shrub the Shannon diversity index was highest (3.21) in close canopy compared to moderate (3.12) canopy and open canopy (3.12) forest. It was significantly varied from open-moderate, moderate-close and close-open canopy ($p<0.01$). For herb species, the Shannon diversity index was highest in open canopy (5.63) compared to moderate canopy (5.40) and close canopy (5.53) forest. Herb diversity was significantly varied from one canopy cover to another ($p<0.01$). While, Simpson index was also found in increasing order (0.336 for close canopy, 0.413 for moderate canopy and 0.903 for open canopy). The evenness index for tree species was found highest in close canopy (0.43) and it was recorded lowest (0.29) at intermediate level of disturbances. The higher the values of evenness index, the more even the species are in their distribution. Thus, close canopy stand has more even distribution than moderate and open canopy, even though they have more species richness than disturbed stand (Table 1). The relationship between the canopy cover and species richness indicated that the shrub richness was negatively related with canopy cover while herb richness was positively correlated with all canopy cover (fig. 1).

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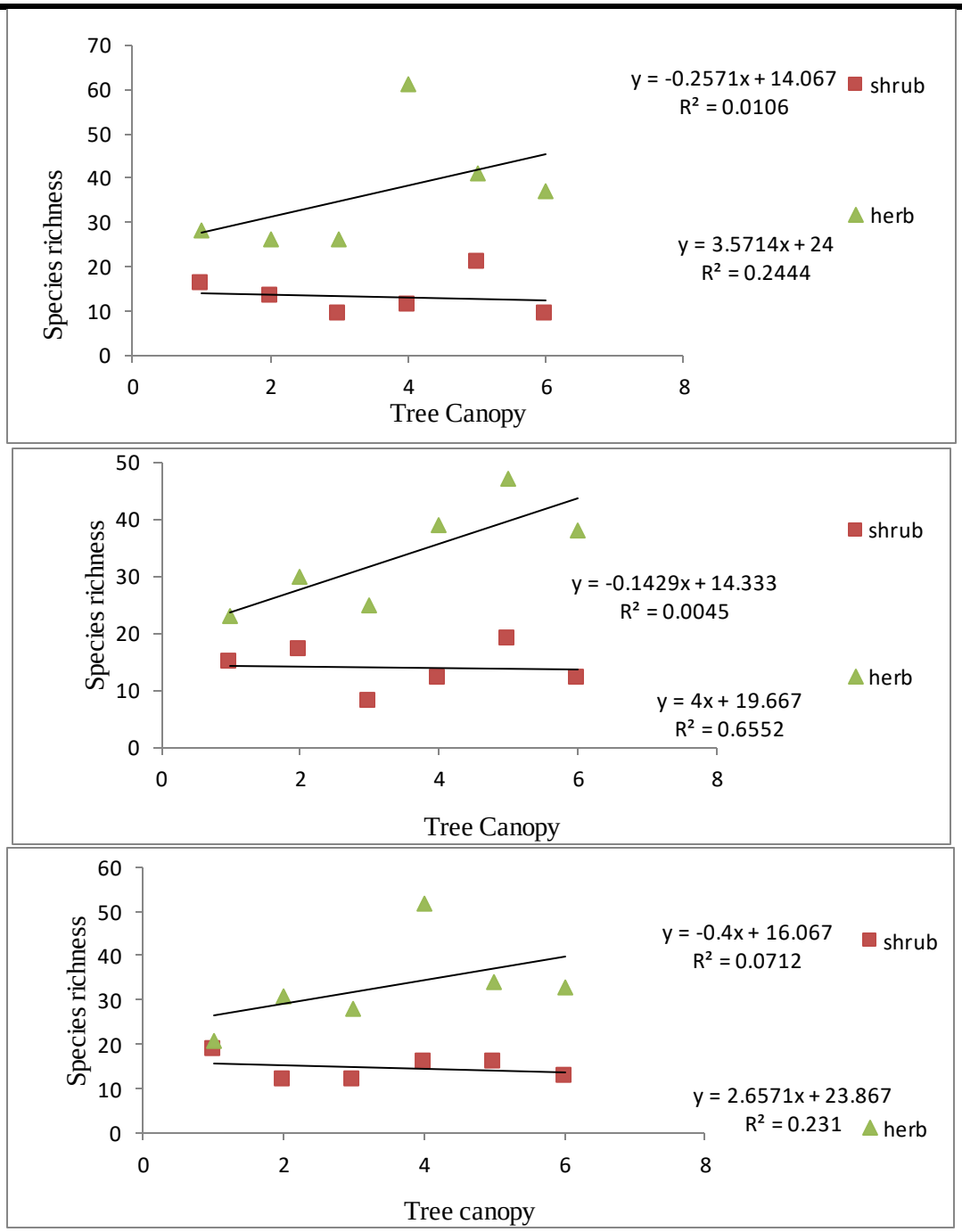

A. Open canopy B. Moderate canopy C. Close canopy

Fig.1: Relationship between tree canopy cover and shrub &herb species richness in mixed broadleaf forest

***Pinus roxburghii* Forest**

A total of 201 species were recorded in pine forest of Nainital, among which 19 were trees, 44 were shrubs and 138 were herbs. Among herbs, *Oplismenus composites* was dominating in close canopy forest, *Polygonum fulgans* was dominating in moderate canopy forest. While *Carex nubigena* and *Justicea simplex* dominate in open canopy forest. It was significantly varied from one canopy cover to another ($p<0.01$). The dominant species were identified on the basis of individual numbers. Maximum tree species (14) were recorded at intermediate level of disturbance followed by close canopy (8) and open canopy (12) forest. In case of shrub, richness was highest in moderate canopy (37) compared to close canopy (35) and open canopy (33) forest. Tree density ranged from 294.0 trees/ha in open canopy to 355.0 trees/ha in close canopy forest. The tree density was significantly varied from one canopy cover to another ($p<0.01$). In case of shrub species *Eupatorium cannabinum*

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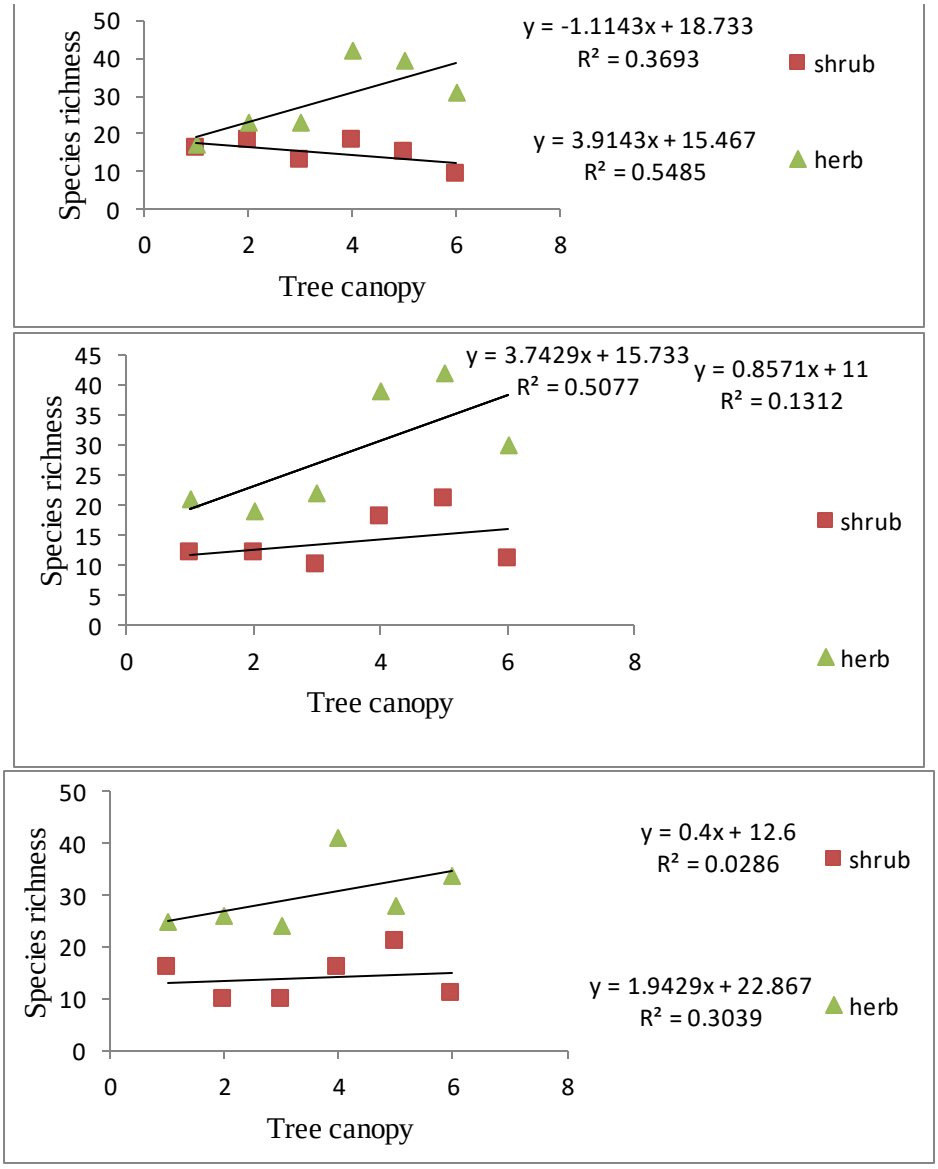
was dominant at all three level of disturbances followed by *Berbaris asiatica, Lantana camara, Rubus ellipticus* and *Myrsine affricana*. Shrub density showed the reverse pattern of trees (density ranged from 27773.4 shrub/ha in close canopy to 30887.1 shrub/ha in open canopy) whereas no trend was found in herb density. The shrub density was significantly varied from one canopy cover to another canopy cover ($p<0.01$) (Table 1).

The Shannon diversity index for tree species was increased (0.69 to 0.89) with decreasing disturbance. Similar results have been shown by herb species as they were highest in open canopy (4.11) forest followed by close canopy (4.06) and lowest in moderate canopy (4.09) forest. It was significantly varied from one canopy cover to another canopy ($p<0.01$). But in case of shrub species, the highest value (3.28) of Shannon diversity index was observed in moderate canopy followed by close canopy (3.22) and open canopy (2.89). It was significantly varied from moderate-close canopy ($p<0.01$). Simpson index for tree species also maximum in close canopy (0.74) forest. Whereas in shrubs, Simpson index was 0.137, 0.162 and 0.194 for close canopy, moderate and open canopy forest. In herbaceous layer, the highest Simpson index (0.083) was recorded in close canopy forest. Evenness for tree species was highest in moderate canopy (0.21) and it was recorded lowest (0.12) at in intermediate level of disturbance. In case of shrub and herb evenness, the maximum values were observed in close canopy forest compared to moderate and open canopy forest (Table 1). The shrub richness was significantly decrease with increasing the tree crown cover and herb richness was increase with increasing the canopy cover (fig. 2).



A. Open canopy B. Moderate canopy C. Close canopy

Fig.2: Relationship between tree canopy cover and shrub &herb species richness in Pinus roxburghii forest

The similarity index of tree indicated that the open canopy of mixed broadleaf forest and close canopy pine forest (77.78%) were more similar as compared to moderate canopy of mixed broadleaf forest and close canopy of mixed broadleaf forest(77.42%). The least similarity was showed in open canopy of pine forest and moderate canopy of mixed broadleaf forest (41.67%) (Table2). In shrub, the similarity index indicated that the open canopy of mixed broadleaf forest and close canopy of mixed broadleaf forest (92.06%) had high similarity compared to moderate canopy of mixed broadleaf forest and close canopy of mixed broadleaf forest (89.23%). Moderate canopy of mixed broadleaf forest and close canopy of pine forest showed least similarity index (74.62%) (Table 3). While, herb similarity index indicated that the moderate canopy of mixed broadleaf forest and close canopy of mixed broadleaf forest (81.72%) have high similarity compared to moderate canopy of pine forest and close canopy of mixed broadleaf forest (68.16%) (Table 4).

IV. DISCUSSION

Anthropogenic disturbances may also directly alter tree species diversity (Cayuela et al. 2006). The forest of Uttarakhand Himalaya are witnesses various disturbances which influence the distribution and composition of species in different canopy openings, forests and elevations. Loss of forest cover associated with human activities and lead to formation of Forest Island within a fragmented landscape.

Both mixed broadleaf forest and *P. roxburghii* forest showed a declined in tree species richness with increasing level of disturbance. The stability increases with the complexity of ecosystem, i.e with the number of species and with the number of interactions between them (Leigh 1965). In these forests, the stem density increased with increasing disturbance level. The high density of trees with close canopy provides moisture and humus, which are essential for the seed germination and growth of most of the shade bearer species. However, establishment and survival of all the seedlings also depends upon several other factors (Samant et al. 2002 and Joshi 2002).

The mixed broadleaf forest and *P. roxburghii* forest behave differently in term of impact of disturbance on species richness, density and diversity. Tree species richness was high in mixed broadleaf forest compared to *P. roxburghii* forest. While, shrub and herb richness were high in *P. roxburghii* forest compared to mixed broadleaf forest. Species richness reported for *P. roxburghii* forest varied from 12-142 (Ram et al. 2004, Rawal 1991 and Chandra 1991). Similarly, 35-160 species were reported for mixed broadleaf forest (Ram 2005, 2004 and Khera et al. 2001). The divers ecological condition like variation in canopy cover, forest types, topography, soil and climate may favour the greater number of species in the area. Pant and Samant (2007) reported that high richness may be of diverse habitats and suitable edaphic and climatic factors supporting growth and survival of the species.

The density was significantly high in mixed broadleaf forest. Singh et al. (1994) have reported that density value ranging from 250-2070 trees/ha across a wide altitudinal range for the forests of Kumaun Himalaya. Shrub density was significantly high in *P. roxburghii* forest because pine is an early sucessional species with spreading canopy support the growth and development few shrubs. The herb density was high in mixed broadleaf forest because the soil moisture favoured the occurrence of large number of herbaceous plant species and their population in the semi-arid climate of western Uttar Pradesh (Sharma and Upadhayay 2002). Srivastava and Singh (2005) have reported that the growth of grasses were predominant at the disturbed site during rainy season. As a result of mild grazing, most graminoids grow by increasing their tillers and persist for long time with annuals and finally maintain higher cumulative density of perennials and annuals in grasslands.

The tree, shrub and herb diversity was high in mixed broadleaf forest compared to pine forest. Monk (1967) and Risser and Rice (1971) obtained 2-3 as the highest value for diversity index of temperate forest on the other hand, tropical forest indicated that higher diversity as calculated by Knight (1975) for Young (H=5.06) and Old (H=5.40) stands. Braum (1950) has reported that 1.69-3.40 value of tree diversity of certain temperate forest were lower than the value reported for tropical forest. The lower diversity of temperate vegetation could be due to lower rate of evolution of diversification of communities (Fisher 1960 and Simpson 1964). The diversity value for tree were in the range of 0.33-2.95 reported for most of the low elevational Central Himalayan forest (Saxena and Singh 1982, Ralhan et al. 1982, Upreti et al. 1985, Bargali et al. 1987, Tripathi et al. 1987 and Rikhari et al. 1991). The disturbance may play an important role in maintaining diversity in these forests. The effect of disturbance and resource availability on the abundance and diversity of herbaceous vegetation have been widely studied (Timothy et al. 1998). In Northern hard woods forests, large scale disturbance increase the abundance and diversity of ground vegetation by increasing resource availability (Bormann and Likens 1979, Hughes and Fahey 1991), but after several decades of stand development, the pattern of abundance of herbs appears to resemble those of the pre-disturbance forest (Reinors 1992).

To conclude the mixed broadleaf forest is more conservative for tree species richness and overall diversity

while shrub and herb richness are more in pine forest. These points should keep in mind while managing such Himalayan forests.

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Population Densities of the Plum scale Insect, *Parlatoria oleae* (Colvee) (Hemiptera: Diaspididae) in relation to the Resultant yield of Mango Fruits

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***Abstract*—** *The main objective of this work is to evaluate the relationship between the pest population, Parlatoria oleae (Colvee), through pest activity peaks in October/November; April and July on the yield loss of seedy Balady mango trees at Esna district, Luxor Governorate, Egypt through two seasons (2016/2017 and 2017/2018). Data revealed that the increment of population density caused gradual decrease of the yield of mango fruits, consequently increased the percentage of yield loss when the data of the yield were colligated with the pest population peaks in October/November; April and July months through 2016/2017 and 2017/2018 seasons. Increasing one individual of pest per leaf caused a reduction of the mango yield by 2.53, 2.51 and 4.59 kg/tree and 2.25, 2.19 and 2.88 kg/tree through the periods of population abundance of the two seasons, respectively. Accordingly, increased yield loss percentages by 1.05, 1.04 and 1.90% and 0.87, 0.84 and 1.10% occurred during the mentioned periods of the two consecutive seasons (2016/2017 and 2017/2018), respectively. These results confirmed that P. oleae population during the peak of April resulted the least expected amount of mango fruits yield and the greatest loss in mango yield. On contrary, the peak during July was less effective, causing the highest expected yield and the lowest reduction in mango fruits yield of the two seasons.*

Generally, the reduction in yield of mango fruits is known to be a summation of many factors including the rate of infestation, time of infestation and variety ability to infestation.

Keywords*— *Parlatoria oleae, pest population, mango yield and reduction.

I. INTRODUCTION

Mango fruits, *Mangier indica* L. (Anacardiaceae) are considered of the most popular in Egypt. Egyptian mango occupied economic importance in the world market for rich flavor and tasty. Mango trees are liable for infestation by several pests. Among which *P. oleae* is considered one of the main destructive pests **(Bakr *et al*., 2009**). This armored scale species injures the shoots, twigs, leaves, branches and fruits by sucking the plant sap with its mouth parts, causing thereafter deformations, defoliation, drying up of young twigs, dieback, poor blossoming, death of twig by the action of the toxic saliva and so affecting the commercial value of fruits where it causes conspicuous pink blemishes around the feeding sites of the scales. A characteristic symptom of infestation by this pest species is the visible accumulations of scales on the attacked mango parts **(El-Amir, 2002 and Hassan *et al*., 2009)**.

The target of this study was to find out the relationship between *P. oleae* population density during three peaks of its seasonal activity (independent factors) on percentage of mango yield loss (dependent factor) during two seasons (2016/2017 and 2017/2018).

II. MATERIALS AND METHODS

This investigation was carried out on mango trees in a private orchard situated at Esna, Luxor Governorate during the period from September, 2016 until mid of August, 2018., to clarify the effect of the rate of infestation by *P. oleae* on the yield of seedy Balady mango variety.

The samples consisted of twenty seedy Balady mango trees (ten almost uninfested and ten severely infested. Trees representing each group were chosen to be of homogeneous infestation rate as far as possible. These trees were of the same age (about twelve years old) and of almost, the same size, shape, height and vegetative growth. All trees in this orchard received the normal agricultural practices, except for being free from any chemical treatment, before and during the period of investigation. Regular bimonthly samples consisted of 20 leaves, were randomly chosen per tree representing the four directions and heights of mango trees. Samples were picked regularly and placed in polyethylene bags and immediately transferred to the laboratory where the leaves of each sample were throughly inspected using a

binocular microscope. Numbers of alive *P. oleae* individuals on upper and lower surfaces of mango leaves were counted and recorded. The monthly mean counts of *P. oleae* scales per leaf was considered in this study to express the population size of pest. The yield of each uninfested and severely infested mango trees was assessed.

Simple regression was used to elucidate the variability of yield loss that could be mostly caused by the pest during the three peaks of seasonal activity. Partial regression was used to find out the simultaneous effects of insect activity peaks in October, November, April and July on mango yield. The equation of linear regression was calculated according to the following formula of **Fisher (1950)** and **Hosny *et al.* (1972)**:

$$Y_{=} = a \pm bx$$

Where:

$Y_{=}$ Prediction value (Dependent variable)

a = Constant (y - intercept)

b = Regression coefficient

x = Independent variable

This method was helpful in obtaining basic information about the amount of variability in the yield that could be attributed to these peaks of activity, together, which was calculated as percentage of explained variance (E.V.%). The partial regression values indicate the average rate of change in yield due to a unit change in any of the three peaks of insect activity. Statistical analysis in the present work was carried out by **MSTATC Program, 1980.** All figures were done by Microsoft Excel 2010.

The amount of yield losses and damage due to scale insect were calculated according to the following equation:

$$\%\text{Yield loss} = \frac{A - B}{A} \times 100$$

Which:

A = Yield from uninfested trees

B = Yield from infested trees.

* Average of yield from mango uninfested trees were 198 and 210 kg/tree during the first and the second seasons of this study, respectively.

III. RESULTS AND DISCUSSION

A- Seasonal activity of *P. oleae* on mango trees:

The half-monthly counts of alive stages of *P. oleae* that infested seedy Balady mango trees by pest at the region of study were recorded during the seasons of 2016/2017 and 2017/2018. Accordingly, it's better to discuss the peaks of seasonal abundance on basis of the monthly mean numbers for every season.

As shown in Table (1), three peaks of total population abundance of *P. oleae* on mango trees, at the region of study, occurred during October, April and July, as the general means of population density were 151.39, 106.46 and 136.59 individuals/leaf in the first season, and were 154.73, 131.46 and 147.19 individuals/leaf during November, April and July in the second season, respectively. The first peak of the pest in October for the first season and November during the second season was the highest, compared to the two other peaks, but the peak of April was the lowest in the two seasons. Also, the peaks of the total population means of the pest through the second season were higher than those recorded in the first season, that might be the attributed to more favorable environmental conditions that occurred during the second season.

B- Relationship between yield and the pest population of *P. oleae*:

Data in Table (1) and illustrated in Figs. (1 and 2) revealed that the mango (seedy Balady variety) yield decreased gradually with the increase of *P. oleae* total population density during the three peaks of seasonal activity in the two seasons. These results confirmed the reciprocal relation between mango yield and the total population density in the three peaks of insect activity during both seasons. However, in the same Table (1), the relationship between the percentages of reduction in mango yield (dependent variable) and the population density of *P. oleae* per leaf of mango as independent factors was positive relations in all peaks of seasonal activities during the two seasons (2016/2017 and 2017/2018). An increase of the percentages of reduction of yield occurred with increasing of the total population density in all peaks during the both seasons (Table, 1 and Figs., 1 and 2).

C- Effect of the total population density of *P. oleae* on the yield:

Statistical analysis of data in Table (2) revealed a highly significant negative correlation between the mango yield and the three peaks of the pest population which were -0.92, -0.96 and -0.96; and -0.96, -0.94 and -0.91 during October or November; April and June peaks during each of the two seasons, respectively. The regression coefficient (b) of the unit effect indicated that an increase of one insect per leaf would decrease the yield of mangos by 2.53, 2.51 and 4.59 kg. per tree through the first season and 2.25, 2.19 and 2.88 kg. per tree during the second season, respectively.

The exact relationship between the peaks of insects abundance and the yield of mango was determined by the partial regression coefficient values (Table, 2). It was insignificantly positive during the peak of October in the first season (P. reg. was +1.13), and negatively insignificant (P. reg. was -0.91) during the peak of November in the second season. While, this relation was insignificant negative (P. reg.; -1.81 and -0.91) during the

peaks of April in the two seasons. However, it was insignificantly negative (P. reg. was -3.32) in the first season and insignificantly positive (P. reg. was +1.97) in the second season when statistical analysis was calculated for the peak of July in relation to *P. oleae* population density through the two successive seasons. Likewise, the partial correlations were + 0.47, - 0.70 and -0.70 during the peaks of October, April and July in the first season and it -0.63, -0.31 and 0.44 through the peaks of November, April and July, respectively in the second season,. The calculated partial regression values indicated simultaneous effects of the three peaks of insect population on the mango yield during the two seasons.

The obtained results showed that the combined effect of the pest activity peaks on the mango yield was highly significant where the "F" values were 49.62 and 27.46 during the 1st and 2nd seasons, respectively (Table 2). The amount of the variability that could be attributed to the combined effect of these peaks on the mango yield was expressed as explained variance percentage (E.V.%), which was 96.13 and 93.21% during the two seasons, respectively. The remaining unexplained variances are assumed to be due to other undetermined factor influences.

These findings are in harmony with those reported by **Hernandez *et al.* (2002)** who found a positive correlation between fruit infestation and yield loss at harvest among consecutive seasons, when they studied the relationship between the population densities of *Aonidiella aurantii* (Mask.) in relation to the yield of citrus trees.

D- Prediction of mango yield and its loss:

Prediction equations for yield of mango and its losses by the action of *P. oleae* infestation were concluded according to the statistical analysis between the two accumulated seasons data. Results of calculations may be presented as follows:

1- The total population density of *P. oleae* during the three peaks versus the yield of mango:

$Y = 526.26^{**} - 3.47\ X_1^{**} + 0.37\ X_2 + 1.16\ X_3$; E.V.% = 86.05%

2- The total population density of *P. oleae* during the three peaks versus the percentages of reduction in mango yield:

$Y = -102.24^{**} + 1.10\ X_1^{**} + 0.12\ X_2 - 0.43X_3$; E.V.%= 87.24%

Where: Y= Prediction value
E.V.% = Explained variance
X_1= Means of peak in October / November altogether
X_2= Means of peak in April
X_3= Means of peak in July
* Significant at $P \leq 0.05$
** Highly significant at $P \leq 0.01$

The aforementioned results on the effect of the three peaks of the pest population peaks of abundance on the yield of mango and its losses during the two successive seasons emphasize that the effect of these factors varied from season to another. This might be due to many factors *i.e.* environmental conditions, rate of infestation, time of infestation and variety ability to infestation.

E- The calculated yield:

The simple linear regression equations were applied to estimate the expected yield of mango Results in Table (3) indicated that the heaviest weight of yield (225 and 240 kg. per tree) was recorded at the lowest values of total density of population of *P. oleae* in all peaks of seasonal activity through the two seasons. While, the minimum yield (168 and 180 kg. per tree) was estimated with the highest values of the total population density of *P. oleae* in the three peaks of activity during the two seasons, respectively (negative correlation).

These results are similar to those obtained by **Mohamed and Asfoor (2004),** in Egypt, the anthors studied the effect of the California red scale, *A. aurantii* infestation on the citrus yield loss and found that the reduction in Valencia orange was higher than that of Navel. They estimated the damage caused as 31.14 and 27.15% reduction in the yield, respectively.

F- The calculated reduction in yield:

The simple linear regression equations were used to determine the expected reduction in yield of mango. Data are presented in Table (4). These data showed that the least loss percentage in yield (6.56 and 7.62%) were recorded with the lowest rates of *P. oleae* population densities in all periods of seasonal abundance during the two seasons. While, the highest loss percentages in yield (30.23 and 30.72%) occurred with the highest values of the total population density by *P. oleae* in the three peaks of the seasonal activity during the two seasons, respectively (positive relation).

These results agree with those obtained by **Salman and Bakry (2012)** in Egypt, they found that the increase in population density of the mealybug, *Icerya seychellarum* decreased the yield by 3.6, 6.5 and 4.3 kg/tree and 2.5, 4.1 and 2.3 kg/tree. Thus confirming the negative relationship between the pest population density and the resultant yield of mango during two successive season, respectively. the same authors found, also, that the percentage of the yield loss by 1.47, 2.64 and 1.77 % and 1.47, 1.97 and 1.08 % occurred when the yield data were correlated with the peaks of insect population in October, May and August, respectively through the two successive seasons. Also, **Bakry and Mohamed (2015)** mentioned that the increase in population density of *A. aurantii* in the four peaks of the pest population decreased the mango yield gradually by 1.37, 1.47, 4.25 and 1.77 kg/tree and 1.45, 1.53, 4.66 and 1.85 kg/tree during two

successive seasons, respectively and increased the percentage of the yield loss by 0.55, 0.59, 1.70 and 0.71 % and 0.60, 0.63, 1.90 and 0.76 %; when the mango yield data were linked with the periods of population abundance in October, December, April and July through two successive seasons, 2016-2017 and 2017-2018, respectively.

G- Expected values in the yield and its loss with increasing the pest population:

Concerning, the comparison between the peaks of the pest population of *P. oleae* and their effect on the yield of mango during the two successive seasons (2016/2017 and 2017/2018), was depending on the total number of the pest per leaf for all periods of population abundance (Table, 5).

The results revealed that the total population density of pest in peak of April was more effective causing the lowest expected values in mango yield with averages of 258.08 and 316.62 kg/tree through the two successive seasons, respectively. While, the peak of total population in July was the least effective causing the highest expected values in mango yield with averages of 446.04 and 395.88 kg/tree during the two successive seasons, respectively (Table, 5).

As regarding, the prospective values with (increase or decrease) in the percentage of yield loss with increasing the infestation rates by *P. oleae* during the two successive seasons (Table, 5). The results showed that the total population density during July peak was least effective causing the least percentages of reduction in mango yield with an average of -85.24 and -52.38% during the two successive seasons, respectively. But, the pest population was more effective during April peak causing the greatest loss in mango yield with an average of -7.17 and -21.87% during the two successive seasons, respectively.

Generally, it seems that the population density of *P. oleae* during April peak was the most serious one, during the two seasons, causing the greatest loss in mango yield which that coincided with the newly spring growth cycle for the vegetative growth of mango trees. These results are accordance with the findings of **El-said (2006)** who found that the high infestation levels by *Icerya seycellarum* and the feeding of this pest species caused a serious damage resulting in early leaves drop and mango yield reduction. **Bakry (2009)** reported that the early season infestation with the Maskell scale insect, *Insulaspis pallidula* during May was more effective than other months causing the greatest loss in mango yield. Also, **Salman and Bakry (2012)** stated that the early infestation with the mealybug, *Icerya Seychellarum* during May was more effective than other months causing the greatest loss in mango yield. **Bakry and Mohamed (2015)** reported that the infestation by *Aonidiella aurantii* (Mask.) (during April was more effective than other time causing the greatest loss in mango yield.

Generally, it could be concluded that the reduction in mango fruits is known to be a summation of many factors including the rate of infestation, time of infestation and variety ability to infestation. These results are similar to those obtained by **Reddy-Seshu (1992)** who found a linear relationship between infestation rate and yield loss, and more increasing in yield loss occurred as a result of the earlier infestation. **Also, Selim (2002)** studied the effect of Maskell scale insect, *Insulaspis pallidula* (Green) infestation on the yield of mango trees. He stated that the yield decreased gradually with increasing the population density of this pest. The same author added that the yield decreased gradually with increasing the population density of *I. pallidula* (Green) in four peaks (September, April, July and August).

Table.1: Effect of infestation by P. oleae total population on the yield of seedy Balady mango variety during three peaks of the seasonal activity of the pest during the two seasons (2016/2017 and 2017/2018).

Season	Inspected trees	Yield (kg)	Yield reduction (%)	Peaks of *P. oleae* total population			Average of population density
				Oct./ Nov.	April	July	
2016/2017	1	225.0	6.56	136.30	93.44	129.79	119.84
	2	224.0	6.98	145.94	99.63	133.28	126.28
	3	210.0	12.79	148.36	102.42	133.48	128.09
	4	208.0	13.62	151.39	104.33	134.97	130.23
	5	196.0	18.60	151.39	106.46	136.29	131.38
	6	195.0	19.02	151.52	106.46	136.97	131.65

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	7	192.0	20.27	154.82	110.24	137.39	134.15
	8	182.0	24.42	156.42	110.80	139.66	135.62
	9	180.0	25.25	158.13	111.08	142.04	137.08
	10	168.0	30.23	159.60	119.73	142.09	140.47
Average		**198.0**	**17.77**	**151.39**	**106.46**	**136.59**	**131.48**
2017/2018	**1**	240.0	7.62	139.06	114.94	133.32	129.11
	2	234.0	9.93	146.31	122.89	143.66	137.62
	3	225.0	13.39	150.31	126.26	144.36	140.31
	4	224.0	13.78	151.63	128.83	146.06	142.18
	5	210.0	19.17	154.73	131.46	146.44	144.21
	6	210.0	19.17	154.73	131.46	146.49	144.23
	7	196.0	24.56	156.20	136.60	149.48	147.43
	8	195.0	24.94	158.76	136.97	149.48	148.40
	9	182.0	29.95	164.77	137.15	154.00	151.98
	10	180.0	30.72	170.78	148.05	158.60	159.14
Average		**210.0**	**19.32**	**154.73**	**131.46**	**147.19**	**144.46**

Average of yield from mango uninfested trees were 198 and 210 kg/tree during the first and the second seasons of this study, respectively.

Table.2: Different correlation models and regression analyses for describing the relationship between P. oleae population density and the mango yield during the two seasons (2016/2017 and 2017/2018).

Season	Tested counts	Simple correlation and regression values				Partial correlation and regression values				Analysis variance			
		r	b	S.E	t-test	P. cor.	P. reg.	S.E	t-test	F values	MR	R^2	E.V.%
2016 / 2017	Average no. of individuals/leaf (Oct.)	- 0.92	-2.53	0.39	-6.48 **	0.47	1.13	0.87	1.29	49.62 **	0.98	0.96	96.13
	Average no. of individuals/leaf (April)	-0.96	-2.51	0.25	-9.92 **	-0.70	-1.81	0.76	-2.39				
	Average no. of individuals/leaf (July)	-0.96	-4.59	0.46	-9.88 **	-0.70	-3.32	1.39	-2.38				
2017 / 2018	Average no. of individuals/leaf (Nov.)	-0.96	-2.25	0.24	-9.24 **	-0.63	-2.79	1.44	-1.94	27.46 **	0.97	0.93	93.21
	Average no. of individuals/leaf (April)	-0.94	-2.19	0.27	-8.02 **	-0.31	-0.91	1.17	-0.77				
	Average no. of individuals/leaf (July)	-0.91	-2.88	0.45	-6.34 **	0.44	1.97	1.68	1.17				

r = Simple correlation; P. cor. = Partial correlation; MR = Multiple correlation; b = Simple regression; P. reg. = Partial regression; R^2= Coefficient of determination; E.V% = Explained variance; S.E = Standard error; * Significant at P ≤ 0.05 and ** Highly significant at P ≤ 0.01.

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Table.3: Gradual decrease in mango fruits yield in relation to population density increase of P. oleae total population of during three peaks of insects abundance during the two successive seasons.

Season	Inspected trees	Yield (kg)	Oct. / Nov. infestation		April infestation		July infestation		Means of	
			No. of insects / leaf	Expected yield	No. of insects / leaf	Expected yield	No. of insects / leaf	Expected yield	No. of insects / leaf	Expected yield
2016/2017	1	**225**	136.3	**236.2**	93.4	**230.6**	129.8	**229.2**	119.8	**233.6**
	2	**224**	145.9	**211.8**	99.6	**215.1**	133.3	**213.2**	126.3	**213.9**
	3	**210**	148.4	**205.7**	102.4	**208.1**	133.5	**212.3**	128.1	**208.4**
	4	**208**	151.4	**198.0**	104.3	**203.3**	135.0	**205.4**	130.2	**201.8**
	5	**196**	151.4	**198.0**	106.5	**198.0**	136.3	**199.4**	131.4	**198.3**
	6	**195**	151.5	**197.7**	106.5	**198.0**	137.0	**196.3**	131.6	**197.5**
	7	**192**	154.8	**189.3**	110.2	**188.5**	137.4	**194.4**	134.1	**189.8**
	8	**182**	156.4	**185.3**	110.8	**187.1**	139.7	**183.9**	135.6	**185.3**
	9	**180**	158.1	**180.9**	111.1	**186.4**	142.0	**173.0**	137.1	**180.8**
	10	**168**	159.6	**177.2**	119.7	**164.7**	142.1	**172.8**	140.5	**170.5**
2017/2018	1	**240**	139.1	**244.9**	114.9	**245.7**	133.3	**249.5**	129.1	**247.1**
	2	**234**	146.3	**228.5**	122.9	**228.3**	143.7	**219.8**	137.6	**226.3**
	3	**225**	150.3	**219.5**	126.3	**221.0**	144.4	**217.8**	140.3	**219.8**
	4	**224**	151.6	**216.6**	128.8	**215.3**	146.1	**212.8**	142.2	**215.2**
	5	**210**	154.7	**209.6**	131.5	**209.6**	146.4	**211.8**	144.2	**210.2**
	6	**210**	154.7	**209.6**	131.5	**209.6**	146.5	**211.6**	144.2	**210.2**
	7	**196**	156.2	**206.3**	136.6	**198.4**	149.5	**203.0**	147.4	**202.3**
	8	**195**	158.8	**200.5**	137.0	**197.6**	149.5	**203.0**	148.4	**200.0**
	9	**182**	164.8	**187.0**	137.2	**197.2**	154.0	**190.0**	152.0	**191.2**
	10	**180**	170.8	**173.5**	148.0	**173.4**	158.6	**176.7**	159.1	**173.7**

Table.4: Gradual increase in yield loss with the population density increase of the total population of P. oleae during three peaks of the seasonal activity during the two successive seasons.

Season	Inspected trees	% Yield reduction	Oct. / Nov. infestation		April infestation		July infestation		General average	
			No. of insects / leaf	% Calculated reduction	No. of insects / leaf	% Calculated reduction	No. of insects / leaf	% Calculated reduction	No. of insects / leaf	% Calculated reduction
2016/2017	**1**	**6.6**	136.3	**1.91**	93.4	**4.2**	129.8	**4.8**	119.8	**2.98**
	2	**7.0**	145.9	**12.06**	99.6	**10.7**	133.3	**11.5**	126.3	**11.17**
	3	**12.8**	148.4	**14.59**	102.4	**13.6**	133.5	**11.8**	128.1	**13.46**
	4	**13.6**	151.4	**17.78**	104.3	**15.6**	135.0	**14.7**	130.2	**16.19**
	5	**18.6**	151.4	**17.78**	106.5	**17.8**	136.3	**17.2**	131.4	**17.65**
	6	**19.0**	151.5	**17.92**	106.5	**17.8**	137.0	**18.5**	131.6	**17.99**

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	7	**20.3**	154.8	**21.39**	110.2	**21.7**	137.4	**19.3**	134.1	**21.17**
	8	**24.4**	156.4	**23.06**	110.8	**22.3**	139.7	**23.6**	135.6	**23.05**
	9	**25.2**	158.1	**24.87**	111.1	**22.6**	142.0	**28.1**	137.1	**24.90**
	10	**30.2**	159.6	**26.41**	119.7	**31.6**	142.1	**28.2**	140.5	**29.22**
2017/2018	**1**	**7.62**	139.1	**5.75**	114.9	**5.4**	133.3	**4.0**	129.1	**4.9**
	2	**9.93**	146.3	**12.0**	122.9	**12.1**	143.7	**15.4**	137.6	**12.9**
	3	**13.39**	150.3	**15.5**	126.3	**14.9**	144.4	**16.2**	140.3	**15.4**
	4	**13.78**	151.6	**16.6**	128.8	**17.1**	146.1	**18.1**	142.2	**17.2**
	5	**19.17**	154.7	**19.3**	131.5	**19.3**	146.4	**18.5**	144.2	**19.1**
	6	**19.17**	154.7	**19.3**	131.5	**19.3**	146.5	**18.5**	144.2	**19.1**
	7	**24.56**	156.2	**20.6**	136.6	**23.6**	149.5	**21.9**	147.4	**22.1**
	8	**24.94**	158.8	**22.8**	137.0	**24.0**	149.5	**21.9**	148.4	**23.0**
	9	**29.95**	164.8	**28.0**	137.2	**24.1**	154.0	**26.9**	152.0	**26.4**
	10	**30.72**	170.8	**33.2**	148.0	**33.3**	158.6	**32.0**	159.1	**33.1**

Table.5: Expected values (increase or decrease) in the yield and its loss with increasing the population density of P. oleae during three peaks of the seasonal activity during 2016/2017 and 2017/2018 seasons.

Season	**No. of insects / leaf**	**Calculated yield**			**% Yield reduction**		
		Oct. / Nov. peak	**April peak**	**July peak**	**Oct. / Nov. peak**	**April peak**	**July peak**
2016/2017	15	543.28	427.35	755.56	-125.62	-77.47	-213.78
	30	505.31	389.73	686.78	-109.85	-61.85	-185.21
	45	467.33	352.12	617.99	-94.08	-46.23	-156.65
	60	429.36	314.50	549.21	-78.31	-30.61	-128.08
	75	391.38	276.89	480.43	-62.54	-14.98	-99.52
	90	353.41	239.28	411.65	-46.76	0.64	-70.95
	105	315.43	201.66	342.86	-30.99	16.26	-42.39
	120	277.46	164.05	274.08	-15.22	31.88	-13.82
	135	239.48	126.43	205.30	0.55	47.50	14.74
	150	201.51	88.82	136.52	16.32	63.12	43.31
Mean	**82.50**	**372.39**	**258.08**	**446.04**	**-54.65**	**-7.17**	**-85.24**
2017/2018	15	524.02	464.15	590.26	-101.70	-78.66	-127.19
	30	490.26	431.37	547.06	-88.71	-66.04	-110.57
	45	456.51	398.58	503.87	-75.72	-53.42	-93.94
	60	422.76	365.80	460.67	-62.72	-40.80	-77.32
	75	389.01	333.01	417.48	-49.73	-28.18	-60.69
	90	355.25	300.23	374.29	-36.74	-15.56	-44.06
	105	321.50	267.44	331.09	-23.75	-2.94	-27.44
	120	287.75	234.66	287.90	-10.76	9.68	-10.81
	135	253.99	201.87	244.70	2.23	22.30	5.81
	150	220.24	169.09	201.51	15.23	34.92	22.44
Mean	**82.50**	**372.13**	**316.62**	**395.88**	**-43.24**	**-21.87**	**-52.38**

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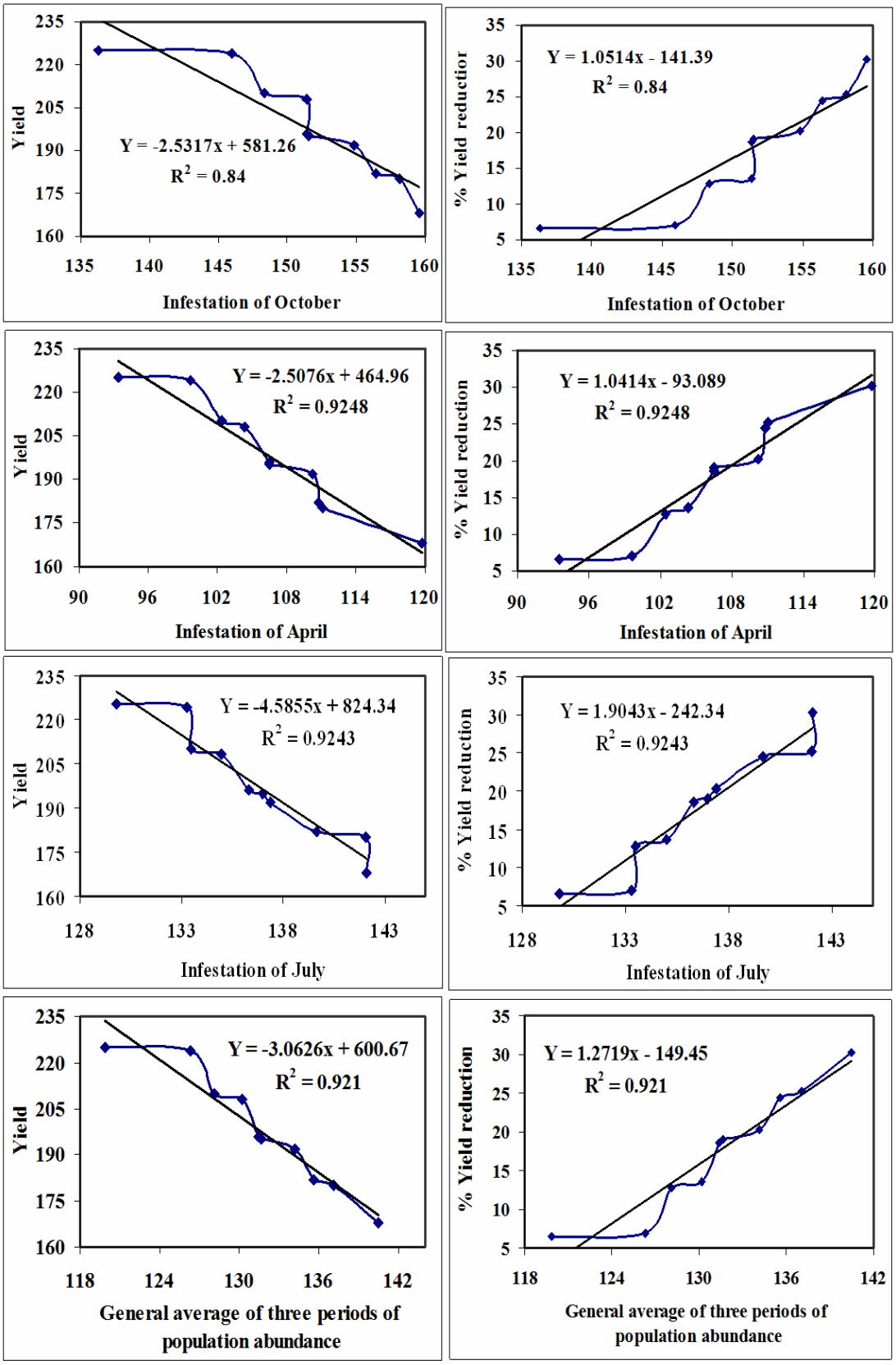

Fig.1: Relationship between the total population of P. oleae and mango yield (seedy Balady variety) and yield reduction during the first season (2016/2017).

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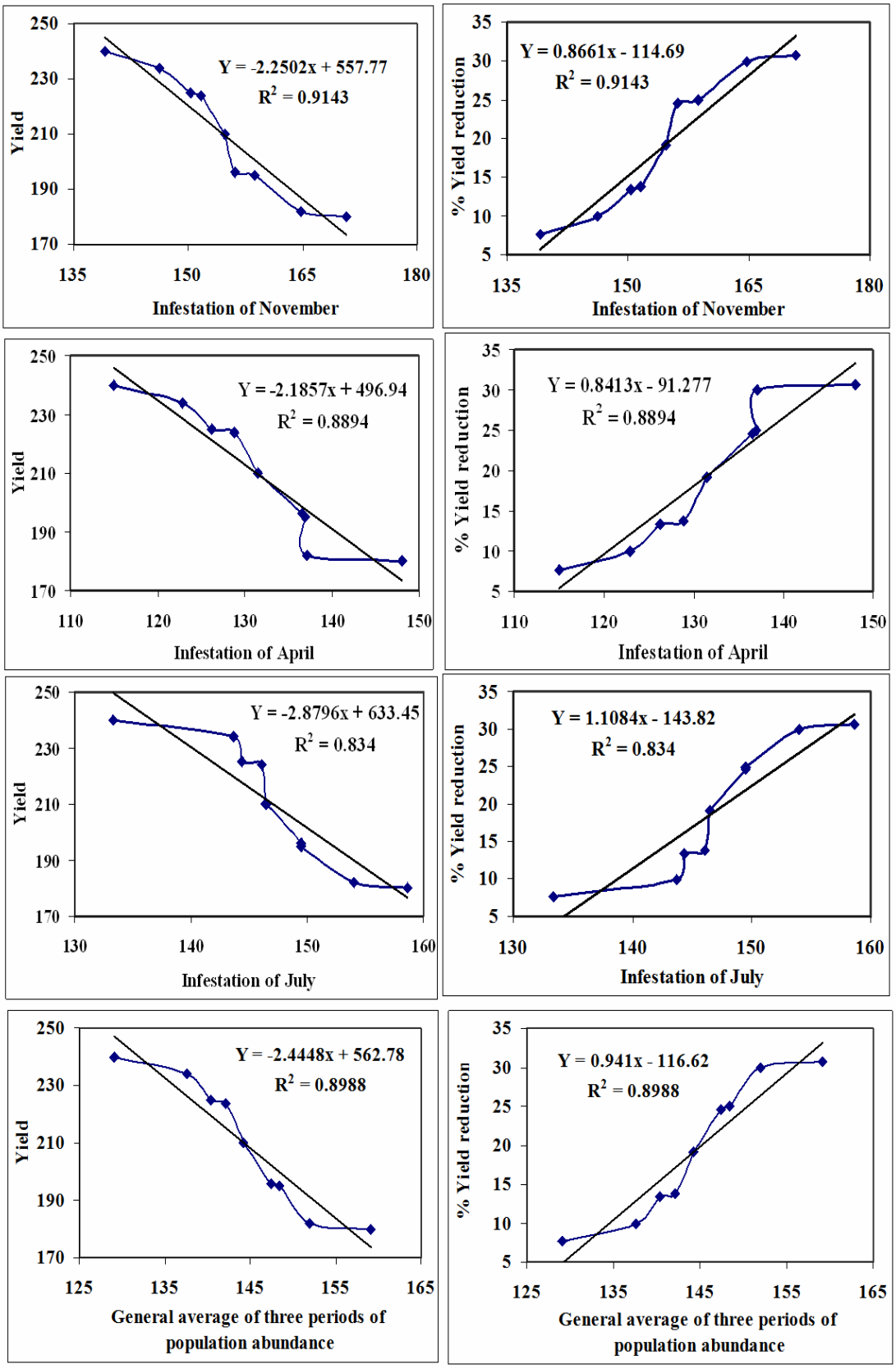

Fig.2: Relationship between population abundance of P. oleae and mango yield (seedy Balady variety) and its reduction during the second season (2017/2018).

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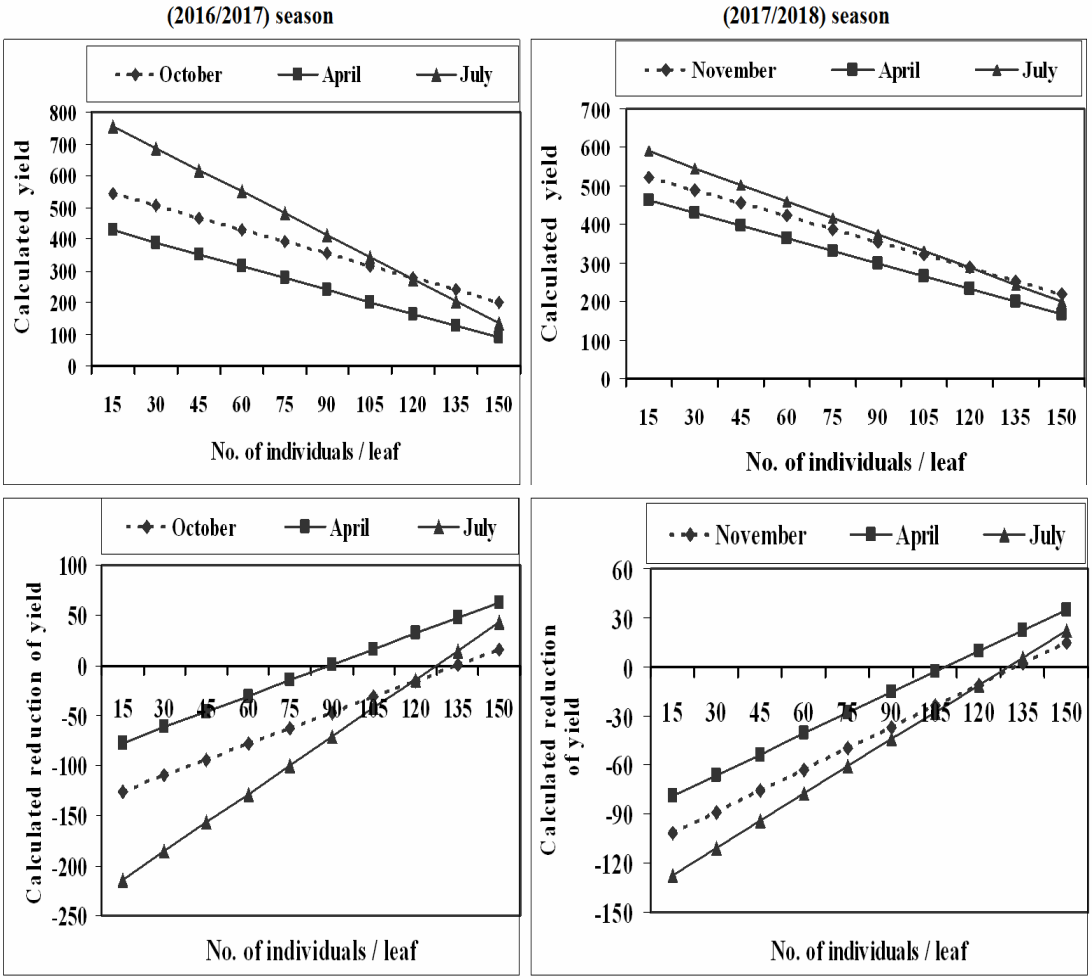

Fig.3: Expected values (increase or decrease) in mango yield fruits and its loss with increasing of population density by P. oleae during three peaks of abundance during the two seasons (2016/2017 and 2017/2018).

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SBR-Based Light Cimental Compositions for use in Sustainable Constructions

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Abstract**—** *The practice of sustainable development is a reality present in various segments of society. In this sense, buildings must be ecologically correct, socially just, culturally accepted, and economically viable. Seeking sustainability, the use of non-conventional building materials is increasing, especially for works of social interest. The aim of the present work was to develop light SBR - based composites based on industrial waste from the footwear sector, aiming at the elaboration of construction elements for the thermal protection of masonry.For the production of the composites, Portland cement type CP II F - 32 and a residue from a footwear industry based on SBR were used. For this, the composition of the granulometry of the light aggregate and the appropriate water / cement ratio were defined, and adequate values of resistance to compression, bending and absorption were considered.Therefore, the composition of the granulometry of the light aggregate and the appropriate water/cement ratio were defined, and adequate values of resistance to compression, bending and absorption were considered. Then, the influence of the incorporation content of the lightweight aggregate and the molding pressure to be used in the composites was evaluated, where flexural strength, absorption capacity, bulk density and visual analysis were performed.In addition, the thermal conductivity was determined in the optimized traces aiming the use of these composites as constructive elements for thermal protection of masonry. The lower values of absorption factors and voids index were reached when molding using the pressure level N1 (0.16 MPa). The bulk density factor of the pressure-molded composites reached the lowest values also under the pressure level N1 (0.16 MPa). The composites with higher contents of incorporation of molded waste without pressure showed no results resistance to bending and absorption.*

Keywords**— industrial waste, thermal insulation, construction elements, sustainability, rural constructions.***

I. INTRODUCTION

Man is increasingly exploiting the natural resources of the planet in an undisciplined way, causing the possibility of their scarcity and consequent degradation of the environment. As in the most diverse areas of science, in search of the development of eco-friendly technologies, research in the construction sector has been intensified in order to obtain high-energy efficiency materials, manufactured with industrial solid waste, that perform well and meet current expectations durability and strength.

Silva (2012) characterized the rubber based butadiene styrene (SBR) residue from a footwear industry in Campina Grande, Brazil, and concluded that this residue is classified as CLASS I - DANGEROUS, as it presents, in its leached extract, heavy metals with values above what are established in standard. In addition, the author considered that because it is coated with a layer of cement paste, the residue has the potential to remain isolated, making it feasible for use in rural and urban construction applications.

Soares et al. (2008) used eight types of mixtures to produce light mortars containing waste from the shoe industry and ceramics: four containing only shoe residue and cement; and another four containing footwear residue, cement and 30% of waste from the ceramic industry in substitution of cement. They concluded that the composite obtained can be used as a safe, ecological and low cost alternative for the manufacture of masonry panels, flat and corrugated boards for use in rural buildings, among other applications.

Rios et al. (2009) found that the composite cement matrix and polymeric reinforcement (SBR) is technically feasible, with a modulus of rupture and compression consistent with the minimum resistances established and

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validated in the market by similar products. These authors stated that when submitted to low pressure energy, the composite shows excellent degree of packaging, lower porosity, better ductility and resistivity.

Thus, this study aimed to elaborate lightweight composites using solid residues based on SBR, originated from the manufacture of footwear, for using in sustainable and low cost constructions.

II. MATERIALS AND METHODS

This study was carried out in the Laboratory of Recyclable Materials of the Rural Constructions area of the Department of Agricultural Engineering of the Federal University of Campina Grande, Campina Grande, Brazil. Different levels of incorporation of solid residues based on SBR from the manufacture of footwear were tested in a cementitious matrix under different molding pressure levels for the development of lightweight plate - shaped construction elements intended for use as insulation thermal, in rural and urban constructions.

For the production of the composites, Portland cement type CP II F - 32 and a residue from a footwear industry of the city of Campina Grande, based on SBR, were used. Table 1 shows the main physical characteristics of the cement.

Table.1: Physical characteristics of Portland cement CP II F-32

Features	Unit	Value
Fineness of the Mesh n° 200	%	≤12
Specific Area (Blaine)	cm^2g^{-1}	≥ 2600
Cement handle start	h:min	≥1:00
End of cement handle	h:min	≤10:00
Simple Compressive Strength at 28 days	MPa	≥32

Silva (2012) evaluated this residue and mentioned that it is hydrophobic, presenting difficulty of interaction with the cement; is very porous, with voids index of 57.06% and pores with a mean diameter of 3.6413µm; however, is not permeable. According to Rios (2008), the composites cement:SBR presented resistance to combustibility, characterized by the absence of flame or incandescence until the fastener, not being perfect fuels, because the presence of the retardant (cement) inhibits the burning.

With the objective of evaluating the incorporation of residues and the pressure levels of molding, the work was divided in three parts.

In the first one, the composition of the granulometry of the light aggregate and the appropriate water/cement ratio were defined. Thus, in the cement matrix, three compositions of light aggregate granulometry (SBR) and two water / cement content were tested. According to the objectives of thisstudy, adequate values of resistance to compression, bending, and absorption were considered.

In the second phase, the influence of the incorporation content of the lightweight aggregate and the molding pressure to be used in the composites was evaluated. In this way, three contents of light aggregate were tested in cement matrices: 25%, 50% and 100% in relation to the amount of binder.

In order to evaluate the appropriate molding pressure, four levels were considered:

- Zero level (N0) - No pressure(0 MPa);
- Level one (N1) – 1.6 $Kgfcm^{-2}$ (0.16 MPa);
- Level two (N2) – 4 $Kgfcm^{-2}$ (0.4 MPa);
- Level three (N3) – 8 $Kgfcm^{-2}$ (0.8 MPa)

Tests of resistance to flexion, absorption capacity, bulk density and visual analysis were carried out.

The tests of resistance to simple compression were realized in the equipment SHIMADZU AUTOGRAPH AG IS 100KN; and those of flexural strength in the SHIMADZU AUTOGRAPH AG-X 50KN electronic press, following the standards of the Brazilian Association of Technical Standards (ABNT) 5739.In order to determine the absorption capacity, an adaptation of standard NBR 9778 (2005) was used, according to Silva (2012).

In the third and final part, the thermal conductivity was determined in the optimized traces, aiming the use of these composites as building elements for thermal masonry protection. In this sense, the thermal conductivity of the composites was determined using the K30 Conductivity Meter, which is based on the protected hot plate method. The results obtained were compared with each other and with already validated thermal insulators in the market.

The physical-mechanical properties of the studied composites were evaluated through the adaptation of Efficiency Factor calculations (Rossignolo, 2003), which defines as a fundamental parameter for the evaluation of

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light concrete, the calculation of the efficiency factor, which takes into account the specific mass dry and the simple compressive strength of the material, expressed mathematically by Equation 1:

$Fef = fc/\lambda$ (Eq. 1)

Where:

fc - compressive strength, MPa;

λ – specific dry mass of concrete, kg dm^{-3} (g cm^{-3})

Adapting the calculations, these factors were determined for other properties of the composites. Aiming at the preparation of lightweight composites, mainly intended for the thermal protection of masonry, low values of some properties and respective efficiency factors are required, such as absorption capacity, voids index and density. Thus, for this study, these parameters were denominated Absorption Factor, Empty Index Factor and Density Factor.

The analysis of variance of the results was done by the Tukey test at 5% of probability.

III. RESULTS AND DISCUSSIONS

Table 3 presents the results of the study for the composition of the particle size of the SBR residue and the a/mc factor, for use in the preparation of the composites.

Table.3: Results of the study for the composition of the granulometry of the SBR residue and the a/mc factor for use in the preparation of the composites.

Granulometry Trace 1:1	a/mc	Simple compression strength (MPa)	Flexural strength (MPa)	Absorption (%)	Void Index (%)
34567	0.3	0.54 b	Did not shape	-	-
3456		0.51 c			
345		0.55 a			
34567	0.4	0.66 c	0.013 c	20.22 a	17.86 a
3456		0.80 b	0.055 b	21.28 b	19.03 b
345		1.02 a	0.073 a	21.97 c	19.40 c

Means followed by the same letters (a, b) do not differ by Tukey test.

When the Tukey test was applied, all values of Flexural Strength, Bulk Density, Absorption and Void Index were statistically different, with 95% confidence.

When using the a/mc content of 0.4, it was noted that the less fines in the particle size composition, the greater the resistance to simple compression. Changingthe granulometryfrom 34567 to 3456, the compressive strength increased by 21.2%. When SBR with 345 granulometrywas used, there was an increase of 54.5% in relation to 34567, and 27.5% in relation to 3456.

As with simple compression strength, flexural strength also increased when using finer grades. Altering the particle size from 34567 to 3456, this resistance increased by 323%, and from 34567 to 345 this increase was 461.5%. Regarding the grain size 3456, the increase in composition 345 was 32.7% in the flexural strength of the composite.

When comparing the absorption capacity, the less thin the material, the higher this characteristic. When changing from 34567 to 3456, the absorption increased only 5.2%. From 34567 to 345, this increase was 8.6%. When changing from 3456 to 345, the granulometry of the light aggregate, the increase in absorption capacity was 3.2%.

With the ratio a/ mc of 0.4, using the adjusted efficiency factor calculations, the best result for compressive and flexural strength was achieved with the particle size composition using particle sizes 3, 4 and 5, that is, retained in the sieves 4.8 mm, 2.4 mm and 1.2 mm, according to Table 4.

Table.4: Effectiveness factor (Fef) of the resistance to simple compression and bending to define the composition of the granulometry of the SBR residue.

Granulometry Trace 1: 1	Fef of Simple Compression Strength (MPa . cm^3/g)	Fefof Flexural strength (MPa . cm^3/g)
345	1.16	0.08

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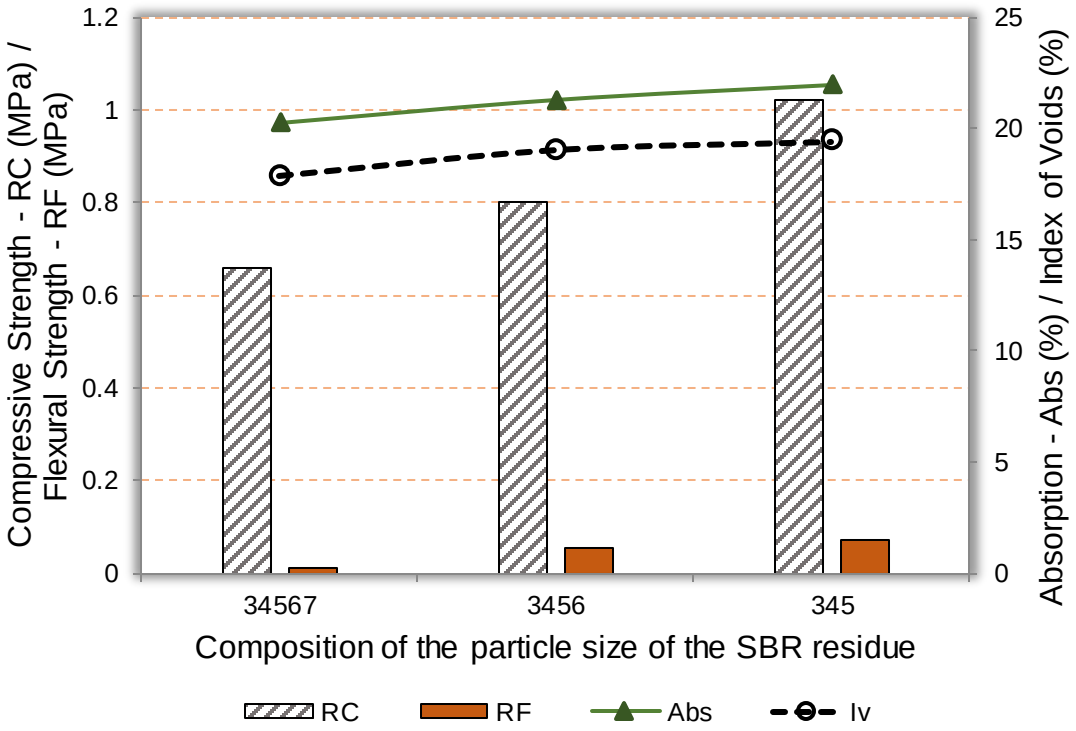
3456	0.89	0.06
34567	0.76	0.01

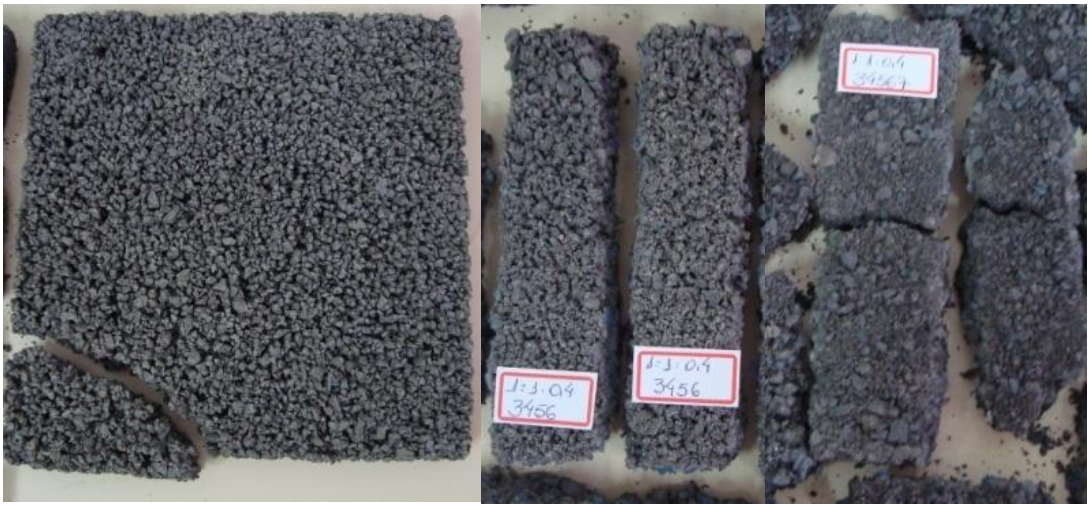
Figure 1 shows the results observed in the properties of the molded composites with a/ mc content of 0.4:



RC: Compressive strength; RF: Flexural Strength; Abs: Absorption: Iv: Index of Voids

Fig.1: Physical-mechanical properties of cement composites and SBR residues, molded with a/mc content of 0.4.

The molded plates with the three granulometries tested and with a / mc factor 0.4 are shown in Figures 2 (a) (b) and (c).Note that the respective granulometries of the lightweight aggregate (with finer ones) leave the material more fragile and brittle.



(a) (b) (c)

Fig.2: Prismatic specimens with trace 1: 1: 0.4 (a) particle size SBR 345 (b) particle size SBR 3456 (c) particle size SBR 34567

In addition to the best results for simple compression and flexural strength, the mixture of cement and SBR residue in grain size 345 showed better workability. Another advantage of this granulometry composition is the fact that it is not necessary to intensify the process of crushing the residue, reducing costs, since they are not fine granulometry.

After the evaluation of the granulometry of the SBR residue, traces and molding pressure levels were tested for cementitious matrix composites. The water content in relation to the binder (a/agl) was adjusted to 0.4

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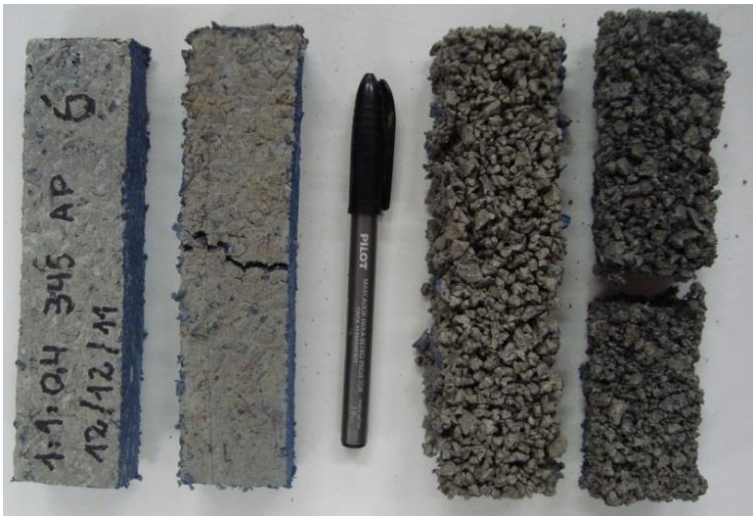
by varying the applied pressure level (N0, N1, N2 or N3) and the trace (1: 1, 1: 0.5 and 1: 0.25) as quoted in the methodology.

Figure 3 shows 1: 1 trace test bodies, subjected to the N3 level molding pressure and without molding pressure (N0), before and after the flexural test.



(a) (b)

Fig.3: Cement matrix test bodies with SBR residues of 1: 1: 0.4 (a) traces of grain size 345 subjected to molding pressure N3 and (b) without molding pressure (N0).

The observed results of the physical-mechanical properties of the composites cement: SBR are in Table 5.

Table.5: Results of the study of the cement composite and SBR residue for use in the elaboration of lightweight construction elements.

Trace Cement: SBR residues	a/agl	Molding Pressure Level	Flexural Strength (MPa)	Tenacity (KJ\cm^2)	Compression Resistance (MPa)	Bulk density (Kg/m^3)	Expelled a/mc	Specific dry mass (g/cm^3)	Absorption (%)	Void Index (%)
1:1	0.4	N0	0.073 ± 0.012 dC	0.07 ± 0.004	1.02	**652.3 ± 6.02**dC•	**0**	**0.91 ± 0.16**bB	31.1 ± 5.57 aA	28.1 ± 3.96 aA
		N1	0.75 ± 0.03 cC	0.67 ± 0.08		**721.1 ± 20.46**cC•	**0**	1.13 ± 0.009 aC	**21.0 ± 0.5**dA	**23.4 ± 0.49**aA
		N2	0.75 ± 0.01 bC	0.87 ± 0.17		947.4 ± 20.5 bC	+	1.10 ± 0.05 aC	22.9 ± 2.3 cA	25.2 ± 1.60 aA
		N3	**0.77 ±0.01**aC	**0.91 ± 0.07**		1062.6 ± 15.31 aC	++	1.11 ± 0.009 aC	23.7 ± 0.32 bA	26.3 ± 0.27 aA
1:0.5		N0	0.66 ± 0.05 cB	0.58± 0.03	1.71	**947.3 ± 42.91**cB	**0**	**0.94 ± 0.16**cB	28.7 ± 8.5 aB	27.0 ± 5.77 aB
		N1	1.30 ± 0.14 bB	0.94± 0.23		**1029.4 ± 15.53**bB	**0**	1.23 ± 0.10 bB	**14.5 ± 0.66** dB	**17.9 ± 0.79** dB
		N2	**1.47 ± 0.06**bB	0.92± 0.05		1058.5 ± 41.37 bB	++	1.22 ± 0.09 bB	17.6 ± 1.17 cB	21.4 ± 2.91 cB
		N3	1.30 ± 0.03 bB	**1.40 ± 0.13**•		1114.1 ± 45.9 aB	+++	1.37 ± 0.02 aB	18.5 ± 1.05 bB	25.5 ± 1.37 bB
1:0.25		N0	1.13 ± 0.15 dA	0.52± 0.07	**4.88**•	**1041.7 ± 8.42**dA	**0**	**1.03 ± 0.076**dA	21.1 ± 3.41 aC	21.8 ± 3.23 bC
		N1	2.02 ± 0.15 cA	0.63± 0.019		**1104.3 ± 29.99**cA	+	1.35 ± 0.04 cA	**13.2 ± 0.37**dC•	**17.8 ± 0.50** dB•
		N2	2.93 ± 0.24 bA	0.67± 0.009		1298.2 ± 21.18 bA	+++	1.43 ± 0.03 bA	14.9 ± 0.70 cC	21.2 ± 0.60 cB
		N3	**3.19 ± 0.34**aA•	**0.79 ± 0.14**		1331.2 ± 20.98 aA	++++	1.59 ± 0.008 aA	15.9 ± 1.01 bC	25.4 ± 1.60 aB

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Means followed by the same lowercase letters (a, b, c, d) for the same trace do not differ by Tukey's test.
Means followed by the same capital letters (A, B, C, D) for the same molding pressure level in different traces do not differ by Tukey's test.
(•) Better results

By evaluating the properties of resistance to simple compression, it is noticed that the smaller the amount of SBR residues, the greater the resistance (Figure 4).

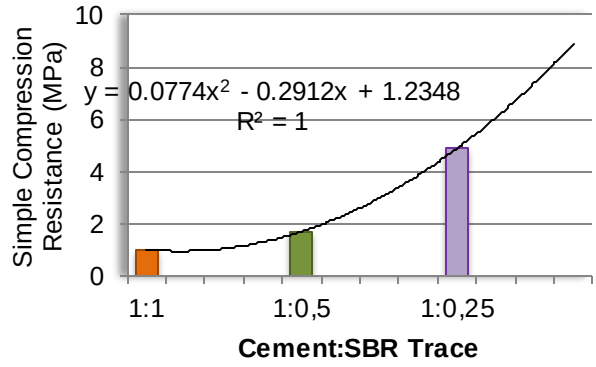

Fig.4: Resistance to Single Compression of the cement matrix with incorporation of SBR residue, in the granulometry 345.

When using the 1: 1 trace of cement and residue, a simple compressive strength of 1.02 MPa was achieved. When the amount of the incorporated residue was decreased by 50% (1: 0.5), the simple compressive strength increased by 67.6% (1.71 MPa). By decreasing the amount of the incorporated residue by 75% (1: 0.25), the increase in the simple compressive strength was 378.4% (4.88 MPa).

No satisfactory flexural strength was achieved using non-pressure molding (N0) for the highest contents of waste incorporation (1: 1 and 1: 0.5). With the application of molding pressure, the flexural strength was increased. The values were compatible with those found by Oliveira (2009), studying composites formed of gypsum or vermiculite and EVA; by Leal (2004) evaluating cement and EVA composites; and by Silva (2012) analyzing traces composed of cement, sand and SBR.

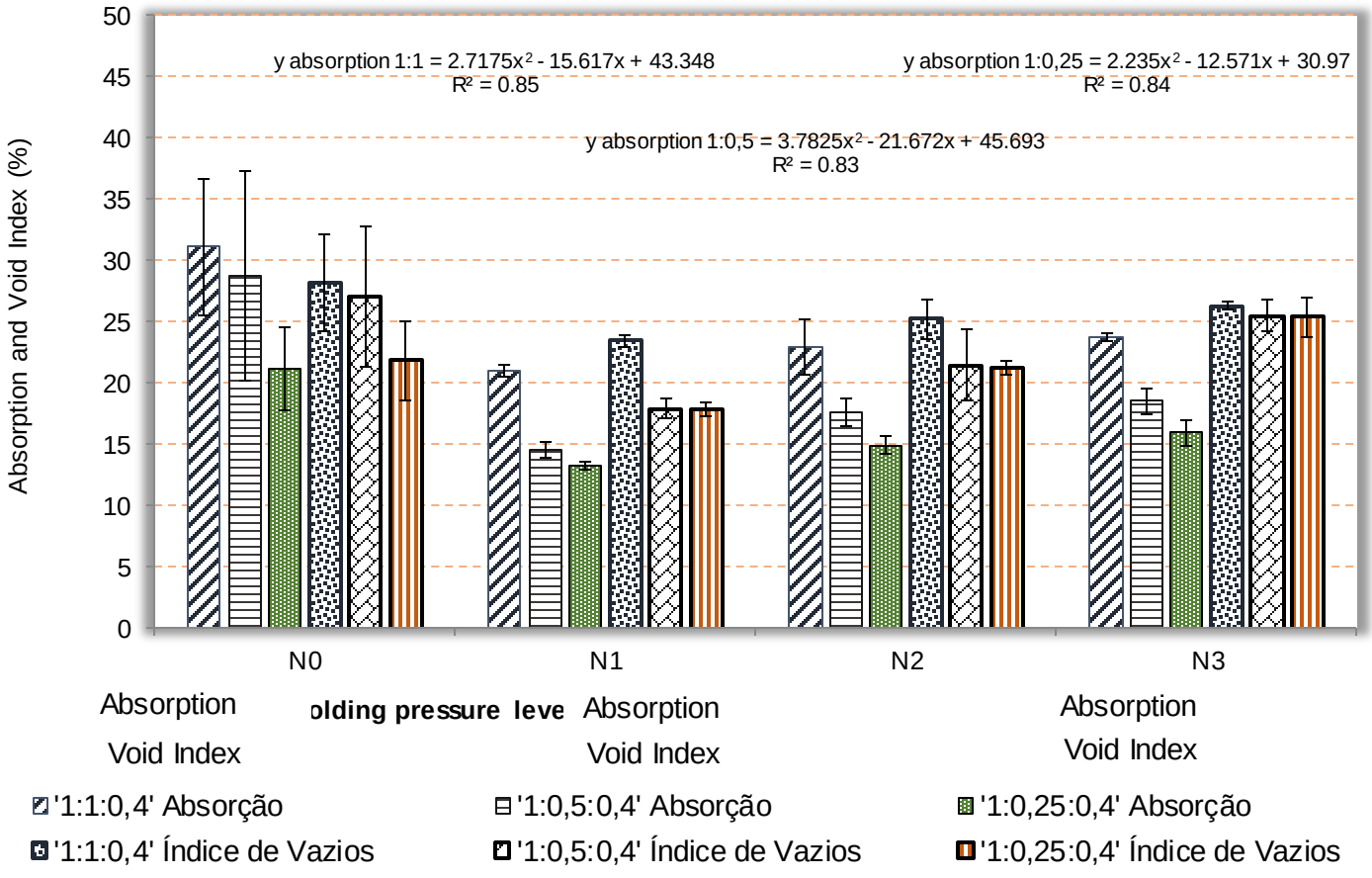

Fig.5: Absorption and Void Index of the cement matrix with incorporation of SBR residue, in the granulometry 345.

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In relation to the specific dry mass, even though an increase occurred due to the application of molding pressure, all the composites could be considered light (910 to 1590 kg/m^3). The application of the molding pressure also caused an increase in the bulk density of the composites, which ranged from 652.3 to 1331.2 kg/ m^3.

Taking into account the purpose of the proposed constructive elements it is necessary that the composites that will be part of its structure have the lowest absorption capacity possible. The lowest results of this property were achieved in the molded composites with N1 pressure level (13 to 21%, according to the residue content). The values of the absorption capacity of the molded composites with this level of molding pressure were compatible with those of Bezerra (2002), studying cement, sand and EVA composites; and by Soares et al (2008), working with cement, ceramic residue and shoe waste.

When N2 and N3 molding pressure levels were used, binder water was ejected from the interior of the composites (highlighted in Table 5), resulting in a higher void index, and consequently a higher absorption capacity. When these properties were applied, the 1: 0.5 and 1: 0.25 trace composites presented similar trend in relation to the void index (Figure 5).

Figure 5 shows the lower absorption capacity and the lower void index of the composites when the N1 (0.16 MPa) level pressure is used. With this level of pressure, there was also a greater uniformity in the results, represented by smaller values of standard deviation.

Figure 6 illustrates the observed trend of the properties of cement composites and SBR residues.

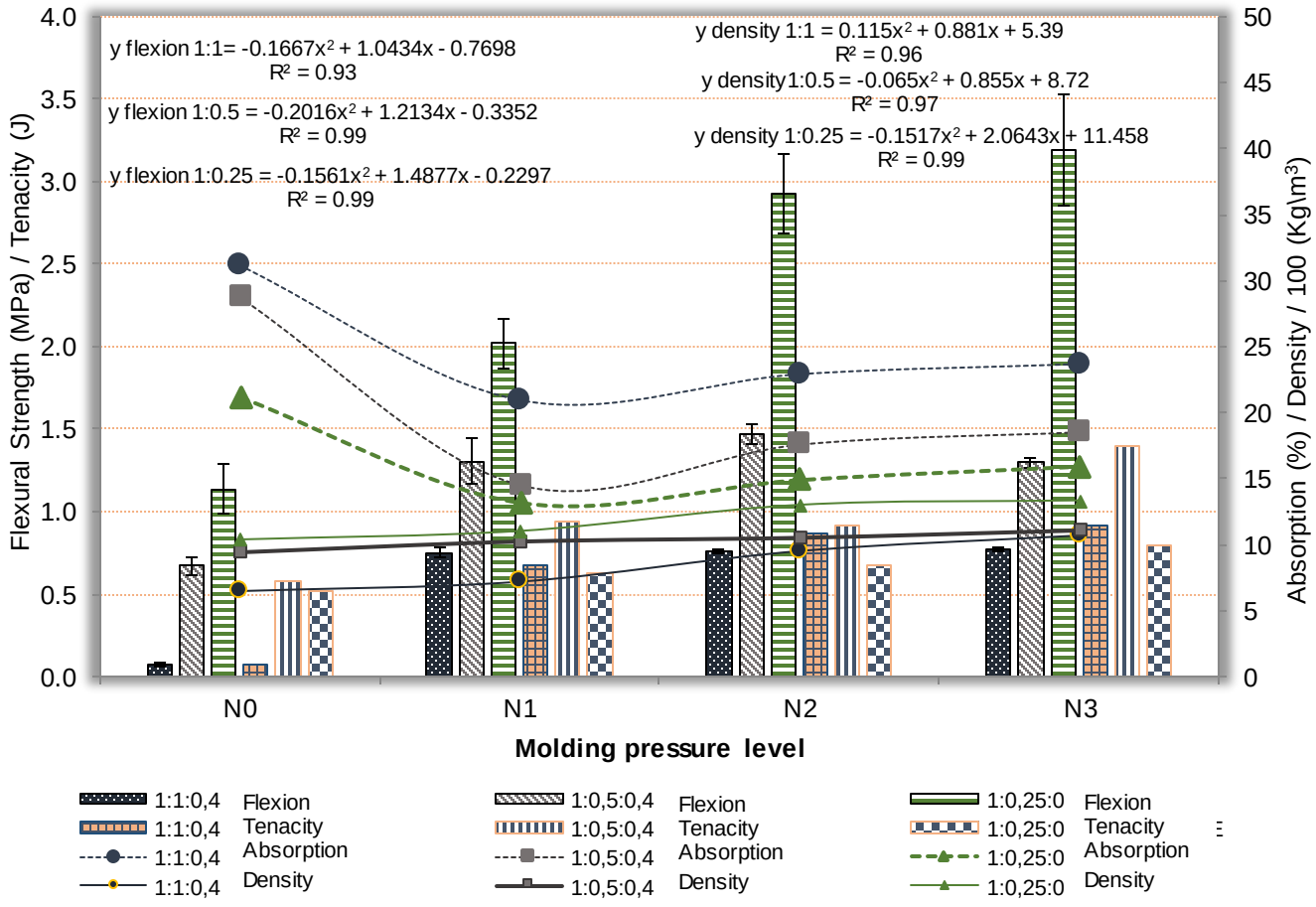

Fig.6: Resistance to flexural strength, Absorption, Tenacity and Bulk density of cement composites and SBR residues.

The results obtained with the calculations adapted from the Efficiency Factor (Fef) of properties according to Rossignolo (2003) are presented in Table 6. For the bulk density and absorption, this factor was denominated "Density Factor" and "Absorption Factor".

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Table.6: Results of the efficiency factors of cement composites and SBR residue for use in the elaboration of lightweight construction elements.

Trace Cement: SBR residues	a/agl	Molding Pressure Level	Efficiency factor ofFlexural Strength (MPa.cm^3\g)	Factor ofBulk density	Factor of Absorption (%.cm^3\g)	Factor ofVoid Index (%.cm^3\g)
1:1	0.4	N0	0.08↓	7.16	34.19	30.97
		N1	0.67	**6.38•**	**18.58•**	**20.77•**
		N2	**0.69•**	8.61	20.85	22.94
		N3	**0.69•**	9.57	21.35	23.71
1:0.5		N0	0.71↓	10.07	30.61	28.76
		N1	1.06	**8.37•**	**11.85•**	**14.56•**
		N2	**1.20•**	8.67	14.44	17.58
		N3	0.95	**8.13•**	13.55	18.63
1:0.25		N0	1.10↓	10.11	20.53	21.17
		N1	1.50	**8.18•**	**9.79•**	**13.22•**
		N2	**2.05•**	9.08	10.43	14.87
		N3	2.01	8.37	10.02	15.97

IV. CONCLUSIONS

The Efficiency Factor of the flexural strength of the 1: 1: 0.4 trace presented higher values with molding pressure levels N2 (0.4 MPa) and N3 (0.8 MPa); however, from N1 (0.16 MPa) this increase was not significant. For the 1: 0.5: 0.4 and 1: 0.25: 0.4 traces the N2 level molding pressure was more efficient for this property.

The lower values of absorption efficiency factors and void index were obtained when the composites were molded using N1 (0.16 MPa) pressure level. The bulk density factor of the pressure molded composites reached the lowest values when the pressure level N1 (0.16 MPa) was also used.

The composites with higher contents of incorporation of residues, molded without pressure, did not present satisfactory results of resistance to the flexion and absorption. Although the 1: 0.25: 0.4 trace presented better values of resistance factors to flexion and absorption, it did not provide a greater incorporation of residues, allowing a greater use of cement.

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Water Quality Management in Cienfuegos Bay (Cuba) with Integrative Approaches

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***Abstract*—** *The aim of this paper is to analyze the contributions of some tools developed with integrative approaches for managing water quality, considering the particular case of Cienfuegos Bay, Cuba. One of the contributions was related to the improvement of a hydrologic monitoring program of this bay with the assimilation of Environmental Totality and Integrated Watershed and Coastal Area Management (IWCAM) approaches. The development of an index for assessing water quality (WQI) of estuarine systems associated with a methodology for implementing a conceptual model on the eutrophication management from systemic and causal approaches, were another developed tools. In addition, a strategic program as a guide for the integrated management of this bay was included considering the participatory and systemic approaches. The assimilation of these integrative approaches for developing scientific tools allowed improving the environmental management at a contextual level and could be generalized in similar aquatic systems.*

***Keywords*— *eutrophication, coastal management, integrative approaches, Cienfuegos Bay*.**

I. INTRODUCTION

At the international level, different scientific tools are used to support coastal environmental management such as environmental monitoring, integrated management programs, indicator systems, among others. Scientific research and environmental monitoring are key components in the decision-making process, as well as for the development and implementation of policies (Vugteveen *et al*., 2014). These contribute to assembling the data and information in useful ways for the managers of the resources.

Watershed and coastal zone to where drain their waters, keep a close relationship. The coastal zone receives direct all processes and phenomena occurring in the basin (González-Díaz, 2015). To contribute to the success of coastal management in an integrated way is a task very important in Cuba. The science using to support this process requires integrative approaches such as Integrated Watershed and Coastal Area Management (IWCAM), systemic, Environmental Totality, causal by means Driver-Pressure-State-Impact-Response (DPSIR) framework, participatory, among others. The integrated management has to be conceived so that national states can develop it voluntarily; adapting it to its ecological characteristics and socio-economic needs and particular cultural contexts (Barragán-Muñoz, 2012).

Cuban scientific institutions related to the environmental management of coastal resources are called to contribute to the strengthening of this process in the provinces, through the development of tools contributing to making decisions based on knowledge. The development of monitoring programs aims to provide an empirical basis for the evaluation of an environmental phenomenon or its trends (Carstensen *et al*., 2011). Consequently, the existence of a program for assessing water quality of the Cienfuegos Bay for more than two decades and projects carried out in the province, all of them related to integrated water and coastal resource management have supported the improvement of this process through the gradual assimilation of integrative approaches in the development of relevant scientific tools. The purpose of this paper is to analyze the contributions of the tools developed with integrative approaches for improving the water quality management in Cienfuegos bay.

II. MATERIALS AND METHODS

2.1. Study area

Cienfuegos bay is situated in the southern central part of Cuba (22°1′ N, 80°20′ W). It is a semi-enclosed bay with an estuarine characteristic. It is connected to the Caribbean Sea

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by a narrow channel 3.6 km long. Its area is 88.46 km^2 and a total volume of 0.84 km^3 with an average depth of 9.5 m (Muñoz *et al*., 2012).

This bay is divided into two natural lobes. The northern lobe has a more anthropogenic impact: e.g. sewage discharges from the city of 161,432 inhabitants (ONEI, 2012) and the incidence from the industrial area. There is also freshwater input from the Damují and Salado rivers and from other aquatic systems such as Inglés, Calabazas and Manacas creeks (Fig.1). In this region, despite actions by the local government to reduce pollution in the bay, the wastewater treatment is still inadequate. The southern lobe is subjected to a lesser degree of pollution arriving from the Caonao and Arimao rivers.

Fig.1: Localization map of Cienfuegos bay (Cuba) and current sampling stations

2.2. Approaches considered to develop tools

The integrative approaches of Environmental Totality (ET) and IWCAM were articulated, enhancing their multidimensional nature to guide and support the need to improve the Monitoring Program of the Cienfuegos Bay (MPCB) that had been carried out since the 90s, which is considered as the first tool developed.

The theory about the environment as a complex whole is important because considers the reality part where coexist and interact the biotic, no biotic and social elements directed in constant development by the social activity. Thus the integration cannot start from the vision of a mechanical sum of elements; but it must be assumed as an organizational quality that results from the historical character of the society-nature relationship, under an integral relationship (Miranda-Vera, 2000).

On the other hand, the need to integrate spaces for understanding the environment as a whole, in correspondence with the systemic nature of the management, based the assimilation of the IWCAM approach, which articulates the watersheds and the coastal areas (León *et al*., 2013).

The MPCB has been implemented to assess its water quality and its design was based on geomorphologic features, as well as on the identification of activities with the highest incidence and distribution of pollutants. However, various socio-economic changes and the training of personnel in new integrative approaches motivated their improvement

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according to the context demands. This task required the analysis of the polluting sources (direct and indirect), the uses of this bay, as well as the basic elements of the monitoring program design (sampling points, frequency, physical-chemical parameters and depth levels considered, etc.) considering the data obtained during ten years.

The evaluation of the MPCB was based on the analysis of its effectiveness to identify the incidence of new socio-economic activities (the activity change of the Fertilizer Factory, the increase of tourist activity in the northern sector of the bay, etc.) using a methodology with four stages (Seisdedo *et al*., 2004). Some of these stages included cluster analysis to identify spatial and month associations by the Euclidean distances and the proposal of the modified MPCB with its argumentation (Seisdedo and Muñoz, 2005). In addition, a flowchart of the information from the MPCB was proposed for its consideration in an appropriate way on integrated management of this bay (Seisdedo *et al*., 2005).

Subsequently, it was necessary to have a contextualized tool to assess the water quality that would eliminate the difficulties related to the large number of parameters and data, and the inadequacies in the technical regulations (NC 22, 1999; NC 25, 1999) on the criteria for systems with estuarine characteristics such as the Cienfuegos bay. In addition, this would be useful for the presentation of results to decision makers and the general public. Therefore, an adaptation was made to the proposal of the Canadian Council of the Ministries of the Environment (CCME, 2001) to developing a Water Quality Index (WQI) for this estuarine system, which is based on the consideration of the quality criteria established in Cuban technical regulations, as well as of the results obtained in the two levels of depth and also, the change from a temporal to spatial focus of the frequency factor, in order to provide a spatial assessment of water quality corresponding to a single campaign instead of a temporal evaluation in a single station. The selection of the parameters to be included in the WQI was based on the evaluation of the correspondence between the anthropogenic incidence and the results of this index considering all the parameters regulated or only some proposed for estuarine systems of South Africa (SOE, 2000), which cover three categories: trophic status (N-nitrate and orthophosphates), convenience for aquatic biota (dissolved oxygen, Biochemical Oxygen Demand and ammonia), for human contact (fecal coliforms) and two others (N-nitrites and chlorophyll *a*), with importance for the assessment of trophic status according to some results reported (Seisdedo *et al*., 2011).

To this was added, the assimilation of integrative approaches such as the systemic and causal (DPSIR) to design and implement the environmental management of this bay with a preventive point of view based on the analysis of eutrophication risk. This was necessary considering the appearance of several events of algal blooms in some areas of this bay since 2005 (Moreira *et al*., 2009). Therefore, an analysis of current trends and the context was carried out for designing the conceptual model considering some aspects (principles, main characteristics and indicator categories) and selecting the indicators. Besides, expert criteria and statistical analyzes (Kendall coefficient, test Wilcoxon and coefficient Rho of Spearman) were used to validate the conceptual model of eutrophication management, its methodology and associated tools (Seisdedo *et al*., 2016).

Also, it was necessary to have an Integrated Management Program of the Cienfuegos bay, to contribute to the implementation of IWCAM approach as a participative and inclusive social process oriented towards the sustainable development of the Cienfuegos province. In its elaboration, relevant information provided by various institutions was considered and this program was conceived from a strategic point of view. It has been pointed out that the formal approach to integrated management should be oriented towards strategic management budgets: flexible, adaptive, broad perspective, participatory, designed for the long term, which assumes adverse scenarios and takes into account both the process and the results (Barragán-Muñoz, 2012).

III. RESULTS AND DISCUSSION

The approach of ET allowed us to assume a philosophical understanding of the environmental, and therefore expand the view, which must be taken into account for any environmental analysis. The comprehension of its existence as an integrated whole leads us to consider the methodological assumptions that on the totality are enunciated from the dialectical - materialist vision. In fact, three basic methodological assumptions served as a basis to consider the entire bay and its social environment as ET (Miranda-Vera, 2003), which are:

1. The necessary articulation and interconnection of the essential parts of the whole. This allowed us recognizing which are the essential parts of the area to be studied as ET, which in this case were: Cienfuegos city and its bay, so that the systemic nature of this relationship is visualized.
2. The internal contradictions of the whole, which define its dynamics of change and development. This allowed us recognizing the environmental problems of the study area considered as ET and

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that must be transformed in all manifestations of the material level: biotic, no biotic and social.

3. The historical character of the whole, insofar as it is a process that is in constant development. This allowed us recognizing the history of the economic, social, cultural and natural development of the study area considered as ET since today is the result of yesterday and the history of tomorrow will depend on what is done today.

The aspects that characterize the ET and that respond to an environmental reality under study, reveals that this totality in construction is a holistic, systemic, continuous, dynamic, historical and concrete expression of the contradictory relationship between society and nature.

The new proposal of monitoring program included the need to quantify the pollutant loads of the tributary watersheds, the incorporation or elimination of some sampling points, the consideration of the vertical sampling (in depth and surface) due to the marked vertical haline stratification of this bay, as well as the decrease of the sampling frequency considering results of an analysis of the seasonal influence on the water quality of this bay (Seisdedo and Muñoz, 2005). As a result, 16 sampling points for the bay, 9 points for the main tributary sources (rivers, creeks and drainages of the Cienfuegos city) (Fig.1) and at least two campaigns per year in correspondence with the wet and dry periods were established.

A Water Quality Index (WQI) was proposed for estuarine systems such as the bay studied (Seisdedo and Muñoz, 2013), which considers some parameters related to the three categories mentioned above. After it's testing during the period 2010-2012, the obtained results showed a correspondence from a spatial point of view with the anthropogenic incidence reported previously (Seisdedo, 2006; Seisdedo and Arencibia, 2010), which allowed consider it as an effective scientific tool for the water quality management in this aquatic system. In addition, it allows numerous data to be summarized in the key information to evaluate the progress made with respect to the established objectives so that its use to date has been important. Besides, this WQI allowed identifying an improvement of water quality based on a change from mediocre to acceptable quality when the nutrient load incorporated from rivers decreased 310 ton of nutrients (N+P) in 2014 (Seisdedo *et al*., 2016).

The design of the conceptual and methodological tools for the water quality management from the trophic point of view in Cuban bays with estuarine characteristics such as Cienfuegos bay was based and characterized in the proposal of 10 identified indicators under the DPSIR framework (Seisdedo *et al*., 2016). These were concretized in contextualized assessment tools that allow orienting this environmental management through four stages: to prepare, to plan, to implement and to control-evaluate, in an appropriate way including participatory conception, which requires the strengthening of the relationships between the stakeholders and the training on IWCAM approach (León *et al*., 2013; Castellanos *et al*., 2009). The conceptual model of this environmental management in the Cienfuegos bay was validated considering the obtained values of Kendall coefficient (W) and significance obtained ($p<0.05$), which showed an appropriate association degree in the expert criteria. The methodology used to implement it included a set of procedures that ensured a logical, practical, simple and coherent sequence. With the application of them during the period 2013-2014, very favorable results related to the improvement of the water quality from the trophic point of view in the studied bay were obtained. These results showed that the nutrient loads incorporated into the bay and its water quality through the WQI showed the effectiveness of the actions designed to manage eutrophication. In addition, the socialization actions related to this process were increased, and stakeholders (government, communities and entities) were involved in this environmental management, as well as the applicability, adequacy to the context and effectiveness of the associated tools were verified.

The Integrated Management Program of the Cienfuegos bay allowed the definition of strategic actions corresponding to the period 2016-2020 based on the achievement of environmental training from decision makers and the general public, the reduction of interest conflicts generated by socio-economic activities, the improvement of participation mechanisms and integration of the key social actors in decision making, the improvement of socio-environmental conditions through the gradual solution of environmental problems identified in the territory from a contextual approach, promoting financial sustainability to guarantee the continuity and dynamics of the IWCAM process.

IV. CONCLUSION

The gradual assimilation of integrative approaches in the development of scientific tools to improve the water quality management in the Cienfuegos bay, for more than a decade, has generated some theoretical and methodological aspects. The importance of them is that have allowed to recognize the complexity of this process for Cuban bays with estuarine characteristics and contribute to the systematization and strengthening of inter-institutional and community relationships, as well as to the links between the

processes of analysis and decision-making, with satisfactory results and possibilities of generalization in other bays with similar characteristics.

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Wealth Generated by Actors in the Livestock-Meat Sector in the Budget of Decentralized Entities in the Department of Bermo, Niger

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Abstract**—** *The self-managed cattle market in Bermo and Wurssena were surveyed during the months of August 2015 to July 2016, among actors in the livestock-meat sector, including butchers (wholesalers and retailers), collectors, conveyors, traders and intermediaries or witnesses to evaluate their contributions to communal pastoral entities. Livestock sales were higher in winter and post harvest periods. Revenues earned by livestock markets are reviewed. Thus, the communes registered respectively 1.920.000FCFA from the self-managed market of Bermo and 21.150.000 FCFA concerning that of Wurssena. These are taxes levied during sales. If we add the annual exercise taxes imposed on butchers, traders, dealers, intermediaries or witnesses, as well as the jobs created, the benefits are far from negligible.*

Keywords**— Livestock sale, Recipe, town, Employment, Livestock market.**

I. INTRODUCTION

Pastoral livestock farming plays an important role in the economies of African countries. Indeed, it represents 25% of agricultural GDP and 10 to 44% of the national GDP of West African countries (IUCN, 2006), 20% of the GDP of various African states (IIED, 2006). Mobile livestock is known for providing goods and services, including livestock, animal products, organic manure, animal traction and job creation, etc. (Steve, 2008).

As a breeding country by excellence, Niger has a considerable workforce estimated at more than 41 million head, or a value of 15,829,818 TLU. The sector accounts for 11% of the national GDP, 35% of the agricultural GDP contributing 3,000 billion FCFA (Atlas on livestock in Niger, 2014). Livestock contributes more than 15% to the household budget and provides huge revenues to towns (INS, 2013).

Three types of livestock systems coexist in Niger. This is the pastoral system, agro-pastoral and urban and peri-urban system(Atlas on livestock in Niger, 2014).

With 76% of the national herd belonging to pastoralists, pastoralism in Niger contributes 84% to agricultural GDP (IIED, 2010). Pastoral farming accounts for over 30% of the livestock population (MEIA, 2002). It accounts for 30% of income of a pastoral household (INS, 2013).

Like other Sahelian countries, pastoralism also provides many products and services at the level of the decentralized entities of Niger; these are animal products (meat, milk and butter), livestock, organic manure, animal traction and jobs. Through the sale of animals, pastoral livestock farming provides important financial resources to rural households and municipalities by levying taxes on livestock markets and small businesses located nearby.

The functioning of the town halls is ensured by the state subsidy and the revenue from taxes levied on some actors (butchers, intermediaries-witnesses, merchants-resellers of cattle, merchants of articles, restorers, etc.).

However, only taxes from livestock markets are counted by the city council as the contribution of pastoral livestock. The resources collected from established businesses thanks to the presence of cattle markets are totally ignored in the economic analyzes. Added to this are the various services created, including the jobs generated by the presence of the livestock market. The situation is the same at the level of the population, technical services and partners; they are unaware of the flow of financial resources involved in livestock market

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transactions and dismemberment, as well as the services provided by livestock farming as an activity.

This explains the disinterest of the communes in the area of livestock, resulting in the lack of investment in the sector. This behavior of the decentralized entities results from the underestimation of the products and services resulting from the functioning of the livestock markets.

The purpose of this study is to evaluate the resources (direct and indirect) offered by livestock markets at the level of communal pastoral entities. This will create renewed interest from municipal authorities and development partners in pastoralists.

II. MATERIAL AND METHODS

2.1 Study area

The area concerned by this study is the department of Bermo in Niger. It covers an area of 6496 km², 210 km from the city of Maradi, the capital of the region. The department is located between the East longitude 006'49.170 ° and the North latitude 15'08.872 ° and has two rural communes including Bermo and Gadabédji. The population is composed of 52274 inhabitants for 6972 households and distributed among the Arabs, Hausa, Fulani and Tuareg (INS, 2012).

The physical environment is characterized by a hot and dry Sahelo-Saharan climate with an average rainfall of 200 to 250 mm per year. At the soil level, soils are dune. The vegetation is composed of clear savannah with a well-developed herbaceous layer. The relief is structured in inter-dune plateaus and valleys which are full of tree concentrations.

The 130-hectare Akadany pond is the main source of surface water in the department. However, there are some semi permanent ponds.

Livestock is the main activity of the population, which is mostly composed of breeders. The department has a herd of 594 642 heads, consisting of 29484 asins, 193082 cattle, 91352 camels, 95643 goats, 1785 horses and 183295 sheep (DDEL Bermo, 2016).

The study covered the self-managed livestock markets of Bermo and Wurssena on an area of 500 m² each (Figure 1).

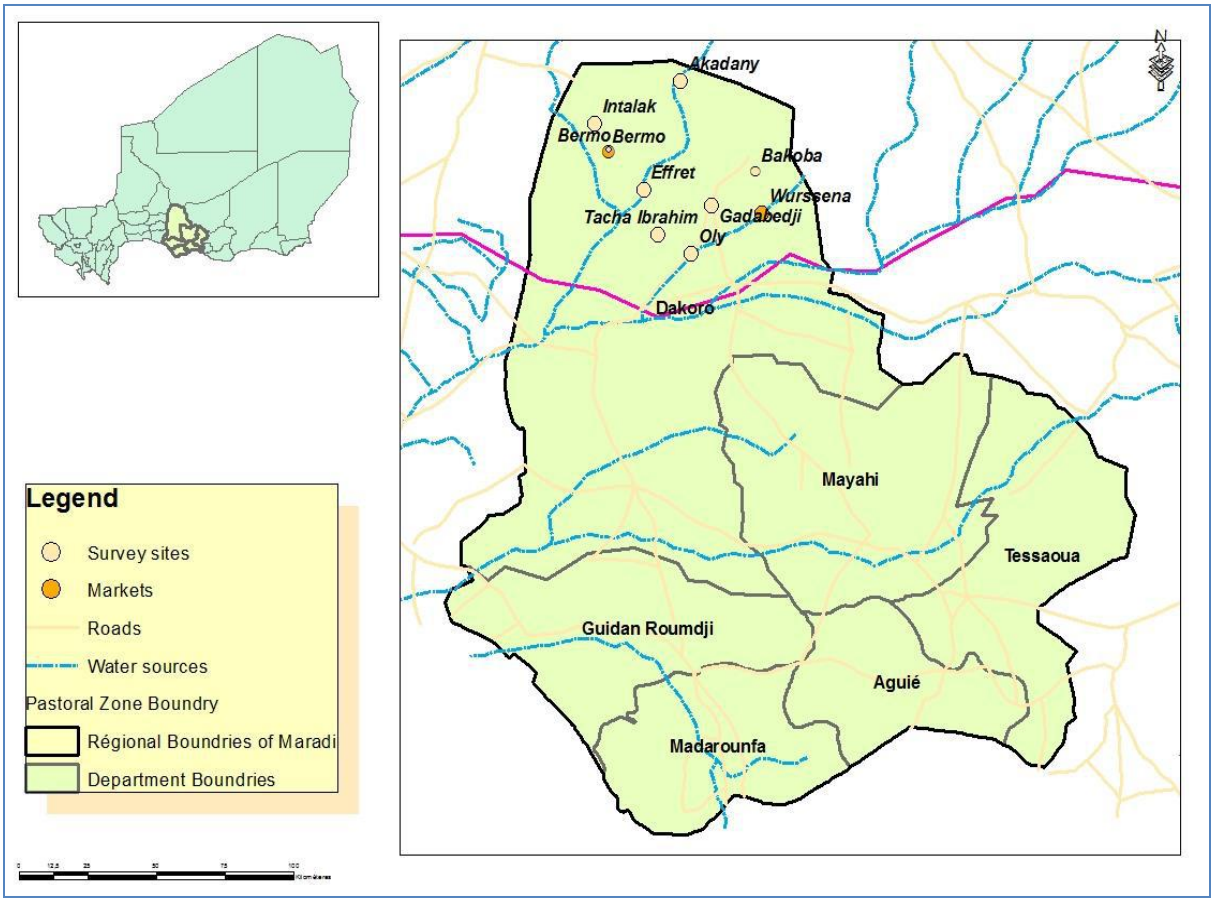

Fig.1: Location map of cattle markets surveyed.

2.2 Data collection and analysis

The survey was conducted at the main livestock market in each of the rural towns of Bermo and Gadabedji. This is the self-managed market of Bermo for the first and Wurssena for the second. The study was spread over the twelve months from August 2015 to July 2016. Both markets are active every Tuesday. The collection of data was done on a frequency of one week out of two, two passages per month.

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Monitoring has affected actors in the livestock-meat sector, including butchers (wholesalers and retailers), collectors, conveyors, traders and intermediaries-witnesses. At each exit a single category of actors is investigated. Municipalities were also called upon to collect the revenue raised by the cattle markets, as well as the distribution of the expenses related to their management.

The data collected focused on the revenue from the sale of animals. The survey cards used for this collection mainly record the number of sales, the monthly evolution of sales prices for the various species (asine, cattle, goat, camel and sheep), the jobs generated and the taxes collected.

The data was processed by the Excel spreadsheet to calculate the reported and sold workforce, as well as the sales amounts based on the period.

III. RESULTS

3.1 Sale of livestock at the level of municipal entities

Pastoralists regularly use the harvest of some animal heads that they sell on the markets, in order to meet the financial needs of their households. Thus, through the sales taxes of cattle on the market, the municipalities earn important revenues.

3.1.1 Revenue from livestock sales

Revenues generated by the Wurssena livestock market were higher during the 2012-2016 period; In fact, they provide more than 70% of the total revenues paid to communities by the two markets combined.

These receipts correspond to the amount allocated to each municipal entity, after subtracting the expenses relating to the management and functioning of the market (salaries, operation, maintenance, etc.).

Table 1 shows the amounts of revenue paid to municipalities for livestock sales at the Bermo and Wurssena markets.

Table.1: Revenue paid to municipalities from 2011 to 2016.

Sales value (FCFA)	Year					
	2011	**2012**	**2013**	**2014**	**2015**	**2016**
MarketBermo	1560000	1800000	1800000	1800000	1920000	1920000
MarketWurssena	710875	11460453	5884400	8125700	19109833	21500000
Total	**2270875**	**13260453**	**7684400**	**9925700**	**21029833**	**23420000**

Figures (2 and 3) describe the distribution of receipts at the respective livestock markets of Bermo and Wurssena.

The salaries of the tax collectors and witnesses, as well as the amounts paid to the town of Bermo, represent respectively averages of 35 and 41% in average of the total receipts. The remaining financial resources of 17% go to AREN through an Economic Interest Group (KLD), which manages the Bermo market.

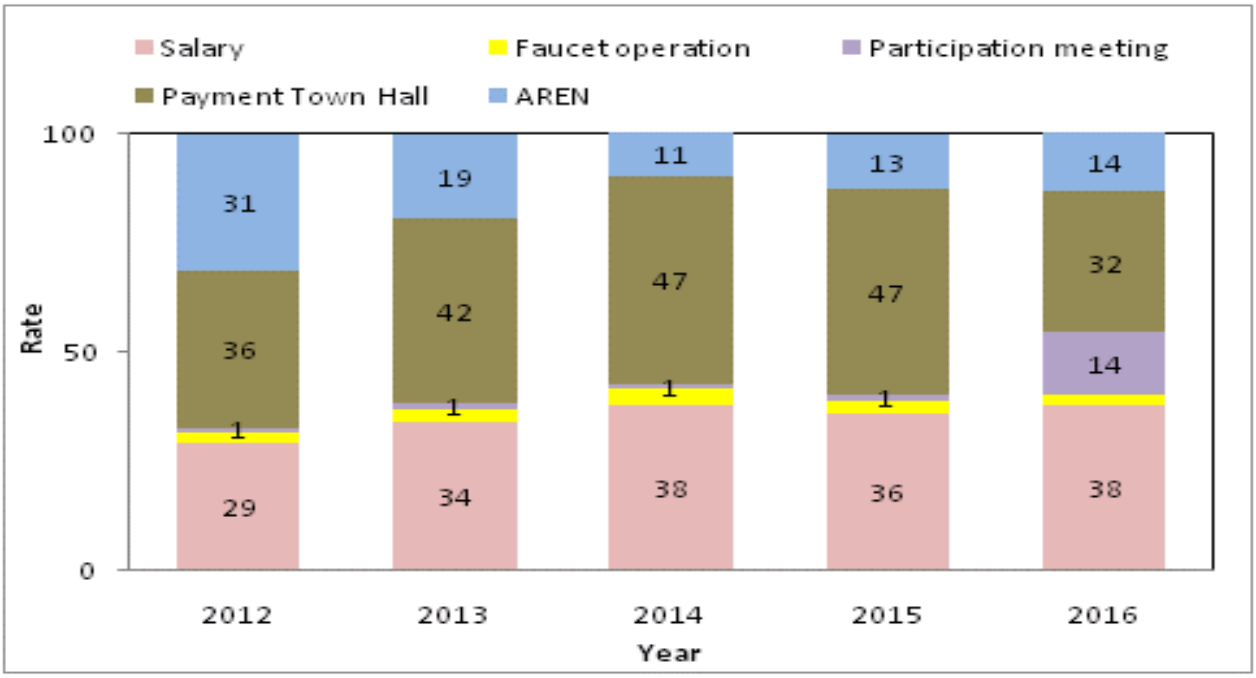

Fig.2: Breakdown of revenue from Bermo self-managed market from 2012 to 2016.

Almost all (91%) of the average earnings obtained at the Wurssena market level are paid to the municipality, which pays the tax collectors' salaries. Except for the editing of receipts used to collect taxes on the amount paid to the town hall, the salaries of the collectors represent the

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municipality's only expense item, at the level of the said market (Figure 3).

Fig.3: Distribution of revenues from the Wurssena market from 2012 to 2016.

Six (6) types of taxes are levied from actors operating on the market; These include identification fees (small and large ruminant) imposed on the owners of the animals, annual exercise taxes for butchers (wholesaler and retailer), intermediaries or witnesses and traders.

Taxes for butchers, intermediaries, witnesses and traders are levied annually; On the other hand, the collection of identification fees is weekly and is collected per capita.

The various taxes mentioned above represent almost all the revenue collected by the communities on livestock markets; they are the main source of income for these communities located in the pastoral zone.

Apart from the support of the state, the main sources of revenue of the municipality of Bermo are the municipal taxes and those of market. The latter come mainly from the self-managed livestock market. The other markets in the locality are almost empty in the dry season and only work in winter after the return of the breeders. Despite the presence of pastoralists, the rate of tax recovery on these seasonal markets is very low. The municipal tax is greatly influenced by the presence of the livestock market; in fact, the installation of small businesses in the municipality follows the transformation of the previously traditional market into a self-managed market.

The Wurssena market provides more than 90% of the revenue of Gadabedji commune. As for the other markets, including that of the town chief (Gadabédji), the revenue generated is low.

Table 2 shows the amounts of taxes collected by the municipalities from actors in the sector.

Table.2: Individual taxes levied on actors.

Nature Tax	Small Ruminant Identification	Large Ruminant Identification	Butcher retailer	Butcher wholesaler	Witness-intermediate	Trader
Amount (FCFA)	500	1500	7500	13000	3500	13000

Identification fees are collected per animal, while other taxes (butcher, intermediary and merchant) are collected annually.

3.1.2 General evolution of livestock sales in the department during 2016.

Sales are spread throughout the year and have been significantly larger in the Wurssena market. They mainly concern sheep, goats and cattle. Peak sales are observed in the months of July for sheep, in january, july and september for goats and in december, march and april for cattle.

Figures 4 and 5 show the evolution of sales during the year.

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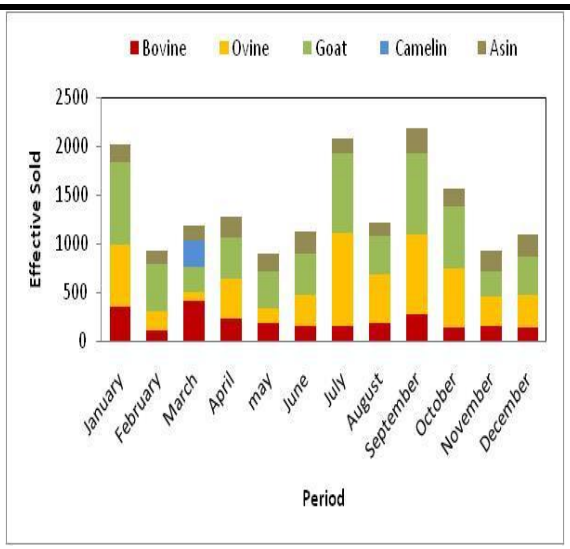

Fig.4: Livestock sales in the market from Bermo

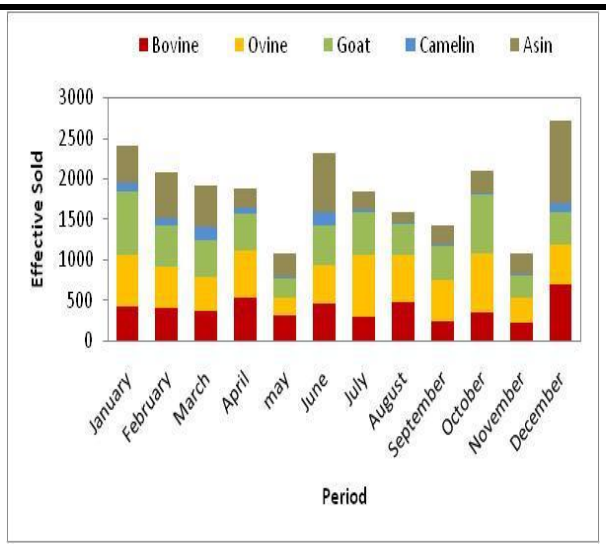

Fig.5: Livestock sales in the market from Wurssena

The best flow rates of the species presented on the market were obtained during the months of march, may and february, respectively in goats (96%), asins (90%), sheep and camels (89%). These record performances have all been recorded at the Wurssena market level.

Figure 6 depicts the evolution of the stocking rate of animals on the market.

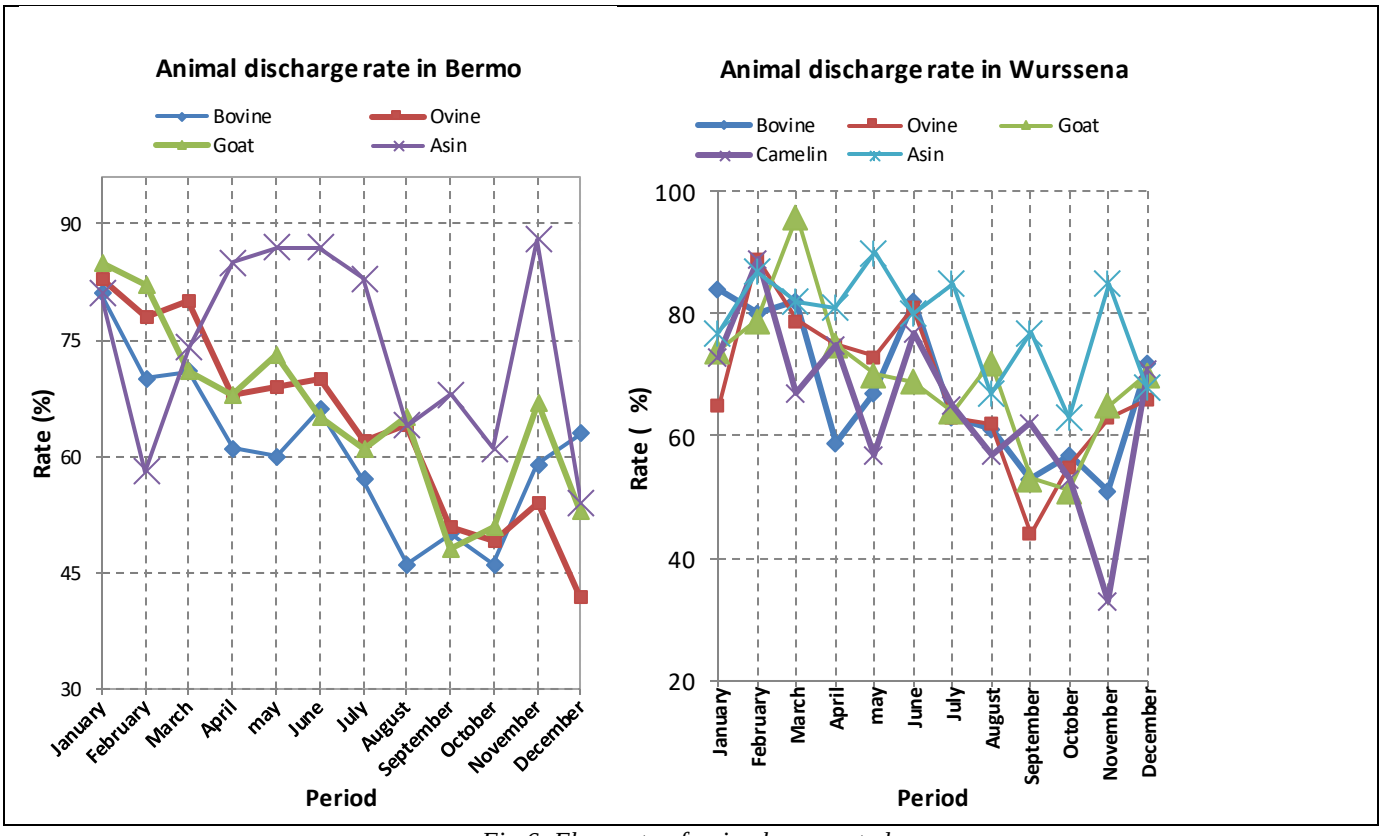

Fig.6: Flow rate of animals presented.

3.1.3 Evolution of selling prices of livestock.

At the cattle level, the highest average prices are recorded in january for bulls, cows and heifers and in september for bulls, as regards the Bermo market. These prices are also recorded in january for bulls and cows, in march for heifers and in august for bulls on the Wurssena market.

The lowest prices are in june for bull and cow and in may for bull and heifer in Bermo's self-managed market. These

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prices are also displayed in june for the cow and the bull, in may for the bull, june and september for the heifer (Figure 7).

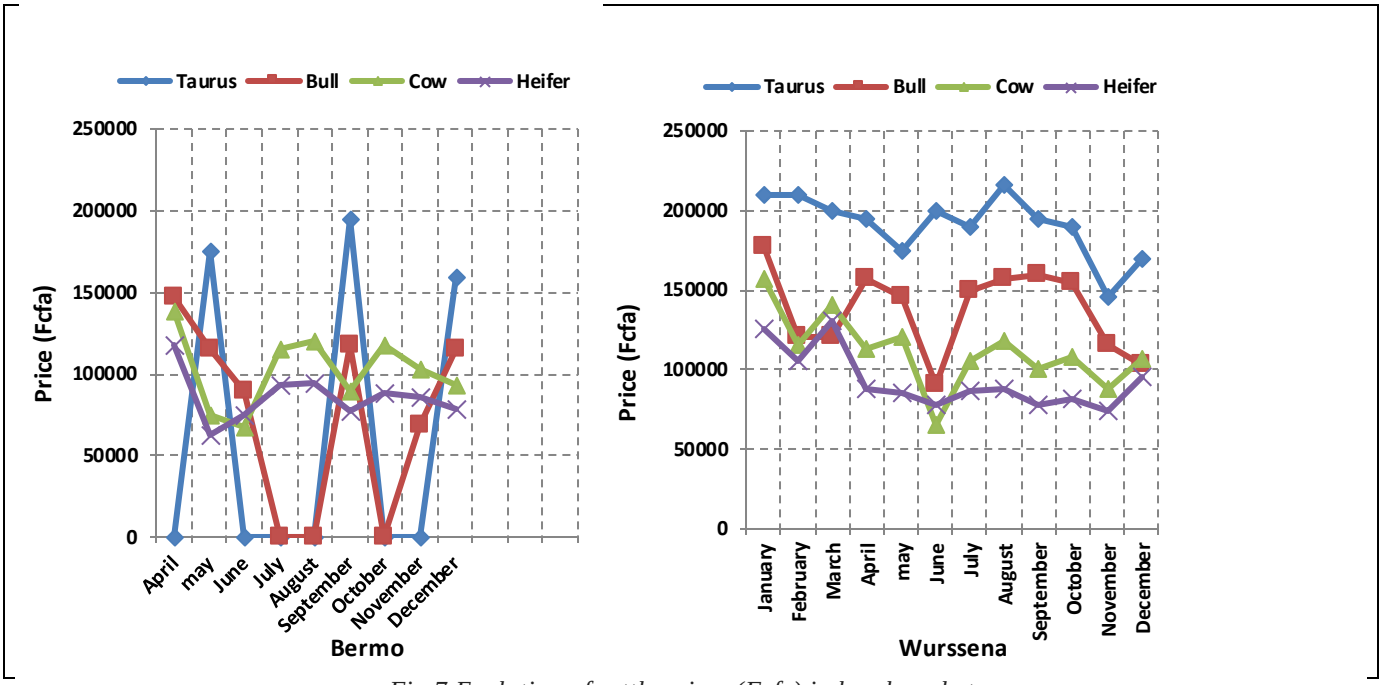

Fig.7:Evolution of cattle prices (Fcfa) in local markets.

Regarding the average prices of sheep, the maximum figures are shown in january for rams, male ewes and sheep under one year old, and in february for female sheep less than one year old on the Bermo market. As for the minima, they are recorded during the months of may to june for the ram, october for the sheep, august for the male sheep less than one year and in august and october for the female sheep of less than a year.

On the Wurssena market, the highest average prices are observed in january for ewes, male sheep under one year of age and female sheep under one year old and in august for ram. The average minimum prices are published in november in the relevant market (figure 8).
Prices are generally higher in the ram, then in the sheep.

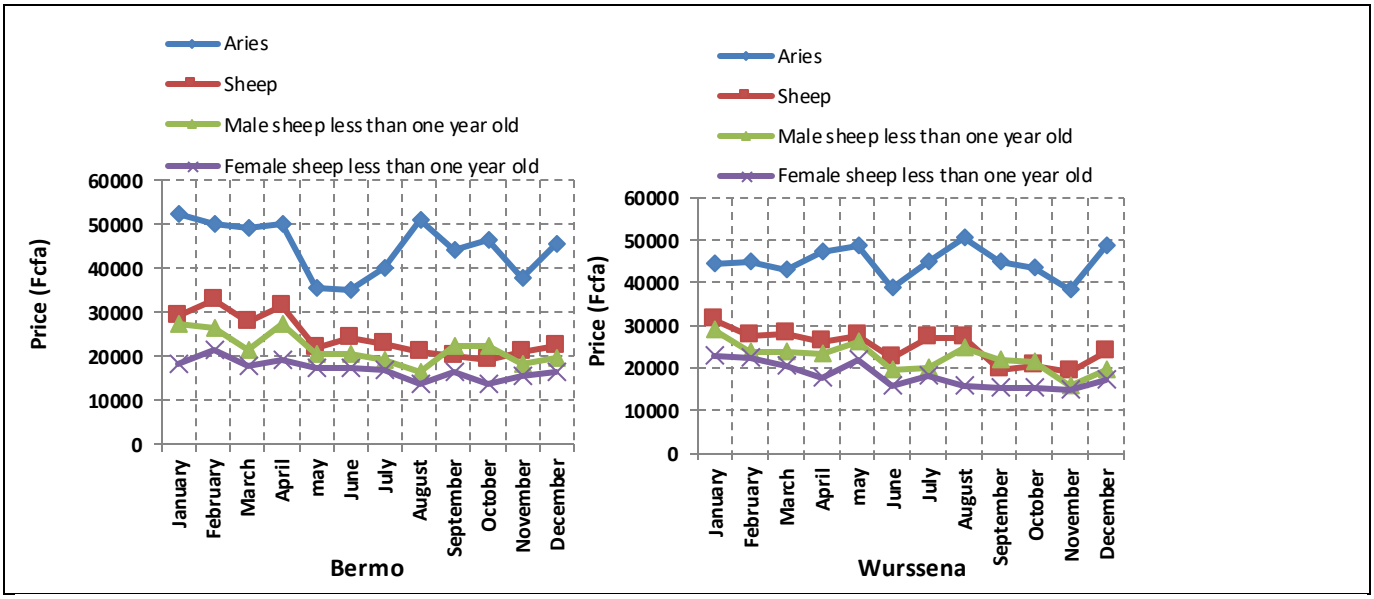

Fig.8: Evolution of sheep prices (Fcfa) in local markets.

In the case of the Bermo market, the highest average goat prices are in January for goats, female goats under one year old, in february for goats and february to april for male goats less than a year. These prices are observed in january for the goat, the male goat of less than one year

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and the female goat of less than one year, in august for the goat on the market of Wurssena.

The lowest prices are recorded in october for the goat, november for the goat on the two markets surveyed. They are displayed in august for male goats less than a year old, in august and september for female goats less than a year old in the Bermo market. In the Wurssena market, these prices are read in november for male goats less than one year old and goats less than one year old (figure 9).

Figure 9 reveals a clear superiority of the selling price of the female relative to the male for the wurssena goat.

In this market, there is also a gradual decline in prices from January to December.

The sale price of the goat is much higher than that of other goats during the year. This is due to the poor overweight condition and small conformation of male goats brought to market. On the other hand, the goat presents an acceptable state of fattening, thus answering the expectations of the customers. In fact, butchers are looking for fat animals, to ensure the satisfaction of consumers.

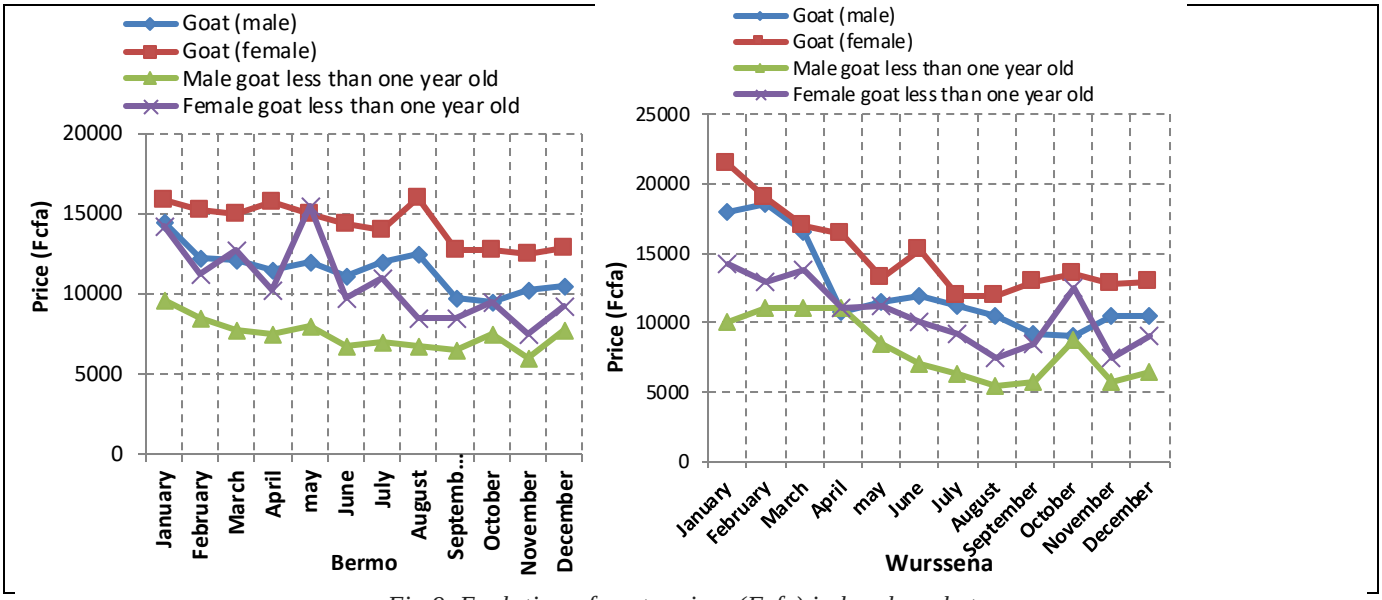

Fig.9: Evolution of goats prices (Fcfa) in local markets.

For camels, sales prices peak in may. They decrease in June and increase in july. After that, prices tend to stabilize until october. They decline from november to december, to grow in january and finally to decrease gradually from february to march. The selling prices of male subjects are slightly higher than those of females. However, no camel sales were recorded in september at Wurssena.

It should be noted that camel sales were only at the Wurssena market level.

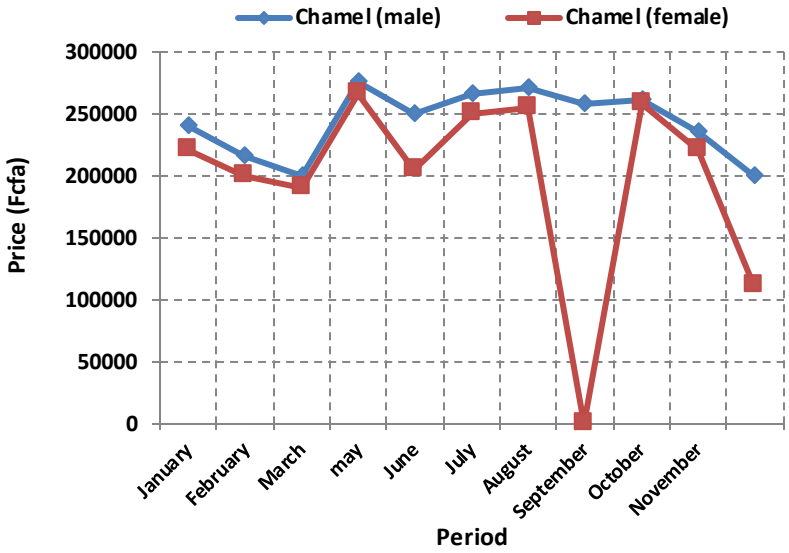

Fig.10: Evolution of the price (Fcfa) of the camels at the wurssena market

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At the level of the insects the price of the sale of the male is everywhere higher than that of the female. All in a ll, the peaks are displayed in may, june and november.

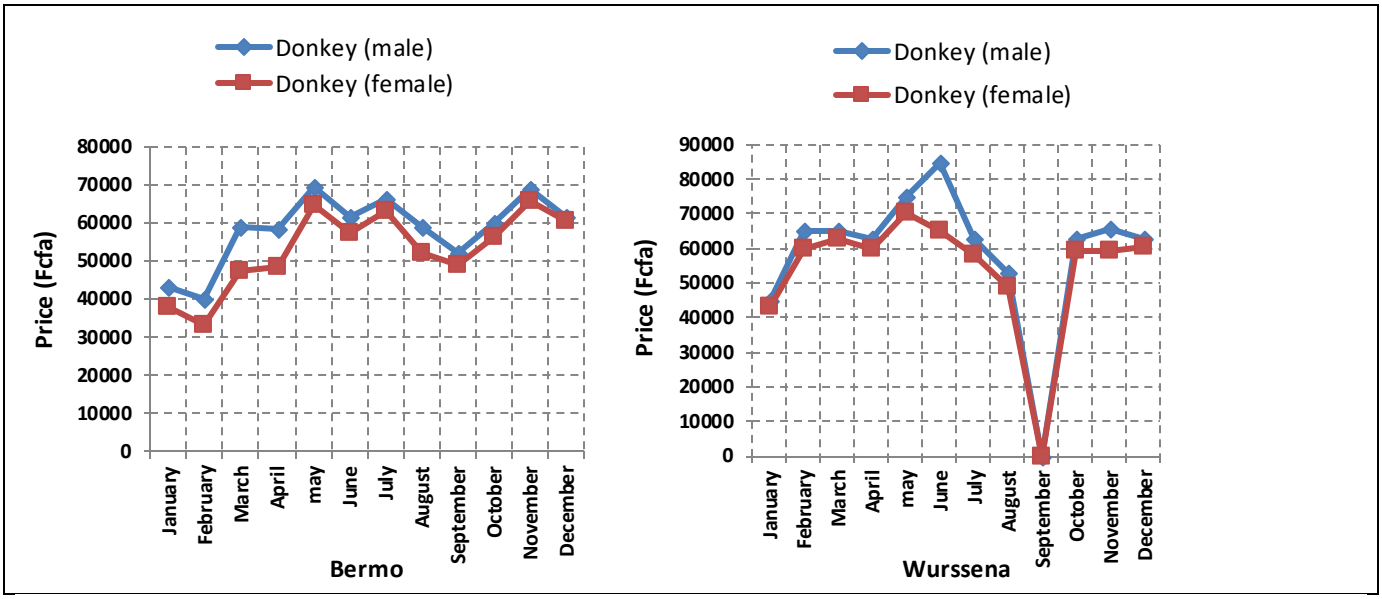

Fig.11:Evolution of donkey prices (Fcfa) in local markets.

IV. DISCUSSION

4.1 Presentations and sales periods of livestock

Animal numbers (insects, cattle, camels, goats and sheep) on the market are almost entirely sold, especially during february, march and may. The best flow rates are obtained with goats (96%) and donkeys (90%) at Wurssena market. Goats are the first species to be sold when needed. As for the donkeys, they are of great importance in this area with pastoral vocation, especially in the activities of dewatering and transport, which explains their strong presence on the markets and the observed selling prices.

Animal sales are mainly in the post-harvest period and during the dry season at the two livestock markets surveyed. They are also important in the run-up to the tabaski festival (aïd el fitre). This explains the high numbers of sheep sales during the months of July-September, the period in which this holiday takes place in 2016.

After harvests, the grain supply is high and selling prices are the lowest. The animals in their turn are in good condition. The period is therefore favorable to livestock farmers, who sell part of cattle to buy good quality cereals at a lower cost. The sales made in the dry season allow them especially to renew the food stock of the household.

A study conducted in the Burkina Faso Sahel, reports the numerical superiority of cattle in the Gorom-Gorom market in 2001. But, between 2002-2003, the numbers of cattle, sheep and goats presented approached (Ouedraogo, 2004). . In the same area, on kiMansila market, the run-off rate of animals presented is 40% for cattle, 23% for sheep and 24% for goats (Ouedraogo, 2004).

In the area of Diffa, sales peaks of small ruminants are recorded from august to september at the N'Guelkollmarket level. A large presence of large ruminants on the relevant market is observed during the monsoon season (July-September), with the return of breeders (Steve, 2008).

The presence of a livestock market allows the farmers to sell their animals to meet their needs. Livestock sales at the Tichift (north Mali) market level are higher than those observed in the two markets monitored; these range from 12645 small ruminants and 1873 camels during the first year (2010) of operation, to 12359 heads of small ruminants and 3452 camels only sold in the first half of 2011 (AVSF, 2013).

Work conducted at the Soubdou market in Niger indicates the sale of 1,000 head of cattle between june and november and 400 head between december and may. Livestock sales are mainly in the post-harvest period (a small analytical and synthetic atlas, 2006).

82% of the animals presented at N'Guelkoll and Kindjandi markets in Diffa area come from mobile pastoralists, including transhumant herders (Steve, 2008).

The sale is more important during the months of november and december.

The livelihoods baseline study of Bororo pastoralists in the former department of Dakoro reveals Peak Sales of livestock before the first rains (May-June) and harvests (Oxfam and*al*., 2008).

Touré and *al* (2005) report higher prices in february and higher sales during tabaski in male sheep.

4.2. Evolution of the price sale of animals.

For most species, sales price peaks are mainly observed during the winter season (June-September) and during the cold dry season (December-January). These two periods

are favorable to the breeders, because of the good state of overweight presented by the animals with the good availability of the natural resources (pasture and water). This results in the reduction of the herd's feed loads. On the other hand household food is facilitated by the abundance of agricultural products on the market. The breeder can therefore quietly negotiate the price in his favor, because the body condition of the animal allows him to negotiate the price in his favor.

Price peaks are also displayed in the hot dry season (March-May), when animals are almost absent from the market, most of the species having moved in transhumance; This allows the farmer to obtain competitive selling prices. This situation is mainly recorded in cattle and goats.

The farmer regularly sells a few heads, after the harvest, to stock food; this situation leads to the price peaks observed during this period, especially with animals in good condition.

The sales price of males generally exceed that of females, with the exception of goats. Most male goats are often slaughtered or sold at a young age. As can be seen, the males brought to the market are sometimes very young or in poor condition. Due to the high harvest in the goats, only a few rare heads for breeding reach maturity.

The work done in the pastoral area of the department of Gouré, report higher sales prices than those observed at the department of Bermo in bulls, cows, rams, goats and camels. On the other hand, the ewe and the camel presented lower amounts (European Commission, 2012). With prices of 280623 FCFA, 182380, 65005, 28947, 35144, 25060, 260674 and 156574 FCFA respectively for the price of the bull, the cow, the ram, the ewe, the goat, the goat, the camel and the camel on the market of Guidigir.

Price changes were also observed during holiday periods and by month (Abba 1998; Moussa and *al*., 1997;Ouedrago 1996).

On the other hand, the selling prices of the animals are higher during the months of June to August; to go lower from october to september (Ministry of Agriculture, Animal Resources and Fisheries of Djibouti, 2015).

At the level of the Tichift market in northern Tombouctou, the selling price is higher than 250000 FCFA for the dromedary and 210000 for the camel. It exceeds respectively 45000 and 20000 FCFA for sheep and sheep (AVSF, 2013). These prices are similar to those obtained in the markets of Bermo and Wurssena, with the exception of the ewe. Kept on the farm for breeding purposes, the ewe is rarely taken to the market. In the case of sales with the exception of diseased or reformed females, those marketed have good market value. Raised only for consumption because it is not whole, sheep are less popular on the market; which gives it relatively low sales prices.

The two markets studied (Tichift and Bermo), being located in pastoral zone; the determination of the price will depend mainly on the nature of the hazards of the pastoral campaign, the state of the animal presented to the market and the demand.

Through its role in meeting social and economic needs, livestock trading is necessary to maintain the survival of pastoralists. It also contributes to the development of rural towns particularly those located in the pastoral zone. It has a significant impact on the economy of municipalities and the creation of jobs and income.

4.3 Impact on the economy of the communes

The budgets of the communal entities located in the pastoral zone are fed mainly by the receipts collected at the level of the cattle markets, through the levy of the taxes on the animals. The recipes consist of identification taxes. Thus, since the creation of the self-managed market, the revenues collected annually at the level of the market of Bermo are never estimated below 1800000 FCFA.

Contrary to the regularity of the performance of this market, the contribution of the Wurssena market varies according to the state of the pastoral campaign. In 2012 and 2014, the department experienced deficits in fodder; which justifies the strong presence of breeders on the cattle market. The years 2011 and 2013, follow periods of crisis, which are characterized by the reduction of the size of the flock, of which a large part is sold. Hence this discontinuous change in revenue from 2011-2014. From 2015, the revenues increase gradually, to reach very important amounts (19 to 21 million FCFA). Despite its traditional character, the Wurssena market dominates in terms of revenue, as local farmers are wealthy, allowing them to bring inputs to the animals during the lean season. Added to this is the presentation of significant staff at the level of this market.

Each of the town halls (Bermo and Gadabédji) also levies taxes from livestock traders, butchers (retailers and wholesalers) and intermediaries / witnesses, which amount respectively to 13,000, 7,500-13,000 and 3,500 FCFA per year.

The revenue of the commune of Bermo results mainly from the existence of the self-managed livestock market. As for Gadabedji, more than 90% of the revenues of the municipality concerned come from the Wurssena market.

The revenue from the sale of livestock, collected by the commune of Chétimari in Niger amounted to 2987010 FCFA in august and 2785860 in november 2007. In the year 2008, the same entity records 2110700 FCFA in february, against 2056050 FCFA in April. The commune of Gueskérou collected revenues of 2004210 and 988380

FCFA in august and november 2007, 587700 FCFA and 389250 FCFA respectively in february and May 2008 (Steve, 2008).

In the sylvo pastoral zone of Senegal, the livestock market provides important currencies to the communes of Dahra, Ranérou and Djibo. At the level of Dahra, He contributes annually for at least twenty-four million; which allows it to be the second source of income of the municipality (Dia, 2014). Revenues from the sale of animals provide more than 20% of family expenses of the population of Ranérou (Dia, 2014). Taxes from the sale of cattle represent 20% of the revenue of the municipality of Dahra. 80% of the revenues generated by the Djibo livestock market are paid to the town hall (Dia, 2014).

The study of Ouedraogo (2004) at the Seytenda secure livestock market (Sahel Burkinabe), distinguishes receipts in the order of 1685850 FCFA, divided between the prefecture and the management committee.

Studies conducted on the Goumori cattle market in Benin, reveal that more than ten million profits from the sale of cattle are paid to the mayor and at least six million reserved to the management committee (Samba and*al*., 2013).

The contribution of the Tichift market in northern Tombouctou amounts to 746.4 million FCFA. Livestock sales account for 59% of household income (AVSF, 2013).

In Burkina Faso, the sale of animals annually provides more than 4 million FCFA to the town hall of GoromGorom (Ouedraogo, 2004).

The sale of animals represents 98.53% of household income in KouroBéri and 97.8% for Dereki (Hafizou, 2017).

4.4 Impact on job creation and income

Part of the revenue collected (35%) on the Bermo market is oriented towards paying the salaries of tax collectors and witnesses. On average 8% of Wurssena revenue is earmarked for tax collectors. The creation of a livestock market allows important actors (intermediary-witnesses, traders, collectors and conveyors) of the cattle-meat sector to obtain a job. The presence of cattle market in this pastoral area, has favored the emergence of new actors, who are responsible for small businesses, restaurateurs, shoemakers, artisans, etc.

In the Diffa region (Niger), 66-86% of Chétimari shopkeepers' turnover comes from pastoralists, compared to 57-83% in Gueskérou commune (Steve, 2008).

In Senegal, the sale of animals has a great influence on transport and trade; indeed during the transhumance the turnover of traders is decreasing; this is also the case for transporters, whose clientele decreases with the departure of farmers to the south. With the presence of the breeders, the traders of Dahra, Linguère and Ranérou record high turnover during the weekly markets. Merchandise trade is mainly maintained by livestock traders, as they represent more than 50% of the clientele in Linguère, 40% for Dahra and 60% at Ranérou. For three quarters of cart drivers, the weekly market (livestock) is driving up their revenues (Dia, 2014).

The Mansila market management committee, the brokers and the administration respectively receive 218,950 FCFA, 1748,000 and 570,400 FCFA per year (Ouedraogo, 2004).

According to the study conducted by AVSF in Mali, the establishment of the Tichift market has greatly improved the exchanges between the actors, the sharing of information concerning the evolution of the pastoral situation and the creation of several jobs (direct sellers, truck loaders and animal conveyors, etc.).

The removal of the witnesses allowed the breeders to be in direct contact with the buyers, to know the real price of the animal and also to sell cash (AVSF, 2013).

V. CONCLUSION

The livestock trade generates significant revenues for the communal entities of the department of Bermo. Thus, taxes collected from market players provide considerable financial resources to the decentralized entities of the pastoral zone. Also, the presence of these livestock markets has favored the establishment of other small businesses, which also provide financial resources to the town halls through the levy of taxes. The sale of livestock allows pastoralists to stock up on food after good harvests. Several species are presented on the market and the best selling periods are the winter season (June-September) and dry season (December-January) for most species because of the good condition of the animals at these times. However, price peaks are also recorded in the hot dry season (March-May), where livestock is scarce on the market because most of the species are transhumant.

In addition to these resources obtained through taxes, the presence of livestock markets leads to the creation of jobs and income for various actors including butchers, middlemen or witnesses, conveyors, collectors, merchant-resellers of livestock, restaurateurs and merchants of various articles.

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Effects of Land use Change on Dung Beetle (Scarabaeinae) Community Structure in South Western Ghats

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Abstract*—Western Ghats in the Indian subcontinent is one of the world's eight 'hottest hotspots' of biological diversity along with Sri Lanka. Land use changes in the Western Ghats caused by agricultural expansion and other anthropogenic activities have resulted in loss of forests and is a major threat to Western Ghats biodiversity. In the present study, Scarabaeinae dung beetles were used as biological indicators to study the effects of land use change on biodiversity in the South Western Ghats. Community attributes such as abundance, species richness, species composition, functional guild composition, temporal guild composition and beetle sizes were compared between a forest and agriculture habitat in the South Western Ghats region. Cow dung baited pitfall traps were used to collect dung beetles in the presummer, summer and monsoon season during 2007-2008 study period. The study showed that dung beetle community attributes were affected due to land use changes. Of the 31 species collected between the two habitats, only 15 species were shared between forest and agriculture habitat accounting for 51.6% species turnover. Low abundance recorded in agriculture habitat resulted from low diversity and amount of dung types available to beetles when compared to forest habitat, while high species richness in agriculture habitat resulted from the presence of heliophiles and synanthropic species that has established in the region owing to decades of anthropogenic disturbance. Functional guild tunneler, dominated both the habitats because of their superior competitive nature. Temporal guild was dominated by nocturnal guild in the forest due to the availability of dung at night from wild animals, and diurnal guild in agriculture habitat owing to the availability of dung during the day as a consequence of agricultural practices. Small beetles dominated both the habitats as a result of decline in large dung pad producing mammals in the region as a consequence of anthropogenic disturbance. Further deterioration of forests in the region is important to conserve the remaining forest specialists.*

Keywords*— *Scarabaeinae, dung beetles, land use, community attributes, biological indicator, South Western Ghats.

I. INTRODUCTION

Population growth and rising consumption exerts continuous pressure on land for increased food production. Higher production is possible either by intensification on existing agricultural land or expansion into new areas (Tilman *et al*., 2011). Conversion of forests into agricultural land is the most widespread method of agricultural expansion and is considered as the leading cause of global forest loss (Kissinger *et al*., 2012). Such land use changes can have serious environmental consequence particularly on biodiversity (Alroy, 2017; Phalan and Balmford, 2014; Wright, 2010). Changes in biodiversity have a strong potential to alter ecosystemproperties and the goods and services they provide to humanity (Hooper *et al*., 2005, Isbel *et al*., 2018).

The Western Ghats in the Indian Subcontinent is a 1,600 km long chain of mountains running parallel to India's western coast. Western Ghats with its exceptionally high level of biological diversity and endemism is recognized as one of the world's eight 'hottest hotspots' of biological diversity along with Sri Lanka. It has profound influence on the rainfall pattern of peninsular India and has high geological, aesthetic and cultural values. The existing forests of Western Ghats are highly fragmented and is facing the prospect of increasing degradation (Bawa *et al*., 2007). Land use changes in the Western Ghats over the last century caused by agricultural expansion, conversion to plantations, non-timber forest product harvest and infrastructural projects have resulted in loss of forests and is a major threat to Western Ghats biodiversity (Jha *et al*., 2000; Kumar, 1993; Menon and Bawa, 1997; Shahabuddin and Prasad, 2004).

Scarabaeinae dung beetles are ecologically important group of insects widely used to study the effects of habitat

modifications on biodiversity. They are cost effective bioindicators as they can be easily sampled, are sensitive to ecosystem changes, are broadly distributed, and their taxonomy and ecology are relatively well known (Nichols *et al*., 2007). Adults and larvae from this subfamily are detritivores and use decaying organic material, such as mammal excrement, dead animal carcasses, rotting plant matter, and other resources, as food (Halffter and Mathews, 1966). Through their feeding habits, they perform important ecological services such as nutrient recycling, seed dispersal, forest regeneration, control populations of disease causing parasites, and reduce carbon emissions (Ardali *et al*., 2016, Forgie *et al*., 2018, Piccini *et al*., 2017; Slade *et al*., 2016).

Scarabaeinae beetles are categorized into three functional groups based on the way they use the resources for feeding and reproduction; they are telecoprids or rollers (food balls are rolled some distance before burial), paracoprids or tunnelers (tunnels are dug next to or below the food source), and endocoprids or dwellers (feed and reproduce inside the food resource) (Halffter and Mathews, 1966). Since dung is an ephemeral source, to avoid competition, dung beetles also exhibit different activity pattern during a day based on which they are divided into temporal guilds (Feer and Pincebourde, 2005). Diel periodicity studies commonly distinguishes two major groups of dung beetle species, nocturnal and diurnal (Krell *et al*. 2003; Krell-Westerwalbesloh *et al*. 2004).

In the present study effects of land use change on dung beetle community attributes in South Western Ghats was studied. Dung beetle community attributes such as abundance, species richness, species composition, functional guild composition, temporal guild composition and beetle sizes were compared between a forest and agriculture habitat in the region.

II. MATERIALS AND METHODS

2.1 Study site

The study region, Nelliampathi is situated in the South Western Ghats just south of the Palghat Gap. The Palghat Gap is a transverse valley about 32 km wide and is the only major break in the continuous mountain range. It sharply divides Wayanad and the Nilgiris in the north from Nelliampathi Hills of the Thrissur district, to the south (Ali, 1999). The study was carried out in Kaikatty in Nelliampathi, located at 10^0 31'N and 76^0 40'E, at an elevation of 960 msl (Fig. 1). It is an ecologically high sensitive area enclosing the Nelliampathi Reserve forest and is bordered by the Parambikulam tiger reserve towards the south and southeast (Nair, 1991; Joy, 1991). The land forms a corridor for the movement of long ranging species such as *Panthera tigris*Linnaeus, 1758 (tiger), *Panthera pardus* Linnaeus, 1758 (leopard), *Bos gaurus* Smith, 1827 (wild gaur), and is also a crucial migratory route for *Elephas maximus* Linnaeus, 1758 (elephant) (Sukumar and Easa, 2006).

The forest in the study site consisted of a 971 hectare reserve forest characterized by West Coast Semi-Evergreen trees (Champion and Seth, 1968). The agriculture habitat consisted of a 372 hectare banana and orange plantations (Fig. 1). The transition between the forest habitat and the agriculture field occurred over the space of five to eight metres.

2.2 Sampling

Dung beetles were collected in May (summer), September (monsoon) and December (presummer) of 2007-2008. Cow dung baited pitfall traps were used to collect dung beetles from the forest and agriculture habitat. Ten traps were placed 50m apart in each of the two habitats during each collection effort. The traps in forest and agriculture habitats were separated by a distance of 100m. The trap contents were collected at 12 h intervals (6:00-18:00h and 18:00-6:00h) to separate diurnal and nocturnal species. Collected beetles were preserved in 70% alcohol and later identified to species levels using taxonomic keys available in Arrow (1931) and Balthasar (1963a, b) and also by verifying with type specimens available in the Coleoptera collections of St. Joseph's College, Devagiri, Calicut.

Species were sorted into the three functional guilds, rollers (telecoprids), tunnelers (paracoprids) and dwellers (endocoprids) (Cambefort and Hanski, 1991). For categorizing temporal guilds, the beetles collected only during the diurnal collections were labelled as diurnal and only in nocturnal collections were labelled as nocturnal beetles. For those beetles collected in diurnal and nocturnal collections, their abundance were tested statistically to designate them as diurnal and nocturnal beetles; those showing no significant variation in abundance between the diurnal and nocturnal collections were labelled as generalist species. Singleton species were excluded from the temporal guild study. Length of the beetles were measured and beetles < 10 mm was designated as small beetles ≥10 mm was designated as large beetles (Barrágan *et al*., 2011).

2.3 Analysis

To show how common or rare a species is in relation to other species, a relative abundance graph was plotted for the two habitats. Since the data was not normally distributed, non-parametric test Kruskal-Wallis was performed to compare the functional guild and temporal guild abundance within the two habitats. Differences with a p-value <0.05 was compared using Wilcoxon-Mann/Whitney Test. Overall abundance between the two habitats was compared using Wilcoxon Signed Ranks Test and in beetle sizes within the habitats was

compared using Mann-Whitney U test. The tests were carried out using SPSS 21.

III. RESULTS

A total of 622 beetles belonging to 21 species and seven genera were collected from forest and 343 beetles belonging to 25 species and eight genera were collected from agriculture habitat (Table 1). The abundance of dung beetles between the two habitats did not vary significantly (p=0.54). Of the 31 species collected between the two habitats, only 15 species were shared by forest and agriculture habitat, resulting in 51.6% species turnover. *Onthophagus pacificus* (37.78%) and *Onthophagus furcillifer* (24.92%) were the dominant beetles in the forest assemblage and together constituted 62.70% of the total abundance. *Caccobius meridionalis* (25.66%) and *Onthophagus fasciatus* (21.57%) were the dominant beetles in the agriculture habitat assemblage and together constituted 46.23% of the total abundance. The Rank abundance plot for the two habitats showed a steep slope as a result of dominance of these two species and a long tail of several rare species (Fig.2).

Functional guild composition showed significant variation in abundance within forest and agriculture habitat (Table 2). Tunnelers dominated the forest (93.41% of total abundance, 18 species) and agriculture habitat (96.50% of total abundance, 22 species). Rollers were represented by two species, *Paragymnopleurus sinuatus* and *Sisyphus araneolus* in the forest habitat and was the second most abundant functional guild (6.43% of total abundance) but was absent in agriculture habitat. Dwellers represented by one species, *Tibiodrepanus setosus* (0.16% of the total abundance) was the least dominant guild in the forest assemblage but dwellers represented by three species *Liatongus indicus*, *Tibiodrepanus setosus* and *T. sinicus* (3.50% of total abundance) was the second dominant guild in agriculture habitat (Fig.3).

Temporal guilds showed significant variation in abundance within forest and agriculture habitat (Table 2). Nocturnal guild was the most abundant guild (60% of abundance) in the forest assemblage but diurnal guild dominated agriculture habitat (66% of total abundance). Generalist species were least abundant in both the habitats (Fig.4).

Small species dominated the assemblage in forest (85.70% of total abundance) and agriculture habitat (82.22% of total abundance). Large species accounted for 14.30% of abundance in forest and 17.78% of abundance in agriculture habitat (Fig.5). There was no significant variation in abundance between large and small beetles in agriculture (p=0.219) or forest habitat (p=0.142).

IV. DISCUSSION

In the present study dung beetle community attributes were affected by land use change in South Western Ghats. High abundance was recorded in forest habitat when compared to agriculture habitat. Similar observations were made in multiple studies done in modified habitats (Nichols *et al*., 2007). Cultivated land often lacks the microhabitat diversity of natural habitats and there are fewer dung types available due to the disappearance of large wild mammals (Nichols *et al*., 2007; Nielsen, 2007). Agriculture habitats in Nelliampathi are relatively small patches amidst vast stretches of plantations and forests and though incursions of wild animals into agriculture habitat has been observed, still the diversity and amount of dung types available is less compared to the forest habitat, which in turn affected the abundance of dung beetles in agriculture habitat.

Higher species richness in agriculture habitat can be attributed to the establishment of heliophiles and synanthropic species in the region as a result of decades of anthropogenic activities such as deforestation, habitat modification and fragmentation in the South Western Ghats region (Sukumar and Easa, 2006; Latha and Unnikrishnan, 2007; Prabhakaran, 2011). These are species capable of thriving in man-made habitats in the region (Vinod, 2009; Sabu *et al*., 2011; Sabu, 2011; Venugopal *et al*., 2012). *Caccobius meridionalis, C. gallinus, C. ultor* which were absent in forest and present in agriculture habitat with preference towards ruminant herbivore dung (Hanski and Cambefort, 1991) are considered as such synanthropic species (Sabu, 2011).Similar presence of synanthropic species were observed in Colombia in studies done in natural and anthropogenic habitats (Escobar, 2004), in guamil patches with secondary successions in Gautemala (Avendano-Mendoza *et al*., 2005) and in pastures of Central America (Horgan, 2007). Such increase in species richness in disturbed habitats associated with species that respond positively to disturbance is not considered a positive attribute, as original species composition is altered to favor disturbance adapted species (Davis *et al*., 2001). *Onthophagus furcillifer* and *Onthophagus pacificus* which were the dominant beetles in the forest habitat and were also well represented in the agriculture habitat are considered as heliophilic species, well adapted to survive in the degraded forests and agriculture habitat of the region.

Tunnelers represented the most speciose and abundant functional guild both in forest and agriculture habitat. Tunneler guild dominated the assemblage in other forests of Western Ghats also (Sabu *et al*., 2006, 2007; Vinod and Sabu 2007; Sabu *et al*. 2011). Aggressive and superior competitive nature of tunnelers in utilizing the dung resource

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(Doube, 1991; Krell-Westerwalbesloh *et al*., 2004) contributed to their success and dominance in the habitats. Rollers though the second most dominant guild in Nelliampathi forest was absent from agriculture habitat. Rollers require firm (less liquid) dung than the tunnelers because of the need to make them into balls (Halffter and Mathews, 1966). The low forest floor temperature and high humidity in these moist forests, keeps the dung moist and in a semi fluid state for longer periods, which makes dung ball making and rolling an energetically costly behaviour (Sabu *et al*., 2007). Thick understorey vegetation in these moist forests also act as a hindrance to ball rolling activities (Vinod, 2009). Their absence in agriculture habitat can be related to their sensitivity to changes in vegetation, microclimate and land use (Nielsen, 2007). Dwellers are strongly associated with large herbivore dung pads and breeds successfully only in undisturbed dung pads with little competition from competitively superior tunnelers and rollers (Hanski and Cambefort 1991; Krell *et al*., 2003; Krell-Westerwalbesloh *et al*., 2004). Low abundance of megaherbivores and their dung pads, in these forests due to extensive human interference (Abraham *et al*., 2006; Joy, 1991; Mathew *et al*., 1998; Sukumar and Easa, 2006) and competition from the competitively superior tunnelers limits the availability of undisturbed dung pads for use by dwellers in both the habitats (Doube, 1991; Krell *et al*., 2003). Moreover, in the agriculture habitat, dung pads are removed by farmers during agricultural practices like tilling, ploughing, manuring, which disrupts feeding and breeding activities of dwellers (Sabu and Vinod, 2005).

Dawn and dusk are two periods when defecation of mammals peak and this corresponds to the increase in activity of dung beetles during these times (Gill, 1991). Dominance of nocturnal guild in the forests of Nelliampathi is probably related to the availability of food resource at night as many mammals void their dung at the end of a feeding day. But in agriculture habitat, the main source of dung is contributed by domestic herbivores which are active during the day and confined to sheds at night. This led to the dominance of diurnal species in agriculture habitat. Similar dominance of diurnal species were observed in pastures, croplands and areas used for raising cattle in Honduras (Halffter *et al*.,1992), Mexico (Horgan, 2002) and Colombia (Escobar, 2004). Diurnal beetles were smaller in size than nocturnal and generalist species (Cambefort, 1991) and this is partially related to thermoregulatory constraints (Bartholomew and Heinrich, 1978). Large beetles dissipate heat more slowly during the day compared to small beetles and may face the problem of overheating. Predation may also play some role in limiting the size of diurnal beetles (Cambefort and Walter, 1991) as small beetles will be less visible to the predator during the day than large beetles.

Studies have recorded local extinctions and abundance declines on large-bodied beetles with increase in anthropogenic disturbance (Feer, 2008; Gardner *et al*., 2008; Jankielsohn *et al*., 2001; Shahabuddin *et al*., 2005). Large beetles prefer large dung pads (Doube, 1990; Hanski and Cambefort, 1991) and also use disproportionately large share of resources, they are therefore negatively affected by reduction in resource availability as in disturbed habitats (Doube, 1990; Larsen *et al*., 2008). Anthropogenic disturbance in the South Western Ghats region (Abraham *et al*., 2006; Joy, 1991; Mathew *et al*., 1998; Sukumar and Easa, 2006) has led to the decline in large dung pad producing mammals like elephant, gaur and the abundance of small dung pad producing mammals in these forests. This has resulted in small sized beetles dominating the forest and agriculture habitats. In addition physiological intolerance to thermal stress in the degraded open forest and agriculture habitat also affects large beetles (Bartholomew and Heinrich, 1978; Chown, 2001). Such dominance of small beetles can negatively affect the ecosystem functions these beetles provide, such as dung removal in a habitat (Kenyon *et al*., 2016).

V. CONCLUSION

In the present study, land use change affected dung beetle community structure in a forest and agriculture habitat in South Western Ghats region. Natural habitat such as the forest supported higher abundance of dung beetles when compared to anthropogenic habitat like agriculture field due to the abundance and diversity of food resource available. But higher species richness in agriculture habitat due to the establishment of synanthropic and heliophilic species dominating the region is of concern. Further studies are recommended in the region to document the general trend in other forests and modified habitats and measures should be taken to protect the remaining forests to conserve the forest specialists.

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Fig. 1: A. Map showing South Western Ghats; Habitats under study in Nelliampathi B. Semi-evergreen forest, C. Agriculture habitat.

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*Table.1: Dung beetle species, abundance, temporal guild (Di= diurnal, N= nocturnal, G= generalist, *= guild not specified); functional guild (T= tunneler, R= roller, Dw= dweller) and beetle sizes (S= small, L= large) in a semi-evergreen forest (SEG) and agriculture habitat (AGR) of Nelliampathi during 2007-2008 study period.*

No.	Species	SEG	AGR	Temporal guild	Functional guild	Size
1	*Caccobius gallinus*	0	5	Di	T	S
2	*Caccobius meridionalis*	0	88	Di	T	S
3	*Caccobius ultor*	0	3	G	T	S
4	*Catharsius molossus*	1	12	N	T	L
5	*Copris repertus*	28	27	N	T	L
6	*Liatongus indicus*	0	1	*	Dw	S
7	*Onitis subopacus*	0	1	*	T	L
8	*Onthophagus amphicoma*	1	3	G	T	S
9	*Onthophagus andrewesi*	8	1	Di	T	S
10	*Onthophagus bronzeus*	29	2	G	T	S
11	*Onthophagus castetsi*	16	0	N	T	S
12	*Onthophagus cavia*	1	0	G	T	S
13	*Onthophagus centricornis*	1	0	*	T	S
14	*Onthophagus ensifer*	3	12	Di	T	S
15	*Onthophagus fasciatus*	0	74	Di	T	S
16	*Onthophagus favrei*	2	5	G	T	S
17	*Onthophagus furcillifer*	155	44	Di	T	S
18	*Onthophagus insignicollis*	1	2	G	T	S
19	*Onthophagus laevis*	18	4	G	T	S
20	*Onthophagus manipurensis*	19	8	G	T	L
21	*Onthophagus pacificus*	235	13	N	T	S
22	*Onthophagus porcus*	0	1	*	T	S
23	*Onthophagus rectecornutus*	0	1	*	T	S
24	*Onthophagus turbatus*	16	12	N	T	S
25	*Onthophagus vladimiri*	7	0	G	T	S
26	*Paracopris cribratus*	40	7	N	T	L
27	*Paracopris davisoni*	0	6	N	T	L
28	*Paragymnopleurus sinuatus*	1	0	*	R	L
29	*Sisyphus araneolus*	39	0	N	R	S
30	*Tibiodrepanus setosus*	1	10	G	Dw	S
31	*Tibiodrepanus sinicus*	0	1	*	Dw	S

Table.2: Statistical analysis of functional and temporal guild abundance of dung beetle species associated with a semi- evergreen forest and agriculture habitat of Nelliampathi during 2007-08 study period.

Parameters	Kruskal-Wallis H test			Wilcoxon-Mann/Whitney Test (*P* value)		
Functional guild	**H**	**DF**	***P***	**T-R**	**R-Dw**	**T-Dw**
Agriculture habitat	19.569	2	< .001	< .001	.042	.003
Forest habitat	21.629	2	< .001	.126	.005	.007
Temporal guild	**H**	**DF**	***P***	**Di-N**	**N-G**	**Di-G**
Agriculture habitat	65.842	2	< .001	< .001	.001	< .001
Forest habitat	49.891	2	< .001	< .001	<.001	.053

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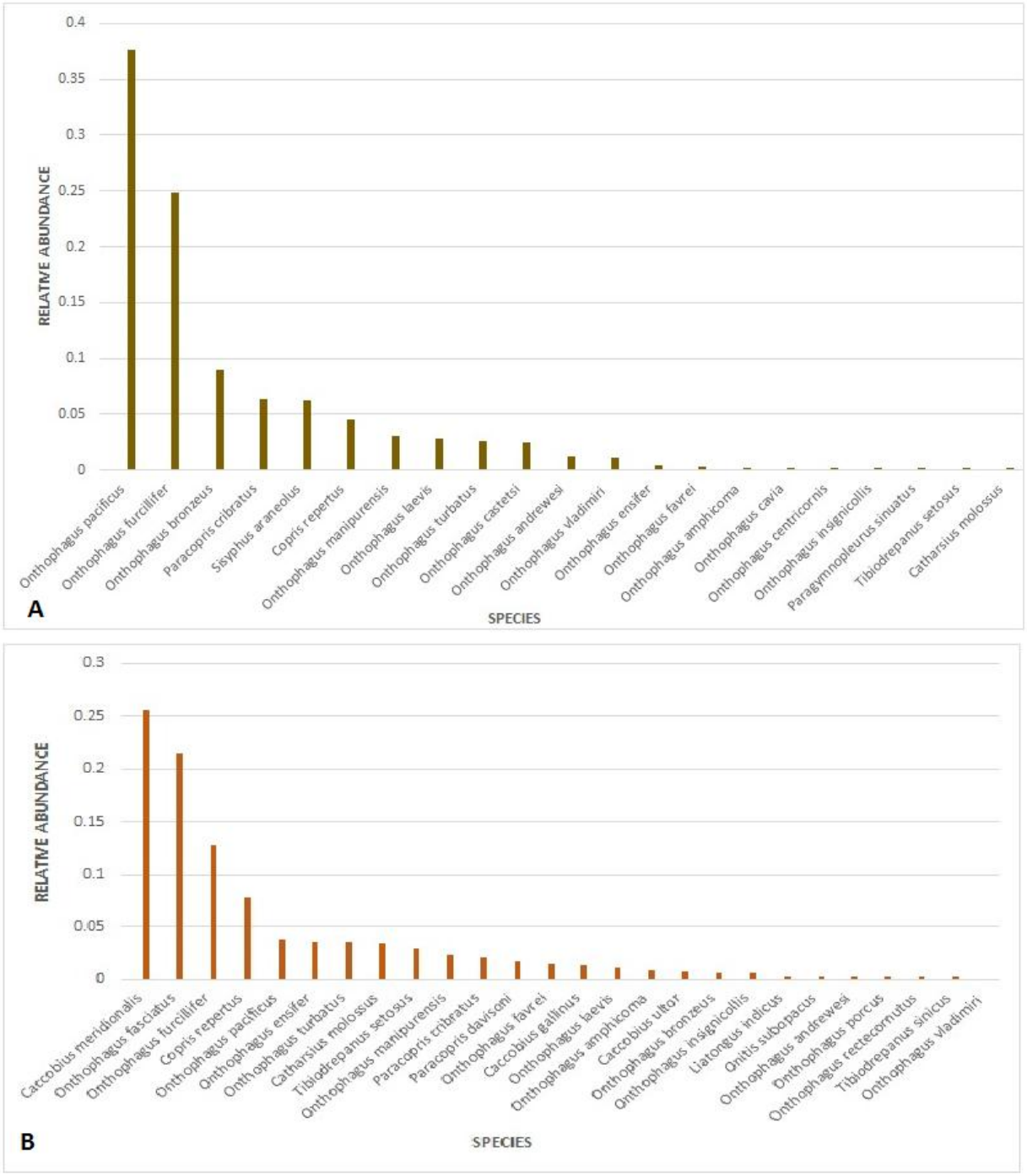

Fig.2: Relative abundance of dung beetle in a (A) Semi-evergreen forest and (B) Agriculture habitat of Nelliampathi during the 2007-2008 study period

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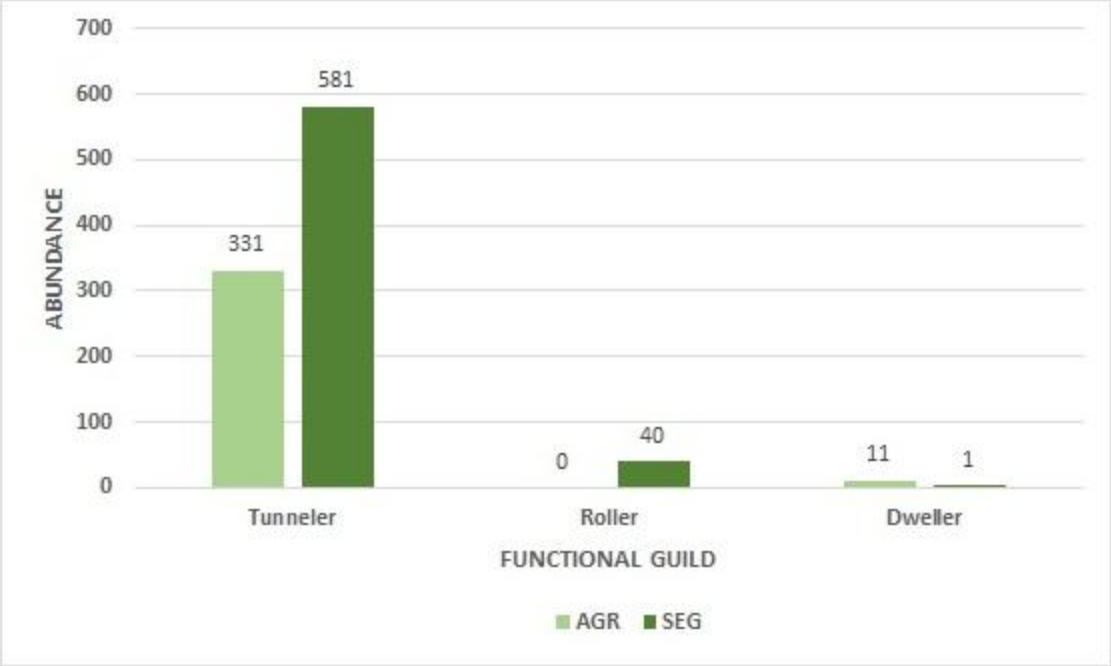

Fig.3: Functional guild composition and abundance of dung beetle species in a Semi-evergreen forest and Agriculture habitat of Nelliampathi during the 2007-2008 study period

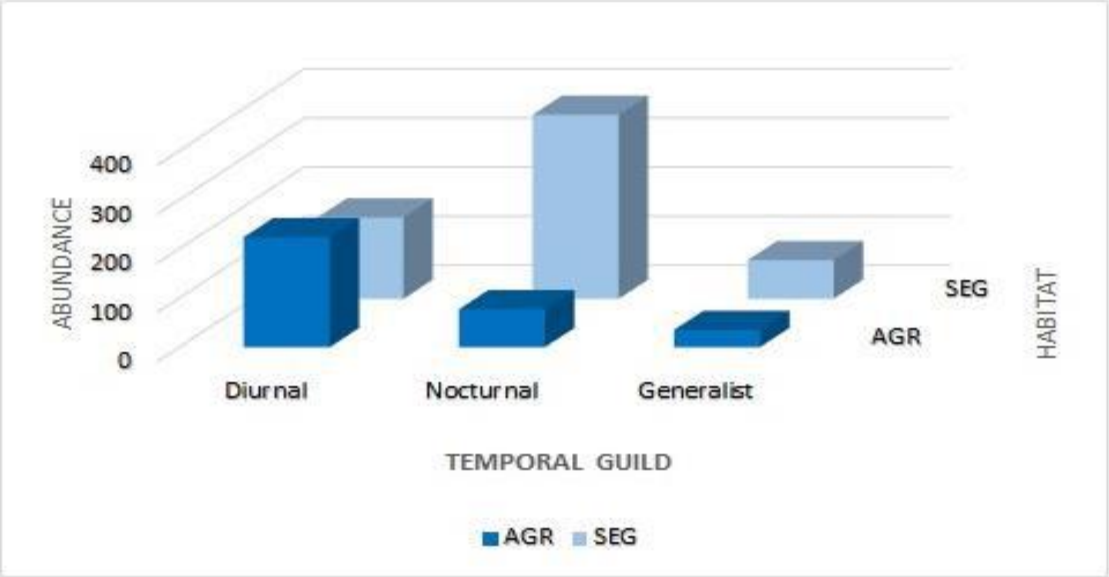

Fig.4: Temporal guild composition and abundance of dung beetle species in a Semi-evergreen forest and Agriculture habitat of Nelliampathi during the 2007-2008 study period

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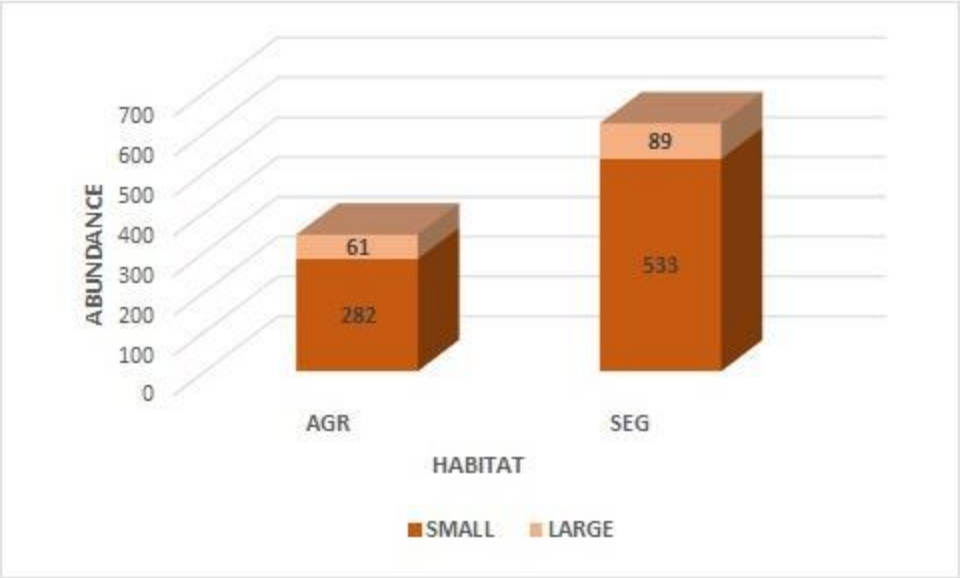

Fig.5: Abundance of large and small dung beetles in a Semi-evergreen forest and Agriculture habitat of Nelliampathi during the 2007-2008 study period

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Occurrence of entomopathogenic nematodes in Golaghat District of Assam

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***Abstract*—** *A survey of entomopathogenic nematodes was conducted in different vegetations of Golaghat district of Assam. Soil samples were tested for the presence of steinernematid and heterorhabditid nematodes by baiting with Galleria mellonella larvae. Out of 100 soil samples studied only one was positive for entomopathogenic nematodes (1%), containing one Steinernema isolate. Morphological and morphometrical studies were undertaken to characterize the isolate. The Steinernema isolate was identified as S. kushidai.*

Keywords*— *Entomopathogenic nematodes, Steinernema species, biocontrol agent, morphological and morphometric studies.

I. INTRODUCTION

Entomopathogenic nematodes (EPNs) of the genus *Steinernema* have been globally used as safe biocontrol agents against soil borne insect pests. Recent years have seen an increased interest in studying entomopathogenic nematodes (EPNs) not only because of their biological control potential but also to answer other research questions in the fields of ecology, biodiversity, evolution, biochemistry, symbiosis, and molecular genetics [1]. These nematodes are symbiotically associated with entomopathogenic bacteria *Photorhabdus* [2] and *Xenorhabdus* [3]. The third-stage infective juvenile (IJ) is the only free-living stage that persists in the soil in search of a host. Following entry, IJs release the symbiotic bacteria into the insect hemocoel, multiply and kill the host, usually within 24-48 h [4,5].In the previous survey, *H. bacteriophora* , *S.tami, S.ceratophorum* and *S.abbasi* have been reported from Assam [6,7] . The current survey is the first to be conducted in Golaghat district of Assam with the goal of finding EPN isolates and to identify the species present which are probably act as a biological control agents against relevant insect pests .

II. MATERIALS AND METHODS

Survey and sample collection: A systematic survey was undertaken in five villages, viz., Changkala senshowa, Alengmuria gaon, Dhekial gaon, Chetia gaon, Sensowa gaon of the development block Kathalguri, Golaghat district of Assam for the presence of entomopathogenic nematodes during 2016 to 2017. A total of 100 soil samples were collected randomly from diverse habitats, *viz.*, rice field, vegetable grown area, sugarcane plantation area, banana plantation area and tea plantation area. Each soil sample was a composite of 5-20 random sub-samples taken in the same location, but at least 10 m away from each other and to a depth of 30 cm, using a small shovel. Between samples, the shovel was thoroughly ringed with water and air dried to prevent contamination of the next sampling unit. Information regarding the location, standing crop in the field and soil type was recorded. Samples were packed in polythene bags. The soil was thoroughly mixed on a plastic sheet and half of each sample was used for extraction of entomopathogenic nematodes (EPNs).

Extraction of entomopathogenic nematodes (EPNs) from soil samples

EPNs were recovered from soil samples using an insect baiting technique described by Bedding and Akhurst [8]. Ten last instar *Galleria mellonella* (L.) larvae were placed in a 300 ml plastic container containing moistened soil obtained from one of the samples and kept at room temperature (22 ± 2°C) for 2 weeks. The traps were checked every two days from the 5th day. Dead larvae from each container were placed in White traps to collect emerging IJ and were replaced by fresh larvae White [9].To verify the pathogenicity of collected nematodes and to establish new cultures, emerging nematodes were pooled for each sample and used to infect fresh *G. mellonella* larvae.

Morphological and morphometric characterization

Nematodes were examined live or heat-killed in Ringer's solution at 60^0C. All nematodes studied were reared in *G. mellonella* larvae. Twenty *G. mellonella* larvae were

exposed to 1000 infective juveniles in a Petri dish lined with two moistened filter papers at room temperature (25 ± 3^0C). Mature females and males of the first and second generations were isolated by dissecting infected larvae in Ringer's solution 3 and 5 days after infection, respectively [10].First generation males and females were collected from 4-5 day post inoculated *Galleria* cadavers (dissected in distilled water).Infective juveniles and second generation adults were obtained during the week after their first emergence from *Galleria* cadavers. Nematodes were killed using hot (50-60^{0}C) Ringer's solution [11], fixed in triethanolamine formalin (TAF), processed to anhydrous glycerin by a slow evaporation method [12] , mounted on glass slides and studied using an Olympus microscope. Morphological identification was done using criteria suggested by Stock and Kaya [13] and Hominick *et al*.,[14].The following characters were measured: total body length; maximum body diam.; anal body diam.; excretory pore position; distance from anterior end to nerve ring position; distance from anterior end to base of pharynx; gubernaculum length; spicule length. In addition to the deMan formula, the other characters studied were: D% (Distance from head to Excretory pore/oesophageal length x 100), E% (Distance from Head to Excretory pore/tail length x 100), F% (Body width/tail length x 100), SW, GS. Morphological and morphometrical data of the isolates were compared with the original description of the type species.

III. RESULTS

Survey data revealed that out of 100 soil samples, only one soil sample from tea plantation area of village Sensowa gaon, Golaghat district, Upper Brahmaputra valley, Assam ($26^0$56988 N and $93^0$99452 E) was positive for entomopathogenic nematodes with 1% frequency of occurrence. The isolate was assigned to the genus *Steinernema*. Morphological and morphometrical studies different life stages (infective juveniles and adults of both the generations) of the isolate revealed it to closely resemble with *Steinernema kushidai* Mamiya,1988 [15] (Table1, Table2) in most of the characters. Body shape and anterior region of third stage infective juvenile heat killed specimen similar to other species of *Steinernema*. They are almost slender, gradually tapering posteriorly, some of them ensheathed in cuticle of second stage juveniles. Lip region is not set off. Mouth opening is closed. Oesophagus is long and narrow, terminating in basal bulb. Excretory pore is at level of mid oesophagus. Nerve ring is distinct, anterior to basal bulb. Cardia is distinct. Intestine is dense, and dark in appearance. Tail conical with pointed terminus. First generation male cuticle smooth, head truncate, not set off , stoma short and wide, procorpus cylindrical, metacarpus swollen and basal bulb pyriform, nerve ring anterior to basal bulb. Testis is single reflexed. Spicules are paired, well curved, spicule head truncate or rounded. Shaft is indistinct. Blade is wide anteriorly, tapering posteriorly to a blunt tip. Spicule tip is slightly curved. Gubernaculum is tapering anteriorly to form a neck then enlarging slightly to a ventrally curved end. Tail is conical, tapering to rounded body terminus. Bursa is absent**.** Second generation male is similar to first generation males but smaller in size with slender body. First generation female cuticle is smooth, lateral fields is inconspicuous. Head is truncate. Stoma is short and wide. Procorpus is cylindrical. Metacorpus is swollen.Basal bulb is pyriform. Cardia is distinct. Nerve ring is just anterior to basal bulb. Gonads are amphidelphic reflexed. Vulva is a transverse slit, protruding slightly from body surface. Tail is tapering rapidly to pointed terminus, shorter than width at anus. Second generation female similar to first generation females, but smaller in size, slender body, tail straight and longer than width at anus.The morphometrics of the infective juvenile of the isolate were almost in similarity with the type specimen of *Steinernema kushidai* Mamiya,1988 (Table 1, 2). The morphometrics of the first generation males of the isolate were almost in similarity with those of the original isolate (Table 1), however differed from the type isolate in some characters such as body length, body width, position of excretory pore, tail length, SW, GS. The morphometrics of the second generation males of all the isolates were almost in agreement with those of the original isolate (Table 2), however differed from the type isolate in some characters such as body length, body width, position of excretory pore and tail length. The morphometrics of the first generation females were almost similar with the type specimen. The second generation females of the isolates were differed from the type isolate in some characters such as body length, body width, position of excretory pore and position of nerve ring (Table 2). Morphometric variation was observed in adult stage of both male and female generations in some characters which were considered as intraspecific variations of *S. kushidai*. The isolates were thus identified as *Steinernema kushidai* which is a new record of this species from Assam, India.

IV. DISCUSSION

The EPN was recovered at a low rate in present study; it may be due to condition of the crop land in terms of irrigation of the field, where the temperature and the soil moisture was suitable for their persistence. One reason for the low recovery rate obtained in the present study, could be

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the fact that only one insect, *G. mellonella*, was used as bait insect and it may not be the appropriate host for all EPN species [10]. EPN distribution depends on temperature, precipitation and soil type and is closely related to vegetation type and presence of insect hosts [16-20].Furthermore, the choice of sampling sites may contribute to differences in EPN recovery percentage [21]. Lower percentage of EPNs probably also due to chemical control of insect pests in fields which partially reduces the abundance of natural biocontrol agents. However, this low recovery percentage is not unusual, and it has already been reported from other surveys [10,22]. Rosa *et al*., [23] reported that most of the surveys showed their recovery rate from soil varies from 6% to 35% in Northern Ireland. Raj Kumar *et al*., [24] reported that out of 105 soil samples collected from Rajasthan, 5(4.76%) were found to be positive for EPNs. Mracek and Becvar [25] and Bruck [26] reported that recovery frequency of EPNs may vary from 0.7% to 70.1%. Akhurst and Brooks [27] and Griffin *et al*., [28] observed that entomopathogenic nematodes were more prevalent in agricultural fields than in natural habitats. The EPN positive soil sample was from sandy loam soil and this finding was in agreement with the findings of the surveys conducted by Ambika and Sivakumar [29] which revealed that the occurrence of EPNs was more in light soils like sandy loam, sandy, loamy sand, loam soils rather than in heavy soils. However EPNs are present in heavy soils like clay soil also as recorded by Shyamprasad *et al*., [30] and Sosamma and Rasmi [31] in the South Andamans and Kerala, respectively. In Sri Lanka, *Heterorhabditis sp*. was reported to be restricted to sandy soils within 100 m of the sea [32].

The nematode *S. kushidai* was isolated from the cadaver of scarabaeid beetle (Anomala cuprea) larvae in Hamakita, Shizuoka, Japan and described by Mamiya in 1988. Nguyen and Smart [11] observed variations in body length, position of excretory pore, tail length and value of E% of *Steinernema glaseri* in relation to time of harvest. It was observed that body length of infective juvenile was 1464µm (1256µm-1610µm) on 3rd day of harvest where as body length was 1306µm (726 µm -1530 µm) on 12th day of harvest. Shishiniova *et al*., [33] observed that the infective juveniles of Bulgarian strains of *S. carpocapsae* differed in some morphometric characters and indexes from the known strains from Europe, USA and South America. Bamel and Waghmare [34] recorded *S. siamkayai* first time from India. The isolate, *S. siamkayai* showed variation with respect to adult stage of both male and female generations in some characters like pharynx, excretory pore, tail length, spicule length and gubernaculum length etc. from the original description.

V. CONCLUSION

Although, entomopathogenic nematodes were recovered only from one soil samples out of 100 soil samples, the recovery of *Steinernema kushidai* highlights the importance of conducting a more intensive survey in Assam. Further studies on characterization and host ranges of these EPN species are necessary to explore and ascertain their possible utilization in biological control programme of insect pests.

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Table.1: Morphometrics of infective juvenile and first generation male of Steinernema sp. from fields of tea plantation, Assam in comparison with original description of Steinernema kushidai.

Measurements in µm and in the form: mean± SD (range)

Character	*Steinernema* sp. (IJ) (n=25)	Original description of *Steinernema kushidai* (Infective juvenile) Mamiya, 1988 (n=50)	*Steinernema* sp. (1st gen male) (n=15)	Original description of *Steinernema kushidai* (1st gen male) Mamiya, 1988 (n=20)
Body length(L)	619.1±61.0 (505-695)	589±39.8 (524-662)	957.3±132 (805-1300)	1400±0.16 (1200-1900)
Body width(W)	39.5±8.1 (26-50)	26±2.5 (22-31)	62.2±96.8 (50-75)	97±19.2 (75-156)
Anterior end to excretory pore(EP)	43.4±13.0 (25-55)	46±2.0 (42-50)	53.2±8.4 (30-63)	84±7.9 (71-105)
Anterior end to nerve ring(NR)	82.9±10.3 (70-100)	76±3.5 (70-84)	101.8±10.3 (80-120)	129±5.3 (120-137)
Anterior end to esophagus base (ES)	115.0±10.0 (95-135)	111±4.1 (106-120)	138.6±8.3 (120-150)	167±7.9 (156-189)
Tail length(T)	37.3±9.3 (20-50)	50±3.4 (44-59)	21.6±3.4 (15-25)	33±3.0 (30-40)
Anal body width(ABW)	21.1±3.8 (15-30)		29.6±3.5 (25-35)	42±5.0 (36-54)
Ratio a= (L/W)	16.0±2.4 (11.0-20.7)	22.5±1.6 (19.3-25.2)		

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Ratio b= (L/ES)	5.3±0.2 (5.0-5.7)	5.3±0.3 (4.9-5.9)		
Ratio c = (L/Tail)	17.8±5.9 (10.1-30.2)	11.7±0.6 (9.9-12.9)		
Spicule length(SL)			68.8±5.6 (60-80)	63±5.5 (48-72)
Gubernaculum length(GL)			43.8±4.7 (35-50)	44±4.9 (39-60)
SW%= SL/ABW*100			2.35±0.3 (1.7-2.8)	1.50
D%= (EP/ES)*100	0.37±0.1 (0.2-0.5)	0.41±0.01 (0.38-0.44)	0.38±0.06 (0.2-0.4)	0.51±0.04 (0.42-0.59)

Table.2: Morphometrics of second generation male; first and second generation female of Steinernema sp. from fields of tea plantation, Assam in comparison with original description of Steinernema kushidai. Measurements in µm and in the form: mean± SD (range)

Character	*Steinernema* sp. (2nd gen male) (n=10)	Original description of *Steinernema kushidai* (2nd gen male) Mamiya, 1988 (n=20)	*Steinernema* sp. (1st gen female) (n=20)	Original description of *Steinernema kushidai* (1st gen female) Mamiya, 1988 (n=20)	*Steinernema* sp. (2nd gen female) (n=15)	Original description of *Steinernema kushidai* (2nd gen female) Mamiya, 1988 (n=20)
Body length(L)	755±33.7 (700-790)	1200±0.09 (1000-1300)	2306±781 (1090-4135)	3500±0.79 (2100-4700)	810±85.5 (730-995)	2300±0.15 (2100-2700)
Body width(W)	55±4.4 (50-60)	58±3.3 (54-63)	125±42.4 (60-210)	175±18.5 (153-210)	51.2±9.1 (40-70)	95±6.7 (84-111)
Anterior end to excretory pore(EP)	47.1±7.0 (40-60)	74±3.1 (66-78)	70±16.5 (50-120)	91±6.6 (78-105)	51.4±16.6 (35-80)	91±11.4 (75-105)
Anterior end to nerve ring(NR)	100±5.4 (95-105)	104±4.0 (96-111)	136±17.0 (100-170)	124±0.9 (111-144)	96.7±11.7 (75-120)	136±9.3 (111-150)
Anterior end to esophagus base (ES)	140.3±3.2 (135-145)	161±6.8 (147-177)	197±23.8 (155-230)	227±15.2 (204-255)	137±16.0 (110-170)	199±9.7 (174-216)
Tail length(T)	23.3±2.5 (20-25)	30±6.8 (27-39)	39±7.1 (30-60)	38±4.4 (30-45)	38.5±7.1 (25-50)	48±8.9 (36-72)
Anal body width(ABW)	29.1±3.7 (25-35)	36±7.4 (36-45)	40.2±9.6 (30-65)	64±7.2 (54-84)	26.0±3.4 (20-30)	41±3.8 (36-48)

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Spicule length(SL)	62.5±11.2 (40-70)	58±4.0 (51-63)				
Gubernaculum length(GL)	35±9.4 (20-45)	40±3.3 (30-45)				
D%= (EP/ES)*100	0.33±0.5 (0.3-0.44)	0.46±0.03 (0.41-0.56)	0.35±0.06 (0.24-0.52)	0.40±0.02 (0.37-0.46)	0.37±0.12 (0.21-0.60)	0.44±0.06 (0.34-0.55)
V%= distance from anterior end to vulva as percentage of length			55.3±4.4 (44-68)	56±1.4 (54-59)	54±4.5 (45-66)	56±2.4 (50-60)

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Nursery Method for Damping - off Disease in True Seed Cultivation of Cluster Onion

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Abstract**—** *Onion (Allium cepa L.) is an important cash crop grown in Sri Lanka. Cluster onion cultivation in Sri Lanka is mainly grown using mother bulbs as planting material. With the introduction of true seed technology, the costs of planting material can be reduced. In general, when using seeds, the plants must be raised in a nursery for 28 days before they can be planted in a field. However, there is a gap in nursery techniques when using seeds and while expanding onion cultivations using true seed. This is a negative factor in the expansion of seed technology for agro-industrial areas such as Jaffna, Kalpitiya, Sri Lanka, since they do continues cultivation in their farm land. Therefore, it is required to maintain an indoor nursery if the onion is expected raise from seeds. Two soil-borne diseases that cause true seed culture in Cluster onions are damping-off (caused by Fusarium spp., Pythium spp., and Rhizoctonia solani) and Fusarium basal rot. These are the main constraint to popularizing the true seed crop. Main objective of this study was to identify the best management method for nurseries to achieve a low incidence of damping – off disease, which will help to obtain a high number of healthy stands. The sterilized normal nursery soil mix used in nursery trays in the shade showed a significantly ($p<0.05$) lowest post-emergence damping – off disease incidences. To avoid the development of an undesirable microclimate, it is essential to use nursery trays with separate holes.*

Keywords**— Cluster onion, Damping – off, Nursery trays, True seed cultivation.**

I. INTRODUCTION

Onion is an important component in Sri Lankan diet. There are two main types of onions: big onion and cluster onion cultivated in Sri Lanka. Big onion cultivation is mainly done with true seeds and cluster onion can be cultivated with mother bulbs as a vegetative cultivation or using true seeds. In the seed crop, the plants must be grown in a nursery for 28 days prior for planting in a field. Current normal practice is to maintain a nursery in a nursery bed in open field. In this situation, transplanting is necessary in field establishments. Transplants are an excellent way to plant onions as healthy and vigorous transplants results in a successful culture. Cook has shown that maintaining root health is a critical condition for achieving high yields in crops (Cook, 1986). Onion seedlings are very susceptible to seedling diseases and need to be protected. The cultivation of the onion is obtained by different methods according to the conditions of the farmers. Some methods involve transplanting, sowing seeds directly and planting bulbs in the fields.

Damping – off is a serious fungal disease caused at the nursery stage. Damping off is caused by *Fusarium* spp. (Lewis J.A., 1977) (Szczech, 1999) *Pythium* spp. (McKellar & Nelson, 2003) (Veeken et al., 2005) and *Rhizoctonia solani* (Papavizas G.C., 1960) (Diab, Hu, & Benson, 2003). *Fusarium* spp can cause pre- and post-emergence damping-off and *Pythium* spp. and *Rhizoctonia solani* have similar symptoms in young seedlings. Both *Pythium* spp. and *Rhizoctonia solani* kill seedlings from the inside, growing through plant tissues and destroying cells and cell walls. This leads to the collapse of the plants or 'Damping – off'. Seeding approaches too much and excess moisture in the soil can cause this disease. With excess moisture, *Pythium* spp. also, the favor with spore swimming will increase the level of the disease. This disease can spread quickly from one plant to another with these conditions. To prevent this disease, cultural practices such as sanitation in the field, sterilization of the soil, use of healthy seeds can be used.

As stated in the recommendation of the Sri Lankan Department of Agriculture, most soil pathogens can be removed by burning of soil or solarizing nursery beds. In addition, if the causative fungus is unknown, broad-spectrum fungicides such as Captan and Thiram may be used [Anon. 2015].

To date, few studies have been conducted on the different nursery methods of other vegetable seeds and big onion seeds in Sri Lanka. Therefore, this study was conducted to determine the best management method in the nursery to achieve a low incidence of Damping – off disease,

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which will help achieve a high number of healthy stands in the true seed cultivation in cluster onion. Nine different nursery methods were tested.

II. METHOD

Nine treatments (Table 1) were tested in this research as follows.

Table 1: Different nursery techniques as treatments

	Treatments
T1	Neem leaves as mulch material (Sterilized soil)
T2	Seed treatment with Captan 50% WP (5g/1kg) (Sterilized soil)
T3	Straw mulch and rain protection in polythene (Sterilized soil)
T4	Sterilized normal nursery soil mixture in nursery trays under shade. (105 holes/tray)
T5	Dapog mixture (in rice) in nursery trays in the shade (105 holes/tray) (Sterilized soil)
T6	Straw mulch and coconut leaves as a shade (Sterilized soil)
T7	Straw as a mulch material (Sterilized soil)
T8	Control (Without mulch or rain cover)
T9	Dapog mixture (in rice) in nursery trays in the shade (without holes in the tray) (Sterilized soil)

To test the true seed cultivation of cluster onion; seeds of Vethalam variety was used in these treatments as well as plots of 3 m * 1 m in the form of Random Complete Block Design (RCBD) with three replicates. Neem leaves, straw used as mulch material and polyethylene, coconut leaves and plant shade used as shading material. Captan 50% WP Fungicide was used as a seed treatment [6]. Soil sterilization was performed by burning the paddy husk and straw in sandwich manner. All of these treatments have been developed with the experience of farmers to select the best method of nursery management. The data were analyzed statistically using SAS version 9.1.3 by the CATMOD procedure.

III. RESULTS

Table 2: Damping off incidences and plant stand count of onion during 1st season

Treatments	Damping off (DI %)		
	WAS 1	WAS 2	WAS 3
Neem leaves as mulch material (Sterilized soil)	4.00 ab	5.00 ab	5.33 b
Seed treatment with Captan 50% WP (5g/1kg) (Sterilized soil)	4.66 a	6.33 a	7.00 a
Straw mulch and rain protection in polythene (Sterilized soil)	3.00 c	3.33 b	3.66 d
Sterilized normal nursery soil mixture in nursery trays under shade. (105 holes/tray)	2.00 d	2.33 c	3.00 d
Dapog mixture (in rice) in nursery trays in the shade (105 holes/tray) (Sterilized soil)	3.01 c	2.66 bc	4.33 c
Straw mulch and coconut leaves as a shade (Sterilized soil)	3.33 b	3.00 b	3.00 d
Straw as a mulch material (Sterilized soil)	4.66 a	2.33 c	5.33 b
Control (Without mulch or rain cover)	5.66 a	3.66 ab	3.66 d
Dapog mixture (in rice) in nursery trays in the shade (without holes in the tray) (Sterilized soil)	4.33 ab	6.66 a	9.66 a

Table.3: Damping off incidences and plant stand count of onion during 2nd season

Treatments	Damping off (DI %)		
	WAS 1	WAS 2	WAS 3
Neem leaves as mulch material (Sterilized soil)	1.66 b	2.66 c	1.66 b
Seed treatment with Captan 50% WP (5g/1kg) (Sterilized soil)	3.33 ab	3.66 bc	4.66 ab
Straw mulch and rain protection in polythene (Sterilized soil)	2.33 b	2.66 c	5.66 a
Sterilized normal nursery soil mixture in nursery trays under shade. (105 holes/tray)	1.00 c	1.66 d	1.33 b
Dapog mixture (in rice) in nursery trays in the shade (105 holes/tray) (Sterilized soil)	2.66 b	5.33 a	6.66 a

Straw mulch and coconut leaves as a shade (Sterilized soil)	3.00 ab	3.00 c	3.66 ab
Straw as a mulch material (Sterilized soil)	2.00 b	4.33 bc	3.33 ab
Control (Without mulch or rain cover)	5.00 a	7.00 a	4.33 ab
Dapog mixture (in rice) in nursery trays in the shade (without holes in the tray) (Sterilized soil)	3.00 ab	6.00 a	4.00 ab

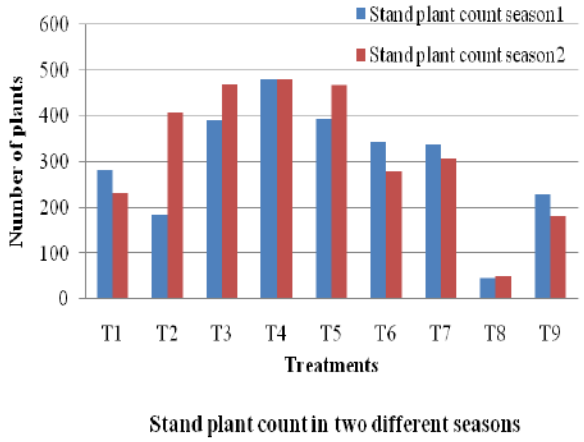

Fig 1: Stand plant counts in two different seasons. The blue color indicates stand plant count of the season 1 and red color indicates stand plant count of the season 2.

IV. DISCUSSION

Among the different nursery methods which were tested in this research, the control showed the highest disease incidences with the lowest plant count in both seasons. It gives an idea about that without mulch and rain cover the fungal disease can be increased. It was observed that the effect of the eight different nursery methods on this research was significant with a $P< 0.05$.

There is a significant in T5 and T9 treatments with the stand plant count. The major difference among them was the tray holes. These trays without holes may have facilitated the favorable micro climate in T9 treatment for pathogen growth. Because; it was as a blanket of dapog mixture without separating as holes compared to T5. Stand plant count in season 1 and 2 respectively 392, 466 in T5 and in T9 it was 227 and 182.

The current recommendation in T2 showed comparatively low plant count in season 1 and it was almost doubled in season 2. Stand plant count in season 1 and 2 were 183 and 406 correspondingly. T6 and T7 had almost similar behavior in both seasons. T6 had 343 and 277 plant stand in respective seasons whereas T7 had 336 and 305 in those seasons.

Our hypothesis was that the neem leaves in-cooperation in to soil will reduce the fungal diseases. Interestingly we found that the neem leaves (Fig: 1, T1) had lowered the plant counts in both seasons, 282 and 232 in respective seasons.

Among the tested treatments Fig. 1 showed that the significantly ($p <0.05$) highest number of plants in three treatments (T3), (T4) and (T5) during both seasons. Among them, the sterilized normal nursery soil mix used in nursery trays in the shade (T5) shows the lowest incidence of post- emergence damping-off with number of plant stands highest.

Table 2 and 3 showed the means of disease incidence percentages during the research. According to the data T3, T4 and T5 had the lowest damping – off disease incidences, making these methods the most effective nursery methods for the damping- off disease. In relevant seasons T3 had 390 and 468, T4 had 478 and 479 and T5 had 392 and 466 plant counts. Among them, the sterilized normal nursery soil mix used nursery trays in the shade (T5) significantly ($p <0.05$) shows the lowest incidence of post- emergence damping-off with highest number of plants.

Due to the use of nursery trays, transplant shock with damage to the root will be zero. But the initial cost with the purchase of trays can be little high. But nurseries on trays can be grown in parallel with the previous season's crop and may not require large fields. It can also be managed in small areas.

V. CONCLUSION

The results of the experiments revealed that it was possible to obtain a higher number of plant stand in the three treatments (T3), (T4) and (T5) during both seasons. Among them, the sterilized normal nursery soil mix used nursery trays in the shade (T5) significantly ($p <0.05$) shows the lowest incidence of post- emergence damping-off with highest number of plants. Based on the results, it can be concluded that the best treatment was the sterilized mixture of normal nursery soil in nursery trays. To avoid the development of an undesirable microclimate, it is essential to use nursery trays with separate holes.

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Typology of Nurseries and Adoption's level of the Technique of Plants Derived Stem Fragment 'PIF' for the Production of Plantain Planting Material (Musa spp.) in Côte d'Ivoire

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***Abstract*—** *One of the major constraints limiting the development of plantain cultivation is the lack of planting material. In Côte d'Ivoire, to overcome this constraint, the FIRCA (Fond Interprofessionnel pour la Recherche et le Conseil Agricole / Interprofessional Fund for Research and Agricultural Advisory) has organized training workshops on PIF technique (Plants Derived from stem fragment). The objective of this study was to take stock of adoption of the PIF technique for seed production of plantain in Côte d'Ivoire, in order to identify the limits. A survey was carried out in the intervention zones of nurserymen during the periods of October-December 2015 and July-September 2016. The informations collected concern the socio-economic impact of PIF on nurserymen and the level of mastery of the technique by nurserymen. A total of 87 nurserymen were visited. About 75% of the nurserymen are men and 48% are over 46 years old. The purchase price of vivoplants fluctuated between 150 and 250 F. CFA. The number of seedlings sold per nurseryman ranged from 6,000 to 180,000 per year. The survival rate in acclimation was at two months between 40 - 85% and at four months between 35 - 60%. The average number of vivoplants produced per explant ranged from 1.87 to 17.01. 28.73% of the nurserymen used suckers of poor quality and 44.82% of suckers of middle quality. 44.67% of nurserymen have limited control of the reactivation technique. In Côte d'Ivoire, the nurserymen improved the availability of seeds for plantain producers in the form of vivoplants.*

***Keywords*— nurseryman, PIF, Plantain, substrate, vivoplants.**

I. INTRODUCTION

Plantain is a food plant cultivated for its fruit which is consumed after cooking, in various forms (cooked, boiled, fried, mashed, cake). It is the fourth most important food crop in Côte d'Ivoire after yams, cassava and rice [5]. Rich in carbohydrates, plantain is the equivalent of potatoes in temperate countries [14]. It is also very rich in vitamins (C, B6 and A), potassium, magnesium and several other nutrients [7].

In Côte d'Ivoire, monoculture of plantain is increasingly practiced because of the development of a sub-regional market [1]; [18]. Global production of plantain in 2014 was estimated at more than 34 million tonnes [5]. Côte d'Ivoire has an estimated production of 1.619 million tonnes for a cultivated area of 425 000 hectares [5]. However, this quantity remains insufficient, because of an increasing demand and a limited production of plantations. A shortage of plantain fruit is even noted between April and September [4].

To adapt supply to demand, the increase of plantain production and the management of off-season plots are essential. Then it is necessary to have optimal management of technical itineraries, and elite varieties. But, above all, the availability of planting material in quantity and quality. Conventional methods of producing plantain material do not guarantee the production of healthy plants and also do not allow to obtain a high quantity of plants. In addition, the use of vitroplants is a more effective way of controlling the spread of telluric pests. However, the high cost of vitroplants hinders its extension in the small producer [15]; [20]. To overcome this constraint, CARBAP (Centre Africain de Recherches sur Bananiers et Plantains / African Center for Banana and Plantain Research) researchers have developed the technique of PIF (Plants derived from stem fragments) to obtain vivoplants of plantain. This plant material is apparently free from nematodes and weevil larvae [13]; [21]. With PIF technique, it is possible to obtain between

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10 and 100 plants depending on the variety of plantain and the size of the suckers [12]; [16]; [9]. The advantage of the PIF technique lies in the fact that the infrastructures required for its implementation are simple, inexpensive and accessible to all producers. As a result, this technique is experiencing an undeniable boom, especially among Cameroonian producers [16].

In Côte d'Ivoire, several nurserymen of plantain and technicians framing were trained in the technique of PIF with the support of the West Africa Agricultural Productivity Program (PPAAO / WAAPP). The activity of these nurserymen aims to ensure the availability of seeds for plantain producers in the form of vivoplants [24]; [3]; [19].

Since the popularization of the PIF technique as a strategy for mass production of plantain material in Côte d'Ivoire, no available report mentions its level of adoption and especially the constraints encountered by nurserymen.

Therefore, the general objective of this study is to take stock of adoption of the PIF technique for the production of plantain seeds in Côte d'Ivoire. Specifically, this study will address the social structuring of nurserymen, the socio-economic impact of PIF technique on nurserymen and the level of mastery of the technique by nurserymen. The analysis of the results from survey data will ultimately allow the research to propose solutions that take into account the constraints faced by nurserymen in Côte d'Ivoire in order to improve the production of plantain planting material.

II. MATERIAL AND METHODS

2.1. Areas and periods of the study

The study was conducted in all areas of nurserymen establishment, whether or not followed by the FIRCA. The producers of vivoplants applying PIF technique in Côte d'Ivoire are located in the southern, central, eastern and western parts of Côte d'Ivoire, which correspond to forest areas and savannah-forest transition zones (Fig. 1). According to [18], the forest areas receive at least 1300 mm of rain per year, while the savanna-forest transition zones receive only between 1100 and 1300 mm per year.

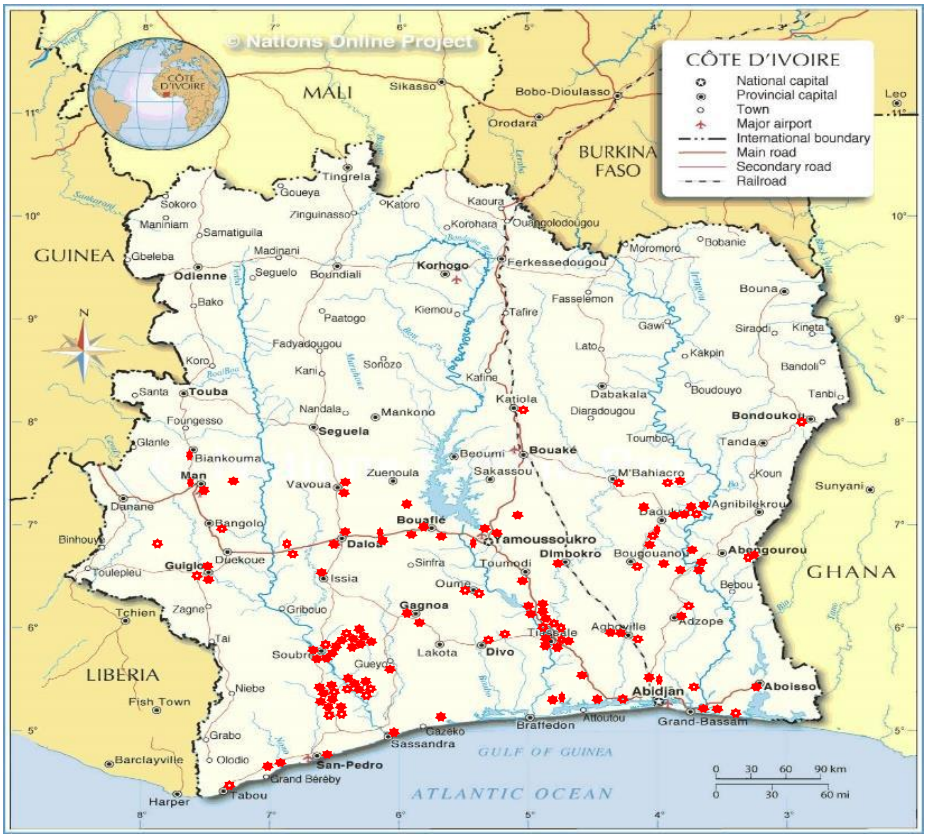

Fig.1: Installation sites (in red points) of plantain nurserymen in Ivory Coast between 2015 and 2016

The execution periods of the study were: (i) October - December 2015 and (ii) July-September 2016. These periods correspond respectively to the beginning (2015) and the end (2016) of the vivoplants production campaign by the PIF technique.

2.2. Plant Material

To practice PIF, plant material recommended by CARBAP is bayonet suckers. This technique allows maximum expression of the offspring potential of a banana plant and to obtain 10 to 100 plants depending on the plantain variety and the size of the suckers.

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2.3. Methodologies

Each nurseryman has been visited at least two times, at the beginning and at the end of the campaign in order to better appreciate the management of the PIF activities. The visits achieve at the beginning of the campaign allow the identification of active and non-active nurserymen. Only active nurserymen were visited at the end of the season.

A survey sheet was designed and provided to the PIF nurserymen. This sheet consisted in three sections (socio-economic aspect, technical aspect and activity evaluation). The sections "socio-economic aspect" and "technical aspect" were realized by semi-structured interview method. The main lines of the interview are detailed in TABLE 1. The "activity evaluation" section was performed following semi-structured interview method and direct observations made in the field. Thus, for each visited nurseryman, an assessment was given according to defined qualitative criteria. The different criteria used for the evaluation are summarized in TABLE 2.

Table.1: Survey parameters based on defined sections

Sections	Information on	Survey Topics
Socio-economic aspect	The nurseryman	Location ; age range ; sex, level of education
	The site	Number of propagator; number of cultivars; number of employees ; choice of cultivar
	The cost of the activity	Cost of inputs; cost of running the site; cost of infrastructure
	The profitability of the activity	Seed transfer price; type of clients; average level of production; number of plants sold
Technical aspect	The seed field	Area; distance between field and site
	The origin of the suckers	Description plot of origin of the suckers
	The life of the propagators	Time of half-life of the propagators; number of weaned seedlings / propagator; number of explants / propagator
	Acclimation	Success rate at 02 months; success rate at 04 months; acclimation time
	Substrate	Substrate state; choice of substrates; formulation of substrates
Evaluation of the activity	Quality of the explants	Quality suckers; suckers treatments
	Quality of infrastructure	Quality of sawdust; shade quality
	Quality of inputs / activities	(re) seeding propagator; weaning; reactivation; quality of acclimation substrate; acclimation quality

Table.2: Criteria and assessments used for the evaluation of the activity of the PIF

Appreciation / Parameters evaluated	Very good (+++)	Good (++)	Fair (+)	Bad (-)
Quality suckers	at least 80% of bayonet, without galleries and necrosis inside the bulb	60 - 80% of bayonet, without galleries and necrosis inside the bulb	40 - 60% of bayonet, without galleries and necrosis inside the bulb	less than 40% bayonet, without galleries and necrosis inside the bulb
suckers treatments	suckers treated within 24 hours after sampling	suckers treated within 24 to 72 hours after sampling	suckers treated within 72 to 96 hours after sampling	suckers treated within 96 hours after sampling
Quality sawdust	medium grind and sawdust new	fine grind and sawdust new	Big grind and sawdust new	copoh of wood and / or old sawdust
Shade quality	about 50% solar infiltration, 30 - 33 ° C temperature, relative humidity over 85%	about 60% or 40% solar infiltration, 30 - 33 ° C temperature, relative humidity over 85%	between 28 - 33 ° C temperature, relative humidity above 85%	temperature below 28 or above 34 ° C, relative humidity below 85%
(Re) seeding propagator	at least 80% of explants well placed	between 80 - 60% of explants well placed	between 60 - 40% of explants well placed	less than 40% of explants well placed

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Weaning	less than 20% of seedlings without bulb and / or small	between 20 - 40% of seedlings without bulb and / or small	between 40 - 60% of seedlings without bulb and / or small	more than 60% of seedlings without bulb and / or small
Reactivation	more than 80% success	between 80 - 60% success	between 60 - 40% success	less than 40% success
quality of acclimatization substrate	fully decomposed and compact	partially decomposed and compact	poorly decomposed and compact	pasty or friable
Quality acclimation	more than 80% success	between 80 - 60% success	between 60 - 40% success	Less than 40% success

2.4. Data analysis

The collected data are registered in the Excel worksheet. They were then grouped together and the various descriptive calculations achieved. The parameters were appreciated and the proportions determined.

For the assessment of suckers and reactivation quality, observations were made on 10 randomly selected suckers in each nursery. Seeding of the explant in propagator was evaluated over an area covering 20 explants. The quality of the acclimation substrate, concerned the appearance (pasty, friable or compact) after watering, when it is strongly pressed in the palm of the hand. As for the quality of acclimatization, the success rate was determined during the propagation under shade at 02 and 4 months after weaning. These parameters were obtained with each nurseryman visited.

The frequencies of citation (FC) of cultivars and substrates, obtained with each nurseryman, are calculated by the following formula:

(FC): NP / NT * 100 with NP: number of times the cultivar / substrate is cited; NT: total number of citations.

III. RESULTS

During this survey, 87 out of 107 nurserymen identified were visited. Twenty were not visited as the activities of the PIF were stopped at their site during the investigation period.

3.1. Typology of nurserymen population

Among the nurserymen population, there are 10 groups or associations, 12 women and 65 men. Of the 77 individuals interviewed, 16% are under 35 years old, 36% are between 35 and 45 years old and 48% are over 45 years old (fig. 2). Only 4.37% of nurserymen have a level of education less than high school.

Of the 87 people interviewed, 85% received training on PIF as part of the West Africa Agricultural Productivity Program (WAAPP/PPAAO) and 15% learned the technique on their own. The number of employees per nurseryman ranged from 1 to 8 with an average of 2.10 employed.

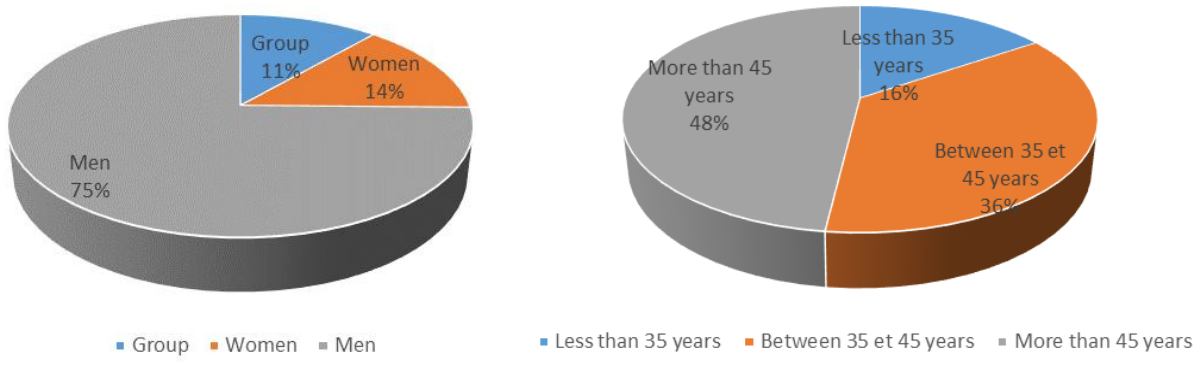

Fig.2: Distribution of nurserymen by sex and age (A: Distribution by sex, B: Distribution by age class)

Out of 87 nurserymen, 33 had seed fields with an average area of 2.63 hectares. The area of the seed fields fluctuated between 0.5 and 10 hectares; the distance between the production site and the point of release was between 0 and 400 kilometers. The most prolific cultivars were " all comers " (FC: 51.9) and Big Ebanga (FC: 32.06). Only 02 nurserymen cultivated FHIA 21 and PITA 3 (FC: 1.53) (fig. 3). The number of cultivars multiplied by nurseryman ranged from 1 to 4 with an average of 1.5 cultivars / nurseryman.

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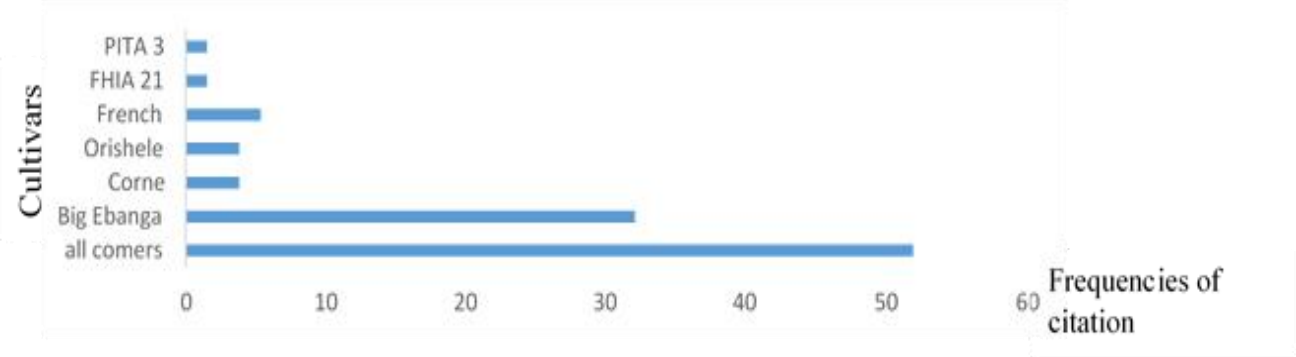

Fig.3: Frequencies of citation of different cultivars

3.2. Economical aspect

The purchase price of the suckers represented in average 42% of the cost of the inputs. The selling price of vivoplant varied from 150 to 250 F. CFA, but that of the Coffee - Cocoa board was set at 175 CFA francs. During the investigation period, 67% of the production of vivoplants was purchased by the Coffee-Cocoa Council, 29% by individuals and 4% by the producers themselves.

Although production data are not recorded by most nurserymen, 22.99% found the activity unprofitable, 70.11% profitable and 6.90% highly profitable (TABLE 3).

Table.3: PIF economic data's

	Minimum	Average	Maximum
Number of propagator / nurseryman	1	15	100
Cost per propagator / year of inputs + labor	180.000 F CFA	265.000 F CFA	336.000 F CFA
Number of seedlings produced per propagator	450	2 257	5 000
Sale price of Vivoplant	150 F CFA	-	250 F CFA
Delivery rate of vivoplants / nurseryman	50%	72%	90%
Number of plants delivered / nurseryman	6 000	35 611	180

3.3. Technical aspects of the PIF technique adoption in Côte d'Ivoire

Suckers used by nurserymen come from plots with different levels of maintenance (fig. 4). The plot considered maintained is regularly weeded, leafless and subjected to phytosanitary treatments (fungicides, nematicides, insecticides). Nearly 50% of nurserymen used suckers from poorly managed (non-irrigated and non-irrigated) plots while 20% used suckers from plots in very good condition.

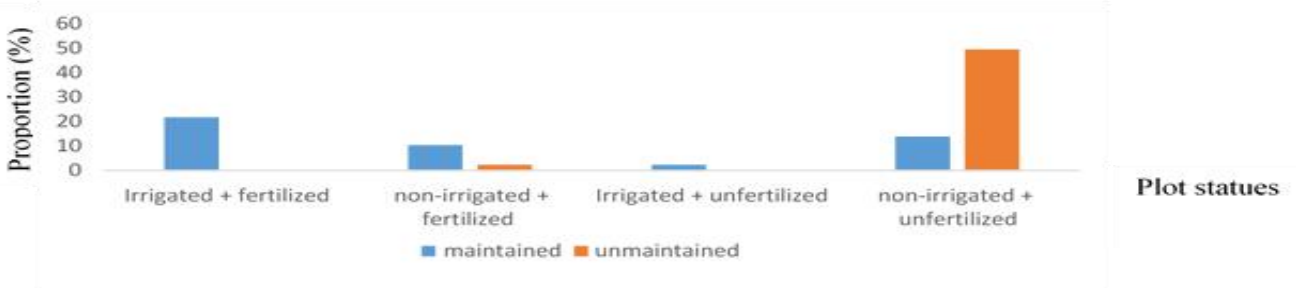

Fig. 4: Proportion of nurserymen according to the state of the suckers sampling plots

The average half-life of the propagator (with at least 50% of the explants still viable) was 3 months. The average success rate in acclimatization was 61.97% at 02 months and 49.85% at 04 months. The level of production is around 6.07 vivoplants per explant put in propagator (TABLE 4).

Of the active nurserymen, 66.66% used a tunnel for the first phase of plant acclimatization.

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Table.4: Evaluation of some technical parameters of the realization of the PIF

	Minimum	Average	Maximum
Half-life time of propagators	2	3	4
Success rate in acclimation (02 months)	40 %	61,97 %	85 %
Success rate in acclimation (04 months)	35 %	49, 85 %	60 %
Acclimatization time (week)	05	08	12
Number of plants produced by explant	1,87	6,07	17,01

Many formulations of acclimation substrates are used by nurserymen (fig. 5). These acclimation substrates were formulated in different proportions (1; 3/2; 1/1; 1/1/1). The most common substrates are sand + coffee parchment (FC: 49,65034) and sand + sawdust + coffee parchment (FC: 16,08391)

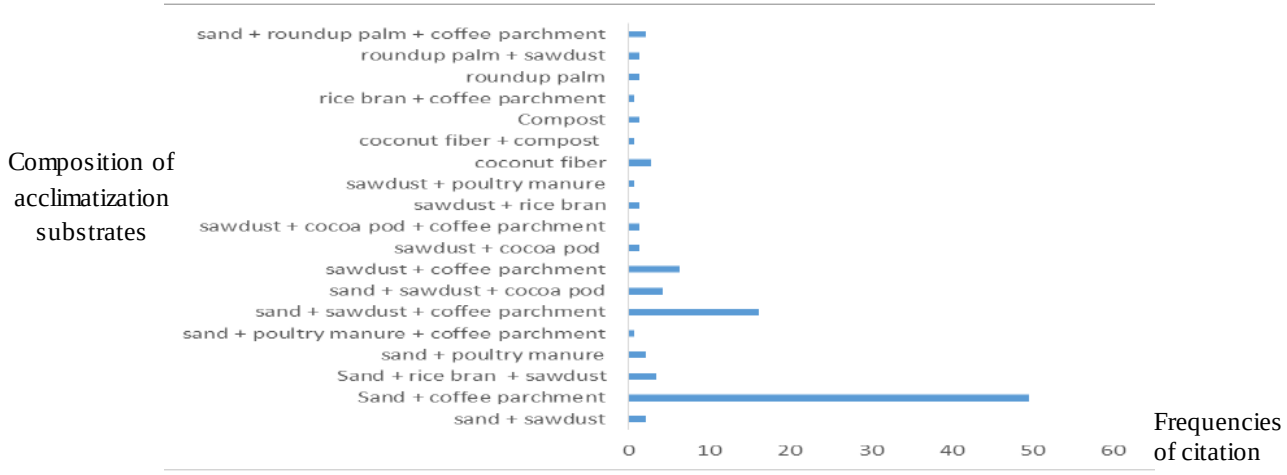

Fig.5: Frequencies of citation (FC) of different types of substrates used during acclimation

3.4. Management of the PIF by nurserymen

The evaluation of the different activities carried out by the nurserymen made it possible to report on the conduct of the PIF.

28.73% of nurserymen used suckers judged to be of poor quality and 44.82%, suckers judged to be of average quality. (fig. 6).

Fig. 6: Different types of suckers used by nurserymen (A: Bayonet suckers of very good quality, B: suckers with a high level of infestation, C: suckers with early necrosis)

Fig. 7 shows the proportions of the different activities carried out by the nurserymen. It also defines the proportions of the modalities of the activities. Thus, many nurserymen have a management of reactivations, acclimatization and suckers considered of average quality. Seeding, weaning, acclimatization, and sawdust generally varied from average to good quality. This resulted in an average proliferation of seedlings of between 2 and 7 seedlings / successful reactivation.

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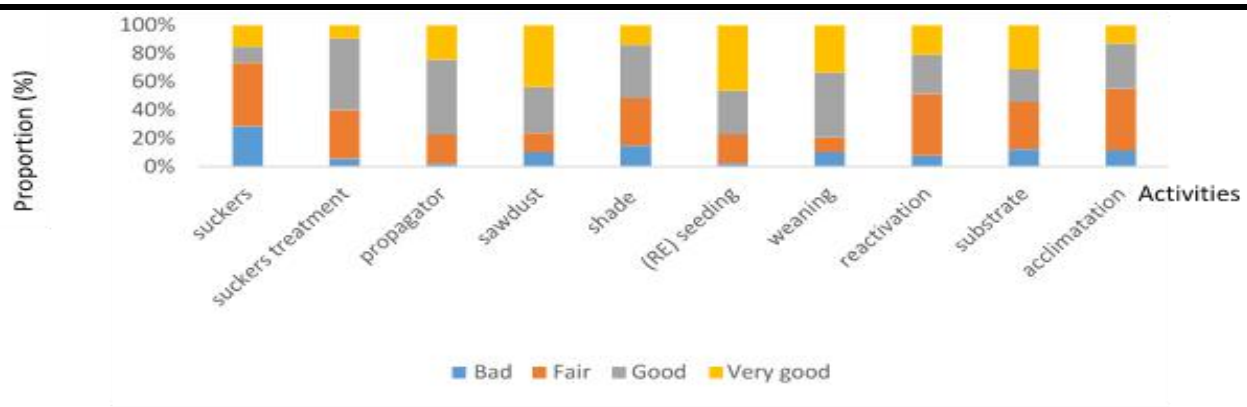

Fig. 7: Proportion of nurserymen according to the modalities of the different activities carried out during the production of vivoplants of plantain.

IV. DISCUSSION

Socio-economic aspect of the PIF technique adoption in Côte d'Ivoire

PIF is a technology introduced in Côte d'Ivoire by the FIRCA (Interprofessional Fund for Research and Agricultural Council) through WAAPP (West Africa Agricultural Productivity Program). This initiative has greatly contributed to the popularization of this technology. The strong demand of the cocoa sector, in plantain, as part of the replanting of the orchard will contributed to develop a market, thus allowing the development of the vivoplant plantain production line. Also, monoculture of plantain is growing up in Côte d'Ivoire [1], increasing the need for vivoplants.

The survey revealed that PIF technique is practiced in all forest areas of Côte d'Ivoire. The location of nurserymen is due to the fact that these areas are the main growing areas for plantain in Côte d'Ivoire [18]; [22]. Indeed, [18] has shown that plantain is grown in all forest areas and, to a lesser extent, in savannah-forest transition areas.

The PIF is practiced mainly by men and the proportion of young people is low. The acquisition of suckers and the management of labor entail a significant expense for nurserymen. This would limit its adoption by women and youth who have limited economic resources.

The majority of nurserymen use "all-comers" (unidentified cultivar and at handy, that is to say consisting of several cultivars), "Big Ebanga", "Horn", "Orishele" and "French (Agninin)" for the production of vivoplants. A small proportion uses the introduced hybrids "FHIA 21" and "PITA 3". These choices are variously justified. The use of "all-comers" would be due to the high demand for seedlings (cocoa board and some plantain producers) and the non-requirement of farmers. These factors would have led nurserymen to base their choice on the availability of suckers, on cultivars strongly appreciated by the populations, resistant and / or high yielding (monoculture of banana). Thus, "all-comers" would be used by nurserymen who do not have a seed field or have small plots of seed fields that fail to meet the need for suckers. The cultivar "Big Ebanga", present at the majority of the nurserymen was distributed by the FIRCA for the creation of their seed field. The cultivars "Horn", "Orishele" and "French (Agninin)" are popular enough, while "FHIA 21" and "PITA 3" have been poorly appreciated.

The majority of nurserymen have found that the planting material production activity using PIF is profitable. The profitability of the activity would be closely linked to the yield (number of plants produced / explants) which varies greatly from one nurserymen to another, depending on the quality of the inputs and the different operations of the PIF. Indeed, many authors [6]; [17]; [2]; [10] report that this technique allows to consider yields of 10 to 100 seedlings per sucker. For example, nurserymen who produce less than 10 seedlings per sucker estimate that the PIF is very unprofitable, and those who have a lot more consider it very profitable.

Technical aspects of the adoption of PIF technique in Côte d'Ivoire

One of the problems facing nurserymen is the absence / insufficiency of seed fields. The number of seedlings produced is linked to the quality of the suckers used. Nearly half of the suckers used come from non-maintained, non-irrigated and unfertilized plots. Suckers obtained on these plots have poor sanitary quality. The poor quality of the suckers is explained by the use of a high quantity of non-bayonet suckers (strains, small suckers), and also of the suckers with numerous galleries and / or necrosis in the bulb. In addition, the suckers are often transported over long distances (up to 400 km from the site). These factors explain the low level of productivity of nurserymen, compared to the potential of the PIF. Indeed, most nurserymen do not have a seed field and for those who own, the exploited area cannot cover

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their need for suckers. They therefore resort to peasant plots to obtain suckers. An investigation conducted by [23] indicates that farmers provide very little care for plantain and their knowledge of pests is limited. The work of [13] and [9] indicate that the physiological state of suckers can influence productivity. [13] found that low production rates of "Kelong mekuitou" and "French clair" cultivars are attributed to a defective starting material condition (suckers attacks by weevils), which would have limited their physiological potential. As for [9], they have shown that the size and physiological state of suckers (under the influence of apical dominance and / or dehydrated for a certain time) strongly influence the number of vivoplants produced.

The level of success of the reactivations is fair. Which could suppose insufficiencies in the conduct of the reactivations. Indeed, the health status of the explant, the type of plantlets reactivated (size and position) and the total number of reactivation on an explant could influence the proliferation of seedlings on the explant. However, according to [13], each suckers on the stem of a banana tree would be at a stage of morphogenetic development and physiological functioning that would confer its own abilities. These gains would therefore occur in the slow or rapid expression of seeded explants. This means that the position of the suckers on the mother foot would influence the response to reactivation. A similar suggestion was also made by [8]. According to this author, the reactivity of the explant is linked to its position on the parent strain, which exerts a more or less strong apical dominance.

During acclimatization, nurserymen face many constraints that are usually adequate shading and the availability of a good quality acclimation substrate. Nurserymen often fail to ensure good temperature management under the shade, due in part to the scarcity of palms in many areas of production. It is also difficult for them to have well decomposed organic substrates in sufficient quantity. Nurserymen choose the substrate according to its availability and / or cost in the area. For example, some nurserymen use several substrate formulations in a single year. This substrate diversity has led to variability in the rate of plant loss during acclimation. In addition, during the survey, many weaned plants were found to be small in diameter and very slender. The works reported by [11] and by [5] showed that plant survival and development during acclimation of plantain vivoplants are influenced by exogenous factors (microclimate around plants and the state of substrate decomposition) and endogenous (diameter of weaned plants). In addition, it was found that a high proportion of plants were lost post-acclimation (04 months after weaning), in the period between the end of acclimation and the removal of plants. The observed loss is explained by the competition for the light between seedlings in nurserymen which becomes more important after 03 - 04 months. This would cause a gradual etiolation of many plants that eventually die.

V. CONCLUSION

In total, this study has shown that PIF activity is widespread in all plantain production areas in Côte d'Ivoire. A large part of the plants produced is used for the banana - cocoa association. The activity of the PIF is a significant source of income for nurserymen. However, the vivoplants market is closely linked to the need for cocoa council in vivoplants.

The nurserymen have a good command of the technical itinerary of the PIF but have difficulties in obtaining quality inputs (suckers, palms). The poor sanitary status of the initial suckers, an average success level of the reactivations and the losses of the plants in acclimation and post acclimation explain the low yield of the explants compared to the estimated yields of the PIF.

Thus, an improvement in the sanitary quality of the available suckers, an optimization of the reactivation behavior and a better technique of conservation of the plants in acclimation could strongly improve the productivity of the nurserymen.

ACKNOWLEDGEMENT

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In-vitro Antibiotic Activity of Dry Ginger Root Extract against Potential enteropathogenic Bacteria isolated from two Weeks old Broiler Chickens

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Abstract**—** *Zoonotic enteropathogenic bacteria of poultry origin is a source of concern to public health. Dependent on various factors, poultry birds may not necessarily fall prey to sub-clinical infections induced by zoonotic enteropathogenic bacteria. A preliminary study was carried out to determine antibacterial activity of dry ginger root (Zingerbain officinalle) extract on Salmonella sp and Escherichia coli isolated from the crop, ileum and caecum of two weeks broiler birds. The in-vitro antibacterial activity was determined by agar disc diffusion method on pre-prepared sensitivity test agar with respective cells inoculated and incubated after 24 hours. Zone of inhibition measured in millimeters after observation gave an inhibition zone of 11.8 ± 2.2, 15.0 ± 2.4, 15.0 ± 2.4 and 14.0 ± 2.9, 13.0 ± 2.3, 8.2 ± 2.6, 15.0 ± 2.2, against Salmonella and E coli respectively, isolated from crop, ileum and caecum. The highest zone of inhibition was observed in the ileum (15.0 ± 2.4) for Salmonella and caecum (15.0 ± 2.2) for E coli. The least was observed in crop (11.8 ± 2.2) and ileum (8.2 ± 2.6) respectively. It is evident that dry ginger root extract has anti-bacterial activity against Salmonella sp and E. coli isolated from the gut of two weeks old broiler birds.*

Keywords**— Antibiotics, Escherichia coli, Ginger root, in-vitro, Salmonella spp.**

I. INTRODUCTION

Enteric infections with pathogenic bacteria can cause mortality in both humans and animals. Such infections are responsible for reduced growth rates and consequent economic losses in animal production. Antibiotics are the main tools for the prevention and treatment of animal infections. Unfortunately, the long term and extensive use of antibiotics for veterinary purpose may eventually result in selection for the survival of resistant bacterial strain [1; 2]. Genes encoding for this resistance can also be transferred to other formerly susceptible bacteria, thereby posing a threat to both animal and human health [3;2]. Consequently, some countries have banned (Sweden) or limited (European Union), total withdrawal (European Union) the general use of in-feed antibiotics as growth promoters in animals [3]. In poultry, the issue of gut health cannot be over emphasized and any attempt to enhance or improve it in the absence of antibiotic growth promoters will be considered an innovation in the right direction. Antibiotics enhance performance of broilers but may not necessarily create an environment that may lead to increased *Lactobacillus* (beneficial bacteria) counts in the gut [4;5]. Although several alternatives to in–feed antibiotics exist, the role of phytobiotic (antibiotic properties of medicinal plants) in the control of certain bacteria in the gut cannot be overemphasized [6]. Many active ingredients from plants are considered as pro–nutrients because of the role they play in enhancing the physiology and microbial load of the animal and as such used in animal feeds [6; 7; 8].

Ginger (*Zingerbain officinale*) roscoe has been exploited for its medicinal value. Some of its pharmacological effect include anti–cancer, antioxidant, potent antibacterial activity, potent anti-fungal activity and anti-helminths' activity [9;10;11]. In–vitro bacteriostatic potential of a commercial ginger paste against *E. coli* has been reported [12]. In view of its potential, a preliminary study was carried out to determine the in –vitro anti-bacterial activity of dry ginger root extract on two potential enteropathogenic bacteria (*Salmonella sp* and *Escherichia coli*) isolated from two weeks old broiler chickens.

II. MATERIALS AND METHODS

Digesta (content of the gastrointestinal tract - GIT) was collected from different sections (crop, ileum and

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caecum) of the GIT of two weeks old broiler chicks. Each bacteria type was isolated on their specific agar on triplicate bases. *Salmonella spp* was isolated on *Salmonella Shigella* agar and *Escherichia coli* on Eosine methylene blue agar. Prior to the isolation of pure cultures of each bacterium (*Salmonella sp* and *Escherichia coli)*, dry ginger was milled and the extract obtained by extracting with petroleum ether in a soxhlet apparatus. The extraction was carried out at the Department of Chemical Sciences, Niger-Delta University.

2.1 Antibacterial activity

The disc diffusion method of [13] was used to determine the in-vitro anti-bacterial activity of the ginger extract. To achieve this Whatman No. 1 filter paper was cut into circular disc of 6mm using a pre–sterilized cork borer. Sterilization of the cork borer was done at 121°C for 20minutes to denature and destroy completely the entire chemical used in its preservation as well as making it sterile before imbedding the extract. The dish was then dipped into the ginger extract using sterile forceps for 15sec and transferred aseptically into sterile glass Petri dish and kept in the oven at 55°C until the following day. Each test bacterium was pre – adjusted to the 0.5 McFarlands turbidity standard in a test tube, dipped with sterile swab stick and used to seed on solidified oxoid sensi-test agar in an inoculating chamber. The prepared disc was carefully transferred onto the inoculated culture plates using sterile forceps. The plates were incubated for 24hr at 37°C, after which the zone of inhibition was measured in millimeters and recorded for each bacterium. The same procedure was repeated for each bacterium using ampiclox as a positive control and distilled water as a negative control. The in – vitro antibacterial activity was done on triplicate basis for each bacterium and the test carried out at the Medical Microbiology Unit, Federal Medical Centre Yenagoa, Bayelsa State.

2.2 Statistical analysis

Data collected on the zone of inhibition (ZOI) by *Salmonella spp* and *Escherichia coli* isolated from different sections of the gut were subjected to statistical analysis using SPSS package volume 17 and significant means separated using LSD (least significant difference).

III. RESULTS

Results on in – vitro antibacterial activity of dry ginger extract on *Salmonella spp* and *E. coli* is presented in the Table below. The antibacterial activity of dry ginger extract was more evident and almost similar to that of Ampiclox (a positive control) against *Salmonella spp* and *E. coli* isolated from the caecum. This was also the case with *Salmonella spp* isolated from the ileum. Distilled water (negative control) had no inhibitory effect on *Salmonella spp* isolated from any section of the gut. Ampiclox was more effective against *Salmonella sp* isolated from the crop with a value of 23 mm recorded against 11.8 mm recorded for dried ginger extract. Antibacterial activity of dried ginger extract against *E. coli* indicated in Table 1 showed an almost similar zone of inhibition (15.0 ± 2.2 mm) with Ampiclox (18 mm).

The negative control (distilled water) also had no antibacterial activity against *E. coli*. The extract was least effective (8.2 ± 2.6mm) against *E. coli* isolated from the ileum compared to ampiclox (20 ± 1.3mm). A value of 13.0 ± 2.3 mm was recorded for *E. coli* isolated from the crop against 23 mm for ampiclox the positive control.

Although the extract was not standardized and dosage determined in this study however, the results indicated a positive inhibitory effect of the extract against each bacterium and in certain cases almost similar effect compared to ampiclox a standard drug in use for humans affected with these bacteria. The two bacteria in the current study are zoonotic enteropathogenic bacteria in poultry gastrointestinal tract and are of public health concern to humans, the ultimate consumers of poultry products.

IV. DISCUSSION

The primary constituents of ginger root are gingerol, zingebain, bisaboline, oeloresins, starch, essential oil, mucilage, protein flavonoids and chemical compounds. According to [14] these constituents are active against a wide array of microorganisms from in–vitro studies. The gingerols have antipyretic, antibacterial and gastrointestinal tract mobility activity thereby capable of eliminating harmful bacteria such as *E. coli* [15]. In the light of this and reports of previous in–vitro studies by means of disc diffusion assay as in this study, an inhibition zone of 9mm [16] or more appearing around the disc indicates presence of antibacterial substance in the extracts tested [17]. Results of the current study agree with these observations even though the least value of 8.2 ± 2.6 mm was recorded against *E. coli* isolated from the ileum. The antibacterial potency of ginger is mainly attributed to the presence of oxygenated mono- and sesquiterpenes, phenolic compounds (shagaol, gingerols) – [18]. They are lipid – soluble phenolic compounds isolated from ginger roots [17;19]. These compounds have different mode of action. They can only attack cell walls and cell membranes (although not tested in this study) by affecting the permeability and release of intracellular constituents (e.g. ribose sodium (Na) glutamate) but they also interfere with membrane functions. Apparently, this could have been one of the likely reasons for the positive zone of inhibition recorded

for both bacterium tested. Apparently, electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity might have been involved in the inhibitory effect of the extract against each bacterium. In a further study, [20] these compounds may have several invasive targets which could lead to the inhibition of bacterial pathogens. An earlier report by [12], a commercial ginger paste exhibited antibacterial activity against *E. coli* (0157:H7 in peptone water at 4°C for two weeks. From our study, supplementation of spices and herbs may have many benefits to broiler health and performance. These include having antioxidant properties [21], antimicrobial activity [8] and enhancing digestion by stimulating endogenous enzymes [22] when included in the diet of broiler chickens. Furthermore, It has been reported that the greater the ZOI, the stronger the antimicrobial effect [23]. The antimicrobial effect, in terms of activity and efficacy of plant extracts on gut microbes can however be quite variable as observed in this study and stated in earlier reports [24]; [25]. This variability can be dependent on the plant source, extraction process, quality and consistency of the product as asserted by [23].

V. CONCLUSION

The ginger extract exhibited inhibitory effect on the bacteria tested. However, further studies are suggested to enable standardization and determination of dosage.

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Table 1: Antibacterial activity (mm) of dry Zingiber officinale (ginger) extract on bacterial isolates from broiler chicken gut

Gut section/ bacteria specie	Ginger extract (mm)	Positive control (mm)	Negative control (mm)
Salmonella spp			
Crop	11.8 ± 2.2^{b}	23 ± 1.2^{a}	0.00±0.00
Ileum	15.0 ± 2.4^{a}	18 ± 1.8^{a}	0.00±0.00
Caecum	14.0 ± 2.9^{a}	16 ± 1.4^{a}	0.00±0.00
Escherichia coli			
Crop	13.0 ± 2.3^{b}	23 ± 1.4^{a}	0.00±0.00
Ileum	8.2 ± 2.6^{b}	20 ± 1.3^{a}	0.00±0.00
Caecum	15.0 ± 2.2^{a}	18 ± 2.2^{a}	0.00±0.00

^{ab}: means along the same row are significantly different ($p<0.05$). N=3

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Cellulase and hemicellulase Activity under Submerged Fermentation of Rice Mill feed by Fungi

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***Abstract*—** *Several agro-industrial by products are utilized in the synthesis of enzymes and production of other value-added products. This experiment was designed to access the cellulase and hemicellulase enzyme activity of three fungi (Aspergillus niger, Trichoderma viride and Rhizopus oryzae) in mono and mixed culture submerged fermentation using rice mill feed as substrate. The cellulase enzyme complex (cellulase (carboxy methyl cellulase, filter paper activity, β-glucosidase activity) and hemicellulase (β-xylanase and β-xylosidase) were determined respectively.*

Carboxy methyl cellulase activity was significantly improved by a mixed culture of all three fungi (4.90U/ml) and a mixed culture of Trichoderma viride and Rhizopus oryzae (4.83U/ml). There was no significant difference ($p>0.05$) in concentration of carboxy methyl cellulase synthesized in mono culture submerged fermentation of rice mill feed but numerically high values were recorded with Trichoderma viride and Rhizopus oryzae. The activity of β-glucosidase was significantly ($p<0.05$) high (4.79U/ml) in mixed culture of the three fungi. Aspergillus niger and Rhizopus oryzae also yielded significantly ($p<0.05$) high β-glucosidase activity of 3.00U/ml and 2.13U/ml respectively. The activity of β-xylanase was significantly ($p<0.05$) enhanced (3.77U/ml) with a mixed culture of all three fungi. Mono culture submerged fermentation resulted in significant ($p<0.05$) β-xylanase activity with Aspergillus niger (1.83U/ml) and Rhizopus oryzae (1.75U/ml) over Trichoderma viride (1.26U/ml).

The fungi utilized in this study were able to produce cellulase and hemicellulase enzyme from rice mill feed in mono and mixed fungi fermentation. The enzyme activity of carboxy methyl cellulase, β-glucosidase and β-xylanase was noticeable.

Keywords*— *cellulase, enzyme activity, fungi, hemicellulase, rice mill feed.

I. INTRODUCTION

Rice mill feed is an agro-industrial by-product (AIB) from the one-step milling of rice. It has a high insoluble non-starch polysaccharide concentration of 576g/kgDM, soluble non-starch polysaccharide concentration of 13g/kgDM and hemicellulose concentration of 211g/kgDM respectively [1]. It is a source of cellulosic waste that can be exploited for the production of value added products from unexploited cellulosic wastes [2] [3]. Cellulase plays a major role in the global enzyme market and is responsible for the hydrolysis of cellulose. It is composed of a complex mixture of enzyme proteins with different specifications to hydrolyze glycosidic bonds [4]. Cellulases are divided into three major enzyme activity classes [5]; [6]; [7]. These are endo-1, 4-*β*-D-glucanase (E.C.3.2.1.4), exo-1, 4-*β*-glucanase (exo-cellobiohydrolase or FPA activity, E. C. 3.2.1.91) and *β*-D-glucosidase (E.C.3.2.1.21). These enzymes together with other related enzymes viz hemicellulase and pectinases, are among the most important group of enzymes employed in the processing of lignocellulosic substrates into various desired products [4]. Xylans are the most abundant hemicellulose. Xylanase hydrolyze the *β*-1, 4 bonds in the xylan backbone to yield short xylo-oligomers which are further hydrolyzed into single xylose units by *β*- Xylosidase. Hemicellulose degradation of AIB is mediated through the action of xylanase and *β*-xylosidase. Complete hydrolysis of cellulose yields glucose while incomplete hydrolysis yields cello-oligomers (cellobiose). The production of cellulase by various microorganisms has been reported earlier ([8]; [9].

Crop residues, crop-processing wastes and sawdust come under agricultural cellulosic wastes previously viewed as valueless and considered for disposal only. The exploitation of cellulosic wastes as substrates has significant environmental, industrial and commercial outcomes. These waste materials can be utilized as raw materials for the production of potential value-added products such as enzymes (cellulase and hemicellulase) as pointed out in this study.

II. MATERIALS AND METHODS

2.1 Experimental site and source of fungi

Fungi utilized for the submerged fermentation of RMF were obtained from the culture bank of the Microbial Physiology Laboratory, Department of Botany and Microbiology, University of Ibadan. They were sub-cultured on Potato Dextrose Agar (PDA). The experiment was also carried out at the Botany and Microbiology Department of the University of Ibadan.

2.2 Preparation of Inoculum

A 1 x 6mm cork borer of 7 days old culture of each fungus (*Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae*) and was dispersed in 15ml sterile deionized water was prepared aseptically. A similar procedure was used to prepare inoculum of mixed culture of *A. niger + T. viride; A. niger + R. oryzae; T. viride + R. oryzae* and *A. niger + T. viride + R. oryzae*. 5ml of each inoculum was used to inoculate the sterile substrate.

2.3 Determination of Inoculum size and Processing of RMF samples

The inoculum used was prepared with a 1× 6 cork borer of each fungus in (mono and mixed culture) from 7 days old culture plates dispersed in sterile deionized water. The inoculum size was determined using a hemocytometer to count the number of spores dispersed as reported by [10]. The RMF was in a ground form when collected from the rice mill and so did not require any further processing.

2.4 Determination of enzymes synthesized

A modified version of [11] medium as developed by [12] was used as basal medium to determine enzyme activity. Fifty (50) ml of the basal medium was added to 0.5gm of RMF in a 150ml Erlenmeyer flask. This was prepared in triplicates for each fungus and their mixed culture. Sterilization was carried out in an autoclave at 121ºC for 15 mins and 15psi. The flasks were inoculated with 3ml of inoculum individually. Inoculation was done after the flask had cooled. They were then incubated for 7 days at 30ºC±2ºC.

The basal medium contained per litre KH_2PO_4 (2.0gm); $(NH_4)_2SO_4$ (2.1gm); $MgSO_4.7H_2O$ (0.3gm); $CaCl_2.6H_2O$ (0.3gm); $MgSO_4.H_2O$ (1.56mg); $ZnSO_4.7H_2O$ (1.4mg); $CoCl_2.6H_2O$ (2.66mg); yeast extract (0.5gm) and was regulated to a pH of 5.0. During the incubation period, the flasks were placed on a rotary shaker at 80rpm for one hour daily. The contents of each flask were filtered through Whatman number 1 filter paper after 7 days and the filtrate taken as the crude enzyme. The filtrate without prior purification was used to determine the following enzymes: cellulase (carboxy methyl cellulase, filter paper activity, β-glucosidase activity) and hemicellulase (*β*-xylanase and *β*-xylosidase) respectively. Filter paper activity (FPA) was taken as a measure of cellobiohydrolase which a component of cellulase enzyme complex. The activity of β-glucosidase activity was determined using *P* - nitrophenyl *-β-D* -glucoside (PNPG) as substrate. Enzyme activity was specified as unit per ml (U/ml) of crude enzyme filtrate. Cellulose activity against filter paper and carboxymethyl cellulase (CMCase) was measured as described by [13]. The *β*-glucosidase activity was determined by using *P*-nitrophenyl-*β-D*-glucoside (PNPG) as substrate [14]. Xylanase: according to the method of [15] and *β*-xylosidase also according to the method of [15].

2.5 Experimental design and statistical analysis

Completely randomized design was used for the experiment having seven treatments and three replicates per treatment. Data obtained were subjected to one-way Analysis of Variance (ANOVA) and significant means separated by Duncan Multiple Range Test using SPSS version 17 (SPSS Inc, Chicago, USA).

III. RESULTS AND DISCUSSION

Results obtained from the hydrolysis of rice mill feed with *A. niger, T. viride* and *R. oryzae* in mono and mixed culture submerged fermentation indicated that RMF is a suitable substrate for the synthesis of cellulase and hemicellulase enzyme.

3.1 Cellulase activity in mono and mixed culture submerged fermentation of rice mill feed

Mono-culture submerged fermentation (SmF) of rice mill feed with *A. niger, T. viride* and *R. oryzae* resulted in varied enzyme activity of cellulase complex (fig. 1). *Rhizopus oryzae* produced numerically higher CMCase activity (4.63U/ml) over *T. viride* (4.60U/ml) and *A. niger* (3.97U/ml). These values were however not significantly different from one another ($p>0.05$). The value of FPA recorded followed a similar trend with values ranging from 2.63U/ml (*A. niger*) to 2.87U/ml with *T. viride* respectively. Significantly higher ($p<0.05$) *β*-glucosidase activity of 3.00U/ml was recorded with A. niger in mono-culture submerged fermentation. The activity of this enzyme was significantly ($p<0.05$) low (1.82U/ml) with *T. viride* compared to a value of 2.13U/ml with *R. oryzae*. The cellulase complex enzyme activity in mixed -culture submerged fermentation of rice mill feed is presented in fig. 2. Unlike mono-culture SmF, a mixed culture of all three fungi resulted in significantly higher ($p<0.05$)

CMCase activity of 4.90U/ml which was followed by a value of 4.83U/ml in a mixed-culture of *T. viride* and *R. oryzae*. The least value of 3.03U/ml was recorded when *A. niger* and *T. viride* were co-cultured. There was no significant different ($p>0.05$) in FPA in mixed-culture of any combination of the three fungi. On the other hand, *β*-glucosidase activity was significantly ($p<0.05$) high with a mixed culture of all three fungi yielding an enzyme activity of 4.79U/ml. this was followed by a value of 3.31U/ml with *A. niger* + *R. oryzae* mixed -culture, 2.65U/ml (*T. viride* + *R. oryzae*) and 2.46U/ml with *A. niger* + *T. viride*.

Cellulose has been reported to be the best inducer of fungal cellulase [16]. Cellulose degradation however, depends on the synergistic action of different components of cellulase enzyme and the induction of synthesis of individual components of the enzyme system is different in different fungi [17;18]. Endoglucanase (CMCase) or endo-1, 4-*β*-*D*-glucanase are proposed to initiate attack randomly at multiple internal sites in the amorphous region of cellulose fibre opening-up sites for subsequent attack by cellobiohydrolase (exo-1, 4- *β*-glucanase) as reported by [19]. Cellobiohydrolase (FPA activity) is involved in the removal of mono and dimmers from the end of the cellobiose chain. The activity of *β*-glucosidase hydrolyses glucose dimmers and in some cases cello-oligosaccharides to glucose. Endoglucanase and cellobiohydrolase work synergistically in the hydrolysis of cellulose, but the details of the mechanism involved were reported to be unclear [7]. Endoglucanase is the major component of fungal cellulase complex accounting for 40 – 70% of the total cellulase proteins and can hydrolyze highly crystalline cellulose [20]. However, a low FPA activity would mean less substrate for *β*-glucosidase to hydrolyze to glucose and a possible substrate induced feed-back inhibition of the cellulase complex. Variations in the CMCase (endoglucanase) and FPA (exo-glucanase) activity recorded in this study may be as a result of multiple distinct variants of these enzymes exhibited by the fungi utilized [21; 22].

An earlier report by [23] identified paddy husk as the best source for maximum enzyme production (endo and exo-β- 1, 4-D-glucanases) by *Hypocrea nigricans* among different lignocellulosic substrates screened. Paddy husk enzyme yield was reported to be maximum under optimal cultural conditions at pH 6.0, incubation temperature 30°C, moisture content 40%, and spore density of 2×10^6 spores/g of substrate under solid state fermentation conditions. Maximum cellulase production using alkali-treated soybean hulls was found to yield CMCase, FPase and *β*-glucosidase activities of 9.91 ± 0.04, 6.20 ± 0.13 and 5.69 ± 0.29 U/g, respectively [24]. According to [25], Maximum lignocellulolytic enzyme activities were obtained on day 6 of cultivation. The authors reported a value of 6.0 U/mL for CMCase activity and 14.5 U/mL for xylanase activity respectively under solid state fermentation conditions. Values recorded for both enzymes under submerged state fermentation in this study were lower compared to the authors report [25]. Notwithstanding this, other published work has also indicated this fact [26]. According to [26], A. niger KKS produced FPase, CMCase, β-glucosidase, xylanase and β-xylosidase activity considerably in submerged fermentation. A mutant version of the same fungi was later reported by [27;28] to produce relatively higher activity of β-glucosidase (514iu/g of ground rice straw in Submerged fermentation and produced xylanase activity higher than 5000iu/g of rice straw under solid state fermentation. Earlier reports have indicated the possibility of inoculum size playing a role in enzyme activity recorded [29;23;30;31].

3.2 Hemicellulose activity in mono culture submerged fermentation of RMF

Hemicellulase enzyme activity in mono and mixed fungi submerged fermentation of rice mill feed is presented in fig 3 and fig 4 respectively. The enzyme activity of *β*-xylanase was significantly high ($p<0.05$) with A. niger (1.83U/ml) and *R. oryzae* (1.75U/ml) mono-culture submerged fermentation of rice mill feed than *T. viride* (1.26U/ml). Conversely, the activity of *β*-xylosidase was not observed to be significantly different ($p>0.05$) across mono-culture submerged fermentation with *A. niger, T. viride* and *R. oryzae* respectively. Nevertheless, the value recorded with *R. oryzae* was numerically higher (2.28U/ml) than that recorded for *A. niger* (2.05U/ml) and *T. viride* (2.21U/ml) respectively.

Mixed -culture submerged fermentation of rice mill feed with *A. niger* + *T. viride* + *R. oryzae* resulted in significantly higher ($p<0.05$) *β*-xylanase activity of 3.77U/ml over a combination of *A. niger* +*R. oryzae, A. niger* + *T. viride* and *T. viride* + *R. oryzae*. The least value of 2.47U/ml was recorded with *T. viride and* +*R. oryzae*. Then again, enzyme activity of *β*- xylosidase ranged between 2.17U/ml with *A. niger* + *R. oryzae* to 2.78U/ml with a mixed culture of *A. niger* + *T. viride* + *R. oryzae*. However, there was no significant difference ($p>0.05$) in enzyme activity of *β*- xylosidase during mixed fungi submerged fermentation of rice mill feed.

Xylans are hydrolyzed by five enzymes: endo-1, 4-*β*-xylanase (E. C. 3.2.1.8), *β*-xylosidase (E. C.3.2.1.37), *α*-L-arabino-furanosidase (E. C. 3.2.1.55), acetyl esterase (E. C. 3.1.1.6) and *α*-glucuronidase (E. C. 3.2.1.139) as reported by [32]. Of which only two, *β*-xylanase and *β*-xylosidase activities were considered in the current study (fig. 3 and fig. 4 below). *β*-xylosidase activity was observed to be high in *A. niger, T. viride* and *R. oryzae* mono cultures compared to their mixed culture

treatments. The *β*-xylanase and *β*-xylosidase activities reported in this study may likely be related to the hemicellulose content of rice husk (205g/kgDM) [33] compared to a high cellulase activity with a corresponding high cellulose content (338g/kgDM). The presence of such a multifunctional xylanolytic enzyme system is found to be quite common among fungi, actinomycetes, and bacteria [34]. The results of this study not only highlight the potential of rice mill feed as a good substrate for the production of cellulase and hemicellulase enzyme but further buttressed the role the nature of a substrate in its hydrolysis by different fungi [36]. Production of cellulase and hemicellulase has been shown to be affected by the nature of the substrate used in fermentation [35]. Cellulase and hemicellulase enzyme activity can differ from enzyme to enzyme according to substrate composition [35]. According to earlier reports [37], *Fuscoporia gilva* and *Pleurotus tuberregium* produced high amount of laccase (440.86 U/L and 480.63 U/L at day 7), as well as carboxymethyl cellulase (CMCase) (4.78 U/mL at day 21 and 3.13 U/mL at day 14) and xylanase (4.48 U/mL and 7.8 U/mL at day 21), respectively. In addition, besides the fact that enzyme yields from solid state fermentation is more over submerged fermentation process other reports indicated that the period of solid state fermentation may also play a role in enzyme yields for CMCase and xylanase, [37].

IV. CONCLUSION

Agro-industrial by-products obtained from rice farming can be quite enormous in rice growing communities and countries. Excess waste can be an issue of concern to such communities. Evidently, rice mill feed has potential as a suitable substrate for the synthesis of cellulase and hemicellulase enzyme under submerged fermentation with fungi. The enzyme activity of CMCase and β-glucosidase was very noticeable in this study.

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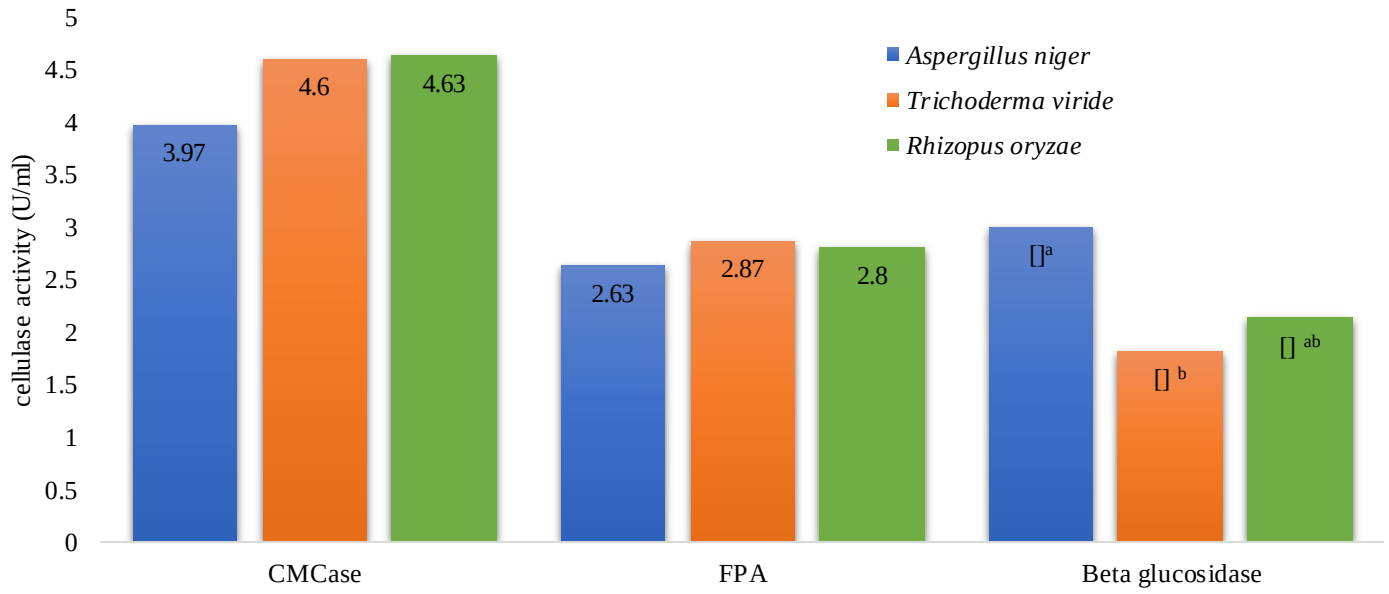

Fig.1: cellulase activity in mono culture submerged fermentation of rice mill feed

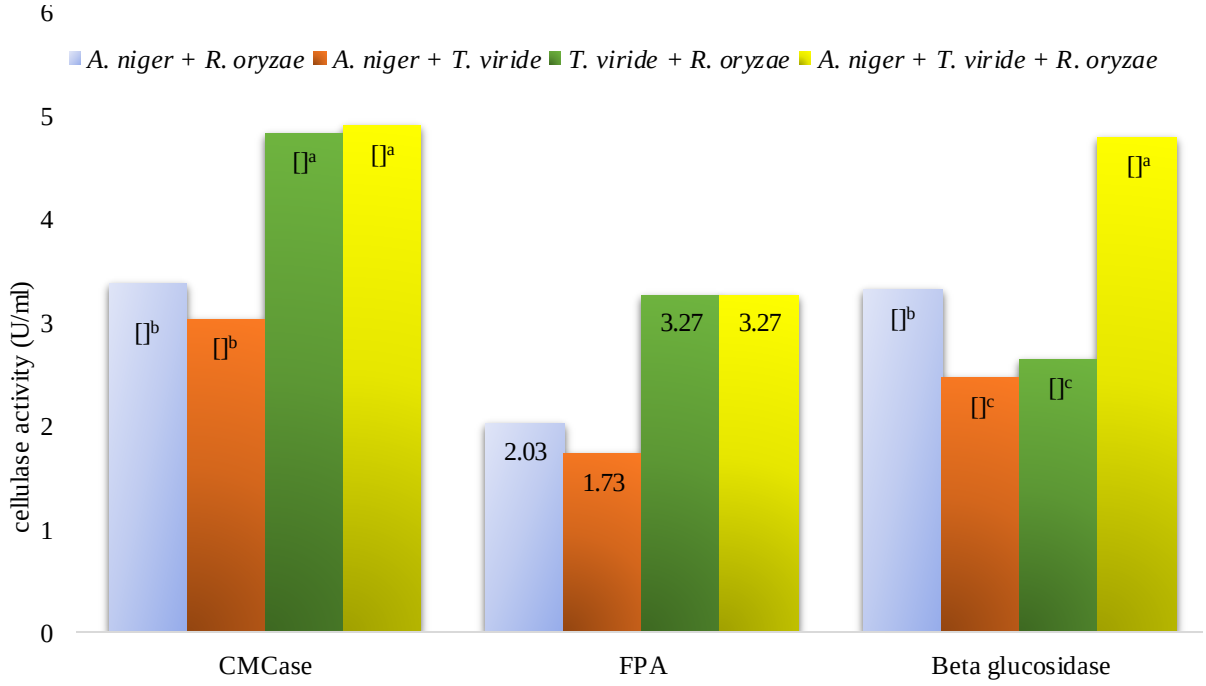

Fig.2: cellulase enzyme complex during mixed submerged fermentation of rice mill feed

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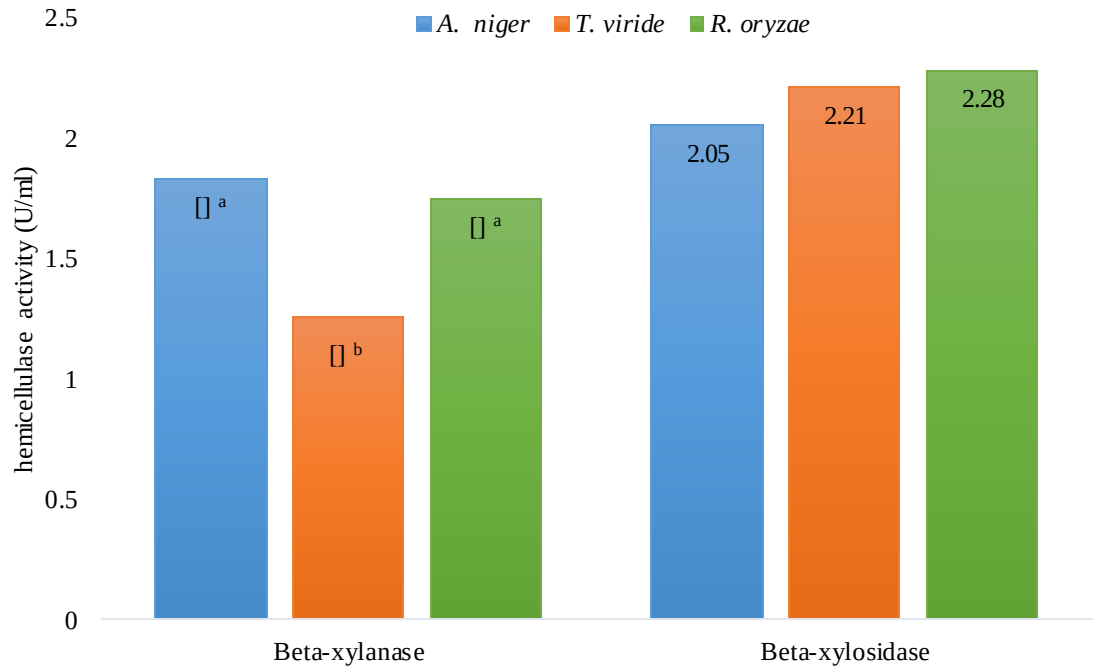

Fig.3: hemicellulase enzyme activity in mono culture submerged fermentation of rice mill feed

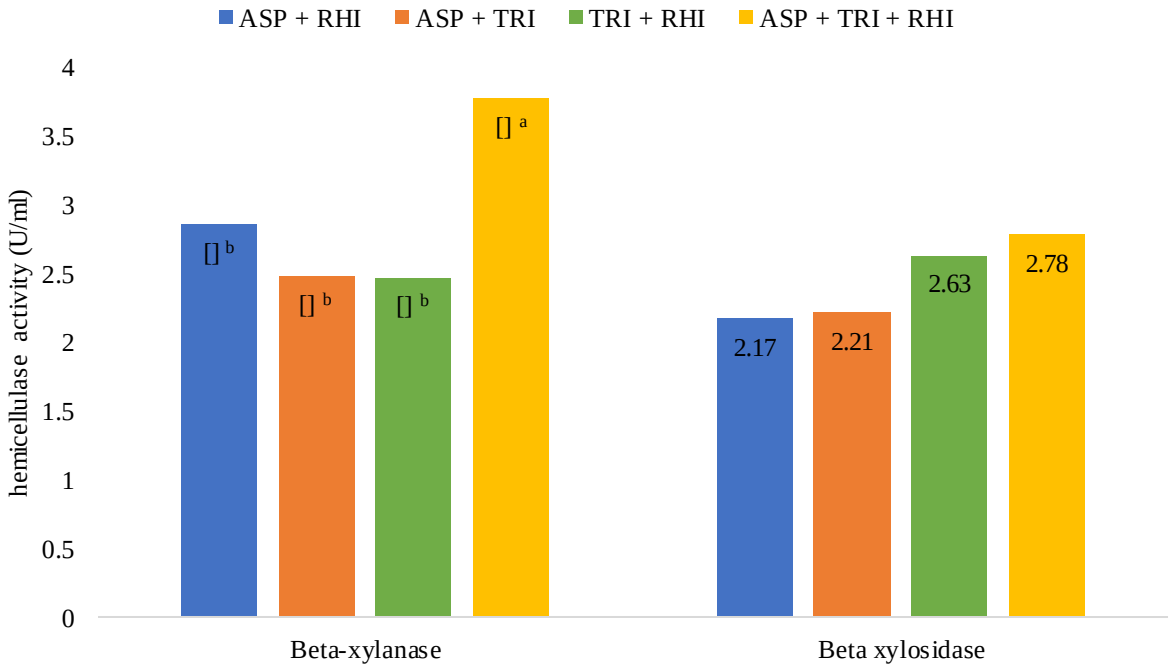

Fig.4: hemicellulase enzyme activity during mixed fungi culture submerged fermentation of rice mill feed

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Effect of biostimulants on the nutrition of maize and soybean plants

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***Abstract*—** *The presence of humic substances in biostimulants composition affect positively plantgrowth by the improvement of the cation exchange capacity of the soils (CEC) and also by theformation of water soluble complexes with ions which can be uptaken by roots. Biostimulantsimprove plant growth due to the cytokinin and humic acids present in their composition. Over theyears the use of these products has been increasing and it is necessary to conduct more studies toevaluate their efficiency in promoting plant growth. The aim of this research was to evaluate theeffect of biostimulants (Brotax Solo®, Naturvital®, PT4-O® and Brotax-5®), applied with andwithout mineral fertilization, on nutrient uptake by maize and soybean plants. A greenhouseexperiment was conducted in Piracicaba, State of São Paulo, Brazil. Three-liter pots were filledwith 0-20 cm depth samples of Quartzipsamment soil. Base saturation was increased to 60% byapplying lime in the samples following incubation for 20 days at 80% the water retentioncapacity. After this period, mineral fertilizers were added to pots of specific treatments. Theapplied doses in L ha-1 were:Brotax Solo®: 0; 150 and 300; Brotax-5®: 0; 8 and 16; Naturvital®:0, 25 and 50; PT4-O®: 0; 0.5 and 1; referring to control, recommended dose by manufacturer and100% higher than this one, respectively. The products were diluted in water and they wereapplied in the pots 21 days after planting. Two months after planting, plant tops were collected,dried, weighted and nutrient contents in plants were determined. Data were subjected to analysisof variance (ANOVA) and means were compared by the LSD test ($\alpha = 0.05$). Nutrient content inplants was higher in fertilized than in non-fertilized pots, for all products and crops. Ca and B;Zn; K; Ca and Mg contents were higher in maize plants treated with Brotax Solo®, Naturvital25®, PT4-O® and Brotax-5®, respectively. In soybean plants only the product PT4-O® showedincrement in Fe content. The amount of nutrients and humic acids in the studied products was notsufficient to increase significantly the amount of nutrients in the maize and soybean plants.*

***Keywords*— nutrients, biostimulants, yield.**

I. INTRODUCTION

Biostimulants are defined by Russo and Berlyn (1990) as products that, when applied to plants, reduce the need for fertilizers and increase their productivity and resistance to water and climatic stress. Casillas et al. (1986) and Zhang and Schmidt (2000) state that these substances are efficient when applied in small concentrations, favoring the good performance of the plant's vital processes and allowing higher yields and higher quality products.Recently, Patrick du Jardin (2015) stated that biostimulantscorrespond 'to any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content'.

The major plant biostimulants are humic and fulvic acids, protein hydrolysates, seaweed extracts, silicon, chitosan, inorganic compounds, beneficial fungi (i.e., arbuscularmycorrhizal fungi; AMF and *Trichoderma*spp.) and plant growth-promoting bacteria (Canellas et al., 2015; Colla et al., 2015a; Rouphael et al., 2015b; Ruzzi and Aroca, 2015).

Biostimulants can increase plant growth and development, stimulating cell division and cell differentiation and stretching; these effects depend on the concentration, the nature and the proportion of the substances present in the products. Biostimulants may also increase the uptake and utilization of water and nutrients by plants (Vieira, 2001). In addition, Colla et al. (2015) citedmany studies conducted on greenhouse and open-field vegetables suggesting that applications of biostimulant substances can promote nutrient uptake and assimilation.

Some factor can be attributed to the increase in plant uptake, such as an increase in soil enzymatic and microbial activities, modifications in root architecture as well as an enhancement in micronutrient mobility and solubility (Ertani et al., 2009; Colla et al., 2013, 2014; Lucini et al., 2015).

Thus, the objective of this work was to study the effects of biostimulants on dry matter production and nutrient uptake by maize and soybean plants submitted to the presence and absence of mineral fertilization.

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II. MATERIAL AND METHODS

In a greenhouse of the Department of Soil Science of the University of São Paulo, in Piracicaba (22°42'30" S 47°38'30" W), Brazil, two experiments were installed using maize and soybean crops for this study.

The biostimulants used in the experiments present the sugarcane residues and the leonardite as raw material (Table 1).

Table.1: Characterization of the biostimulantsused in the greenhouse study

Parameter	Biostimulant			
	Brotax Solo®	Brotax-5®	PT4-O®	Naturvital 25®
pH	4.7	5.6	1.5	13.0
TotalN (g L^{-1})	98.0	138.2	14.0	4.6
P_2O_5(g L^{-1})	19.4	2.0	233.8	0.06
K_2O (g L^{-1})	22.0	23.0	5.6	63.0
Ca (g L^{-1})	0.40	0.32	0.41	4.9
Mg (g L^{-1})	1.1	0.33	0.7	1.9
S (g L^{-1})	52.1	26.8	1.1	10.1
Cu (mg dm^{-3})	47.0	15.0	3.0	2.0
Fe (mg dm^{-3})	360.0	261.0	162.0	1890.0
Mn (mg dm^{-3})	235.0	12.0	7.0	18.0
Zn (mg dm^{-3})	98.0	1100.0	6000.0	6.0
Organic Matter (g L^{-1})	384.6	343.2	145.5	102.5
Total C (g L^{-1})	213.7	190.6	80.8	56.9
Humic acid (g L^{-1})	71.3	58.6	N.A.*	163.6
Fulvic acid (g L^{-1})	120.3	114.7	185.4	84.3
C/N ratio	2/1	1/1	6/1	13/1
Density (g L^{-1})	1.25	1.25	1.24	1.16

* Notavailable

The experimental design was completely randomized using a 3x2 factorial scheme with three replicates: three doses of the product in the absence or presence of mineral fertilization, totaling 18 experimental plots for each product and crop. The doses of the biostimulants used are shown in Table 2. Treatments 1 and 2 correspond to the dose recommended by the manufacturer and a dose of 100% higher, respectively.

Table.2: Doses of the biostimulants used in the experiments

Bioestimulant	Doses						
	Control	----- Treatament 1 ----			---- Treatament 2 ----		
			maize	soybean		maize	soybean
		L ha^{-1}	--- mL/pot ---		L ha^{-1}	--- mL/pot ---	
Brotax Solo®	0	150	5.0	1.2	300	10.0	2.4
Brotax-5®	0	8	0.3	0.06	16	0.6	0.12
Naturvital 25®	0	25	0.8	0.2	50	1.6	0.4
PT4-O®	0	0.5	0.02	0.004	1	0.04	0.008

For field application, field doses were calculated based on the number of plants of each crop per hectare and then related to two plants per pot.The experiments were carried out in three-liter pots where samples of the 0-20 cm layer of a QuartzarenicNeosol of the city of Piracicaba, whose chemical characterization, according to Raij et al. (2001), is shown in Table 3.

Table.3: Chemical and physical characterization of the soil used in the experiments

Parameter	Value
pH $CaCl_2$	4.1
P (mg dm^{-3})	2.8
K ($mmol_c$ dm^{-3})	1.3
Ca ($mmol_c$ dm^{-3})	2.0
Mg ($mmol_c$ dm^{-3})	1.5
Al ($mmol_c$ dm^{-3})	6.0
H+Al ($mmol_c$ dm^{-3})	28.0
S. B. ($mmol_c$ dm^{-3})	4.8
C.T.C ($mmol_c$ dm^{-3})	32.8

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V (%)	14.6
M.O. (g dm^{-3})	1.4
Cu (mg dm^{-3})	0.3
Zn (mg dm^{-3})	0.5
Mn (mg dm^{-3})	7.1
Fe (mg dm^{-3})	22.3
B (mg dm^{-3})	0.2
S (mg dm^{-3})	17.7
Areia (g kg^{-1})	840
Silte (g kg^{-1})	40
Argila (g kg^{-1})	120

As the soil showed high acidity and low base saturation, the samples were treated with 0.6 mg dm^{-3} of high purity calcium carbonate; and 0.2 mg dm^{-3} of high purity magnesium carbonate, the doses of which were calculated to raise the base saturation to 60%. After incorporation of the carbonates, the samples were incubated for 20 days with humidity referring to 70% of the water retention capacity.

After this period, the sampleswere submitted to mineral fertilization in those plots where it was intended to evaluate the effects of the interaction between the mineral fertilization and the doses of the conditioners. 150 mg dm^{-3} of N as ammonium sulfate (with 20% N), 100 mg dm^{-3} of K (KCl with 60% K_2O) and 300 mg dm^{-3} of P (single superphosphate with 18% of P_2O_5), following recommendations of Raij et al. (2001) to raise these nutrients from low to medium-high in the soil. The micronutrients, as a solution, were applied together with the irrigation water, namely: 0.5 mg dm^{-3} of boron (H_3BO_3 with 17.7% of B); 0.8 mg dm^{-3} Cu ($CuSO_4$.$5H_2O$ with 25.6% Cu), and Zn ($ZnSO_47H_2O$ with 22.6% Zn).

After the mineral fertilization, sowing was done using five seeds per pot, leaving two plants per pot after thinning.

Biostimulantswere diluted in water at the established doses and applied to the soil twenty-one days after sowing. As the doses had low volumes to be applied per pot, the following application criterion was adopted: each pot received 100 mL of the dilution made with the dose of each product, in order to allow the soil to receive the doses in a more homogeneous way.

Two months after sowing, the aerial part of the plants was harvested, oven dried at 65oC, weighed and submitted to determination of macro and micronutrients contents according to Malavolta (1997).

Statistical analyzes were performed using the statistical software SAS version 8.2 (SAS INST., 2002). The effect of the treatments was evaluated by analysis of variance (ANOVA) and the means compared by the minimum significant difference at the 5% level of probability by the Tukey test.

III. RESULTS AND DISCUSSION

The biostimulant Naturvital 25® showed a significant effect among the doses applied only to the zinc content of maize plants, not causing a significant effect on the other nutrients. The dose recommended by the manufacturer of this product was the one that favored the highest zinc content in these plants.

The biostimulant PT4-O showed a significant difference between the doses only for the potassium content of maize plants. The dose recommended by the manufacturer of the biostimulant PT4-O was the only one that significantly increased the potassium content in the plant, showing no significant effect for the other nutrients.

For the biostimulantBrotax 5®, there were statistically significant differences for the calcium and manganese averages of maize plants, but the variations were not very significant in both cases. For calcium, doses 1 and 2 of the biostimulantBrotax 5® were effective, whereas for manganese, only dose 1 exceeded the control.

For the Brotax Solo®, Naturvital 25®, PT4-O® and Brotax-5® products, the average between the fertilized plots exceeded the average of the non-fertilized plots at the 5% probability level in the soybean plants.

In the comparison between the doses applied in the experiment for soybean, none of the products showed significant effect, except for the biostimulant PT4-O that showed significant effect only for the iron content. However, this effect was not consistent, since none of the doses was superior to the control.

Chen et al. (2004) and Marschner (1995) state that the plants respond to the application of humic acid products by increasing the availability of soil micronutrients to plants, especially iron and zinc, by complexing or complexing these metals with humic substances.

In general, the results obtained in this study showed that the application of four commercial biostimulants to the soil resulted in little or no significant increase in the content of the nutrients in the plants. These values were below the range of values suitable for maize and soybean crops (Malavolta, 1997). In some cases, the results were similar to those found by Csizinszky (1990) who observed higher nutrient content in control plants than in those submitted to biostimulant applications. Canellas et al. (2015) did not obtain effects of humic substances on the growth parameters of lettuce plants. Dry matter production in the presence of mineral fertilization was higher than that obtained in the absence of this fertilization at the 5% probability level.

Table 4 shows the dry matter yield of maize and soybean plants subjected to the application of the doses of the products

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Table.4: Dry matter yield of aerial part of maize and soybean plants in response to the application of biostimulants in association or not with mineral fertilization

Treatment	Dose	Maize			Soybean		
		Mineral Fertilization			Mineral Fertilization		
		Presence	Absence	Average†	Presence	Absence	Average†
	L ha^{-1}	---------- g ----------					
				Brotax Solo®			
Control	0	49.4	2.2	25.8 ab	7.8	1.4	4.6 a
1	150	54.0	3.2	28.6 a	9.7	1.5	5.6 a
2	300	47.6	2.6	25.1 b	7.2	1.4	4.3 a
Average†		50.4 x	2.7 y		8.2 x	1.4 y	
				Naturvital®			
Control	0	50.5	1.9	26.2 a	9.6	1.4	5.5 a
1	25	49.2	2.4	25.8 a	10.6	1.4	6.0 a
2	50	51.3	2.9	27.1 a	9.2	1.4	5.3 a
Average†		50.3 x	2.4 y		9.8 x	1.4 y	
				PT4-O®			
Control	0	50.7	2.1	26.4 a	10.6	1.4	6.0 a
1	0.5	51.1	2.3	26.7 a	9.3	1.5	5.4 a
2	1	49.2	2.4	25.8 a	10.9	1.2	6.1 a
Average†		50.3 x	2.3y		10.3 x	1.4 y	
				Brotax-5®			
Control	0	52.5	1.9	27.2 a	9.1	1.4	5.2 a
1	8	48.4	2.2	25.3 a	6.8	0.9	3.9 a
2	16	48.6	3.0	25.8 a	6.9	1.2	4.0 a
Average†		49.8 x	1.8 y		7.6 x	1.2 y	

† Averages followed by the same letter (a or b in the column and x or y in the row) do not differ significantly from each other at the 5% level by the t test

Among the average doses of biostimulantsapplied there was no statistically significant difference for both maize and soybean plants, except for the Brotax Solo® product applied to maize. However, the differences were small and none of the doses differed from the control.

Delfine et al. (2005) did not find positive results in the yield of wheat submitted to the application of humic acids. In addition, the authors state that the application of products with the presence of humic acids in their composition does not increase the nutrient content in wheat plants and, therefore, its application is not necessary in order to improve the mineral nutrition of the plants.

The amounts of nutrients present in these doses supplied to the plants were very low, as shown in Table 5. These quantities are not sufficient to reflect a significant increase of nutrients in the plant tissue when compared with the necessary amounts of fertilizers recommended by Raij et al. (1997) for maize and soybean.

Table.5: Amounts of nutrients supplied to the soil with the application of the doses of the products

Nutrient	Bioestimulants							
	Brotax Solo®		Naturvital 25®		PT4-O®		Brotax-5®	
	dose 1	dose 2	dose 1	dose 2	dose 1	dose 2	dose 1	dose 2
N (kg ha^{-1})	14.7	29.4	0.11	0.23	0.00	0.01	1.11	2.21
P (kg ha^{-1})	1.27	2.53	0.00	0.00	0.05	0.10	0.01	0.01
K (kg ha^{-1})	2.74	5.48	1.31	2.61	0.00	0.00	0.15	0.31
Ca (kg ha^{-1})	0.06	0.12	0.12	0.25	0.00	0.00	0.00	0.01
Mg (kg ha^{-1})	0.16	0.32	0.05	0.09	0.00	0.00	0.00	0.01
S (kg ha^{-1})	7.82	15.6	0.25	0.51	0.00	0.00	0.21	0.43
Cu (g ha^{-1})	7.05	14.1	0.05	0.10	0.00	0.00	0.12	0.24
Fe (g ha^{-1})	54.0	108.0	47.2	94.5	0.08	0.16	2.09	4.18
Mn (g ha^{-1})	35.2	70.5	0.45	0.90	0.00	0.00	0.09	0.19
Zn (g ha^{-1})	14.7	29.4	0.15	0.30	3.0	6.0	8.8	17.6

The application of higher doses of these products to the soil could be a way to provide greater increases of nutrients in the plants. In addition, the products could present higher concentrations of nutrients in their composition in order to allow greater supply to the plants at the recommended doses.

According to the conditions of this experiment, the application of the products used does not present advantages to increase the yield of maize and soybean, representing another cost to the producer.

IV. CONCLUSIONS

The use of the biostimulants selected for this study did not increase the nutrient content in maize and soybean plants at the applied doses.

The weight of dry matter of aerial part of the plants was not increased with the application of the doses of the biostimulants.

The concentrations of nutrients present in the biostimulants and the recommended doses result in nutrient amounts much lower than those required to obtain satisfactory yields.

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