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Dr. Pietro Paolo Falciglia

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FOREWORD

I am pleased to put into the hands of readers Volume-4; Issue-5: Sept-Oct 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: Nov, 2019

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Evaluation of Allelopathic Property of *Lantana camara* Vegetative Parts on Seed Germination Attributes of Maize [*Zea mays* L.]

E.N Ngonadi[1], C.W Worlu[2], S.O Onyeyirim[3]

[1]Department of Crop Science & Horticulture, Chukwuemeka Odumegwu Ojukwu University, Nigeria
Email: emmasunrise@yahoo.com
[2]Department of Plant Science & Biotechnology, Rivers State University, Nigeria
Email: cworlu1@gmail.com
[3]Department of Plant Science & Biotechnology, University of Port Harcourt, Nigeria
Email:samuelonyeyirim@yahoo.com
corresponding author: *email: emmasunrise@yahoo.com*

***Abstract*— *Some weeds that have been reported to have allelopathic potentials are always found in association with field crops. This association might be inhibitory or stimulatory, some of these weeds however have the potentials of becoming invasive under certain cropping system and environmental situations. The objective of this study was to evaluate the most effective aqueous extract from three vegetative part of Lantana camara on seed germination of maize. An in vivo, experiment was conducted to study the effect of water extract of leaf, stem and root of Lantana camara on the germination of maize, in a completely randomized design with three replicates. Three concentrations (10%, 20% and 40%) of each ground plant parts were studied with distilled water used as control. The aqueous extracts of the plant parts and control were used to germinate seeds of Maize (DTE-YSTR) in petri-dish with 5ml of extracts administered to each petri dish. Data were collected on number of germinated seeds daily, from 3 Days after Sowing (DAS) to 8DAS. Also length of plumules and radicles were measured and recorded at 8 DAS on five randomly selected germinated seeds. Data were analyzed by ANOVA and the means separated using the Duncan's Multiple Range Test (DMRT). Results showed that germination percentage, mean radicle length and mean plumule length decreased with increasing concentrations. Reduction in germination, length of radicles and plumules was more pronounced in the leaf extract.*
Keywords*— *Lantana camara, allelopathy, maize, leaf, root.

I. INTRODUCTION

Generally, plants interact with others in natural environment; sometimes an individual plant can have a depressive effect on its neighbors. [12] described the adverse effect of a neighboring plant in association with others as interference. Interference is the association between two organisms in which one or both suffer(s) some set back, this includes Competition, Parasitism and allelopathy. According to [17] the potential causes of interference include; Allelospoly (competition) the depletion of one or more resources required for growth while Allelopathy is the addition of chemical toxins by one or more species in association. The term "allelopathy" was proposed for expressing the harmful, stimulatory effects that one plant species has on another through the formation of chemical retardants escaping into the environment [11]. The International Allelopathy Society [7] defined allelopathy as any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems, including positive and negative effects. Chemicals released from plants that impose allelopathic influences are termed allelochemicals or allelochemics or allelotoxins [5]. These chemicals are present in different parts of plants like stem, leaves, roots, flowers, fruits and seeds [15]. These allelochemicals are released from the plants by volatilization, leaching, exudation and decomposition of plant residues [16]. *Lantana camara*, one of the world's 10 worst weeds was introduced in the Indian subcontinent during the early part

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of the nineteenth century [3]. The weed is aggressively growing in forest, tea garden and wastelands of the country [1]. This obnoxious weed poses a serious problem to flora and fauna because of its toxic substance and it contains certain allelopathic compounds [8]. Although several researches have so far worked on the invasion and allelopathic effects of *Lantana* on various agricultural crops [3].

II. MATERIALS AND METHODS

2.1. Receptor crop

The maize seeds variety DTE-YSTR was the test crop and was collected from Institute of Agricultural Research and Training (IAR&T) Ibadan.

2.2. Donor plant

In the experiment, *L. camara* was the donor plant, while stem, leaf and root aqueous extracts were used as the allelochemical.

2.3. Preparation and application of aqueous extracts

The aqueous extracts were prepared following Edrisi method [4] with modifications. The collected plant materials were sorted into three parts (root, stem and leaf) and air dried in the laboratory at 25^{o}C for twenty one days. The air dried plant materials were then ground with Thomas, bench top milling machine and stored away in well labeled envelopes. The ground plant materials were weighed 10g, 20g and 40g then soaked separately in distilled water made up to 100 ml in beakers to have 10%, 20% and 40% (w/v) concentrations respectively. The beakers were covered with aluminium foil and extraction was kept on at room temperature (25^{o}C) for 24 hours, when extracts were obtained by filtering with a muslin cloth. The experiment was laid out in a Completely Randomized Design (CRD), with the control treatment being distilled water. The treatments were replicated three times. The allelopathy was separately compared by plant parts. Ten (10) seeds of the test crop were placed in petri-dishes lined with Whatman No 1 filter paper and 5ml (milliliter) of each treatment was applied to each petri-dish using a syringe. The seeds were observed for germination, by the protrusion of radicle and plumule. Germination was observed on daily basis for eight days. On 8 DAS the number of seeds that germinated was counted in each of the treatment for the test crop. Also, at 8 DAS the length of plumules and radicles was measured (using meter rule). Five germinating seeds were randomly picked in each petri-dish for the later measurement.

III. RESULTS AND DISCUSSION

3.1. Germination of maize seeds exposed to varying concentrations of leaf, stem and root aqueous extracts of *Lantana camara*.

Germination of maize seeds exposed to varying concentrations of plant part aqueous extracts, comparing the extracts to control (distilled water), germination decreased with increasing concentration of aqueous extract. Germination varied from 56.7% in the leaf extract, to 73.3% at 10% concentration in stem and root vegetative part (Table 1). Inhibition increased with increase in concentration, leaf extract shows higher inhibition at 20% and 40% concentration level (26.7% and 16.7%) compared to stem and root extracts.

Table 1: Germination percentage of maize seeds treated with varying concentration of stem, root and leaf extracts of Lantana camara at 8 DAS.

	GERMINATION (%)		
TREATMENTS	LEAF EXTRACTS	STEM EXTRACTS	ROOT EXTRACTS
Control	100a	100a	100a
L. camara 10%	56.7d	73.3c	73.3c
L. camara 20%	26.7e	60.0e	61.2e
L. camara 40%	16.7f	46.7g	47.0g

Percentage values with the same letters under a column are not significantly different according to Duncan's Multiple Range Test (DMRT), at 5%level.

3.2. Effects of aqueous extract of plant parts on radicle length (cm) at 8 das

The treated maize seeds at different concentrations of aqueous extracts have significant difference on the radicle length, recording 6.05±0.44 cm in leaf extract at

10% but varied from 8.04±0.60 cm and 7.80±0.60 cm in stem and root extracts respectively (Table 2). At 20% and 40% concentration, the leaf extract radicle length was not statistically different from the stem and root extract. Reduction in radicles length increased with the increase in the concentrations of the extracts thus suggesting that the effect of the extracts is concentration-dependent.

Table 2: Mean radicle length of germinating maize seeds treated with varying concentrations of leaf, stem and root aqueous extracts of Lantana camara at 8 DAS.

GERMINATION (%)			
TREATMENTS	LEAF EXTRACTS	STEM EXTRACTS	ROOT EXTRACTS
Control	12.00a+0.00	12.00a+0.00	12.00a+0.00
L. camara 10%	6.05c±0.44	8.04b±0.60	7.80b±0.60
L. camara 20%	5.00±e0.44	5.74e±0.05	6.10e±0.44
L. camara 40%	4.04d±0.44	5.30d±0.44	5.16d±0.44
CV	13.61	14.57	13.72

Values with the same letters under a column are not significantly different according to Duncan's Multiple Range Test (DMRT), at 5%level

3.3. Effects of aqueous extract of plant parts on plumule length (cm) at 8 das

The plumule length of the germinated seeds of maize varied among the various vegetative parts at different level of concentrations, recording 4.00±0.00 cm in leaf extract to 5.60.00±0.33 cm and 5.52±0.33 in stem and root respectively (Table 3).

Less reduction in length was recorded in stem aqueous extract at 20% and 40% concentration.

Table 3: Mean plumule length of germinating maize seeds treated with varying concentrations of leaf, stem and root aqueous extracts of Lantana camara at 8 DAS.

PLUMULE LENGTH (cm)			
TREATMENT	LEAF EXTRACTS	STEMEXTRACTS	ROOT EXTRACTS
Control	7.77a.±0.00	7.80a.±0.00	8.00a.±0.00
L. camara 10%	4.00c.±0.00	5.60b.±0.33	5.52b.±0.33
L. camara 20%	3.50d.±0.88	4.55c.±0.33	4.35c.±0.33
L. camara 40%	2.06e.±0.88	3.46d.±0.88	3.44d.±0.88
CV (%)	11.37	9.50	11.16

Values with the same letters under a column are not significantly different according to Duncan's Multiple Range Test (DMRT), at 5% level.

IV. CONCLUSION

Many plants and their root residues have been reported to have allelopathic effect on agricultural crops [18]. Studies have been carried out on the effects of allelochemicals released by root, leaves, stem, fruits and other parts [16]. The present study revealed that aqueous extracts of the selected weed specie *L. camara* contained water soluble allelochemicals which cause inhibitory effects on germination and on germination attributes. The aqueous leaf extracts showed higher inhibitory effect on the seed germination with increase in concentration. Effects of leaf extracts could be due to the large amount of allelochemicals present in the leaf [9]. Inhibitory effects increased with increase in concentrations. This study shows that the leaf extracts of *L. camara* showed significant inhibition of maize seed compared to the control treatment at all concentrations. This is a confirmation of observation of [1] on allelopathic effects of *L.camara* on some agricultural crops.

Results obtained from this work are similar to that of other researchers, in relation to inhibitory effects of leaf extracts of *Ageratum conyzoids* on seed germination of rice [10]; [14]. [6] reported that *Chromolaena odorata* allelochemicals inhibit the growth of many plants in nurseries and plantations. [13] have demonstrated that aqueous extracts of leaf and shoot extract of *T. diversifolia* was inhibitory to the germination and growth of *Amaranthus cruentus*. However, results suggest that reduction in geremination, radicle and plumule length was more pronounced in the leaf extracts from *L. camara* than stem and root aqueous extracts. Similar observations were made by [2] on wheat. Based on the result obtained from this research on the allelopathic potential of the selected weed specie, *Lantana camara* leaf extract showed higher allelopathic potency, even at low concentrations and should be carefully removed during land preparation and cultivation, to avoid high deposit of residues of various vegetative parts.

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Crop Diversification among Rural Farm Households in Kwande Local Government Area of Benue State, Nigeria

Z. T. Nyiatagher[1], P. A. Abiyong[2] and N. U. Aniah.[3]

[1]Department of Agribusiness, University of Agriculture, Makurdi, Nigeria.
[2]School of Agricultural Technology, Samaru-Kataf Campus, Nuhu Bamalli Polytechnic, Zaria, Nigeria.
[3]Cross River Basin Development Authority, Calabar, Nigeria.
Corresponding author: nyiataghertz@gmail.com

***Abstract*— *This study analysed crop diversification among rural farm households in Kwande Local Government Area of Benue State, Nigeria. The study drew a sample of 360 rural farm households through a multi-stage sampling technique from 12 communities in four districts of Kwande LGA (Ikyurav-ya, Turan, Nanev and Shangev-ya) and the primary data obtained were analysed using the descriptive statistics, Simpson index and Tobit regression model. Results indicated that an average farm household head was 42.8 years old and had 4.2 years of schooling. Ninety three percent (93 %) of the farm households diversified their cropping activities with 51.7 percent diversified into three or more crops. The Tobit regression results revealed that there were marginal increases in crop diversification with increase in farm size, gender, access to credit, membership of co-operative and educational level but crop diversification decreases as farmers grow older in age and farming experience. Policy implications were drawn for provision of functional social amenities and encouragement of the rural farmers to join cooperative societies for easy access to loans that promote crop diversification and hence improve the standard of living of the rural populace.*
***Keywords*— Crop Diversification, Rural populace, Simpson Index, Cropping activities, Household.**

I. INTRODUCTION

Agriculture is an important sector of Nigeria's economy, to the extent that the livelihoods of the majority of the population depends on it and serves as the main source of income for the rural population (FAO, 2012). It accounts for 30.9 % of Gross Domestic Product (GDP) and some 5 % of earning from non-oil exports. There is an agreement throughout literature that Nigeria's large potential in agriculture has not yet been fully exploited (CIA World Fact book, 2012). Thus, if well managed, the sector would potentially contribute to substantial improvements in GDP, employment and tax collections (Food and Agriculture Organization, FAO, 2005). It is in this regard that the Nigerian government positions agricultural sector as one of the driving forces for the anticipated economic growth that is required to reduce poverty (Delgado and Siamwalla, 1999; Delgado and Siamwalla, 1997).

The agricultural sector in Nigeria can be disaggregated into three categories; large, medium and small scale. Large (commercial) farmers cultivate areas of 10 hectares and above and are characterized by extensive mechanization, use of modern technology and management, and the rearing of exotic breeds of livestock. They also rely on extensively hired labour. However, nearly two-third of agricultural land and a large share of the national herd are held by smallholder farmers. The smallholder farmers are classified as either small-scale or medium-scale. The former cultivates land areas of less than 5 hectares, while medium scale farmers are those that cultivate areas between 5 and 10 hectares. Themajority of smallholder farmer rely on rain-fed hoe cultivation and the use of unpaid family labour and focus much of their crop production on rice (especially in Kwande Local Government Area). Their production also is characterized by the low use of modern inputs (FAO, 2012; World Bank, 2012).

Following several years of crop failure due to adverse weather conditions and poor prices, the government of Nigeria, through the Ministry of Agriculture introduced a

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programme to promote crop diversification. Diversification in agriculture could be classified into the following three categories (Ryan and Spencer, 2001): shift of resources from farm to non-farm activities, shift of resources within agriculture from less profitable crop or enterprise to more profitable crop or enterprise and use of resources in diverse but complimentary activities. Crop diversification strategy belongs to the second category and it involves shifting from less profitable to more profitable crops, changing of variety, cropping system, increasing exports and competitiveness in both domestic and international markets, protecting the environment, and making conditions favourable for combining Agriculture-Fishery-Forestry-Livestock (Pingali and Rosegrant, 1995; Kumar and Chattopadhyay, 2010; Okali, Okpara and Olawoye, 2001). This is a silent revolution within crop production sector. The motives behind this silent revolution are livelihood sustainability through raising the income levels, urbanization expansion, infrastructural development and trade liberalization policies. Households diversify as a strategy for coping with an unexpected shock, or to minimize risk ex-ante by participating in activities that generate imperfectly correlated returns (Kumar and Chattopadhyay, 2010).

The crops considered in the diversification programme included cassava, groundnut, soyabean, sesame (beniseed), sunflower, bambara nuts among others. The programme was implemented with the objective of enhancing income levels, increasing food security and nutrition status of farm households. It was anticipated that this, in turn, would enhance the living standards of farm households, while offering various cropping alternatives to farmers, as opposed to relying on a single crop namely rice. Among the additional advantages to the farm household growing more than one crop is an opportunity to mitigate risks associated with crop-specific failure due to adverse weather conditions, pests and diseases (Ibrahim, Rahman, Envulus and Oyewole, 2009).

It is against this background that this study looked at the nature and determinants of crop diversification in rural Nigeria especially Kwande Local Government Area of Benue State where the Guinea Savannah vegetation and tropical climate allow varieties of crops to be grown.

Objectives of the Study

The broad objective of the study was to analyse crop diversification among rural farm households in Kwande Local Government Area of Benue State, Nigeria.

The specific objectives were to:

i. describe the socio-economic characteristics of farm households' in the study area;
ii. identify the pattern of farm households' crop diversification in the study area;
iii. analyse the determinants of crop diversification among the farm households' in the study area;

II. METHODOLOGY

The Study Area

This study was carried out in Kwande Local Government Area of Benue State. The Local Government was created in 1976 with Adikpo being the Local Government Headquarters. The Local Government is predominantly agrarian and comprises four districts (Ikyurav-ya, Turan, Nanev and Shangev-ya) with 15 council wards. The districts are divided on the basis of their socio-cultural and historical peculiarities.

It covers a geographical land area of 2, 891 square kilometers. It has a population of 248, 697 (NPC, 2006). The Local Government is bounded by several other LGAs. On the West, it is bounded by Vandeikya LGA, Ushongo LGA on the North and Katsina-Ala LGA on the North-West. On the South, it is bounded by Cross River State and in the East by the Republic of Cameroon. Kwande LGA also shares a common border with Takum LGA of Taraba State. The LGA has abundant land estimated to be 391,500 hectares. This represents 7.7 % of the state land mass. Arable land in Kwande LGA is estimated to be 292,300 hectares (BNARDA, 1998). The LGA is predominately rural with an estimated 80 percent of the population engaged in rain-fed subsistence agriculture and is popularly known as the "Ancestral Home of Tiv Nation". Cereal crops like sorghum and maize are produced in abundance. Roots and tubers produced include yam, cassava, sweet potato and cocoyam. Oil seed crops include pigeon pea, soyabeans and groundnuts while tree crops include citrus, mango, oil palm, guava, cashew and paw-paw. Other crops commonly grown include pepper, tomato, ginger, okro etc. Livestock such as goats, pigs and poultry are reared in the Local Government at small- scale and medium scale levels. There are about 40, 000 farm families in the local government (BNARDA, 1998). The weather is marked by a single rainy season (April – October) and dry season (November – March). The mean temperature range is 31 °C to 38 °C. As a result of its mountainous nature and proximity to the Cameroonian range of mountains, Kwande Local Government Area usually has cold weather which makes it very conducive to traders and investors. The Local Government also has big streams which could adequately take care of agricultural and industrial needs.

Population and Sampling Procedure

The population of this study comprised all rural farm households in Kwande Local Government Area involved in food crop farming. Multi-stage sampling technique was used to select 360 farm household heads used for the study. The first stage involved the purposive selection of four (4) districts based on the population of food crop farmers in the study area. The second stage involved random selection of three (3) farm communities from each of the four districts selected in stage one. The third stage involved the selection of thirty (30) farm households from each of the 12 farm communities selected in stage two.

Data Collection and Analysis

The data collected for this study were analysed using both descriptive and quantitative (inferential) techniques.The quantitative techniques employed in the study were Simpson index and Tobit regression model.

Simpson Index

Simpson index was used to determine the pattern of crop diversification among farm households. The technique has been previously used by Roonnaphai (2005), Bhattacharyya (2008) and Ibrahim *et al*. (2009) in assessing crop diversification among farm households. The Simpson index is presented as follows;

$I = 1 – i=1nAi^2$ (1)

Where Ai= XiXi (2)

Where:

Xi = planted area of ithcrop, i = 1, 2, 3 ……6

Ai = proportionate planted area of ith crop in the total planted.

When I shows a value of zero, it means that the farmer is least diversified while a value of one indicates the most (highly) diversified.

The crops planted by farmers in the study area under consideration include; rice, yam, cassava, groundnuts, sorghum and bambara nuts. Most farmers cultivated at least two of these crops (diversified) while those that did not diversify cultivated only one crop.

Tobit regression model

The Tobit model was considered the most appropriate in this study because some farmers that highly diversified in specified period may not diversify during the period covered by the survey because of the prevailing crop price, pressure from farm work, health and many other possible factors. Also, conventional regression methods fail to take into account the qualitative difference between zero and continuous observation. Therefore, Tobit model assumes that all zeros are attributable to standard corner solutions. As such, zero observations are accounted for and the censored regression provides a more accurate estimation.

The Tobit model for the analysis of the determinants of crop diversification takes the following specifications;

Ii*= Li + ii ~ N (O, 2) (3)

Ii= Ii* if Ii*> 0

Where Li is the explanatory variable, i is the standard cumulative normal with mean zero and variance 2.

Where Ii = crop diversification (Simpson index values, representing the crop diversification index, where 0 I 1; as provided in crop diversification result.

According to Dougherty (2007), the dependent variable in this kind of model is subject to both the lower bound D_L and upper bound $D_{U.}$ in the case of both lower and upper bounds, the model can be characterized as;

Ii*= 1 +2Li + i

$I = I^*$ for $I^* > I_L$

$I = I_L$ for I^* I_L

The model is known as a censored regression model because I* is unobserved for $I^* < I_L$ or $I^* > I_U$. It is effectively a hybrid between a standard regression model and a binary choice model, and OLS would yield inconsistent estimates if used to fit this model.

The explanatory variables used include;

L_1 = Age of the household head (years)

L_2 = Household size (number of persons)

L_3 = Gender (Male = 1, Female = 0)

L_4 = Farm Experience (Years)

L_5 = Farm size (hectares).

L_6 = Dependency ratio (number of non-working members/total household size)

L_7 = Membership of Cooperative Society (Member = 1, Otherwise = 0)

L_8 = Average distance between land parcel (km)

L_9 = Access to credit (Yes = 1, No = 0)

L_{10} = Nearest to market (km)

L_{11} = Education (years)

L_{12} = Availability of good road (Yes = 1, No = 0).

The i is the model errors which are assumed to be independent N(O, 2) distributed, conditional on Li's.

III. RESULTS AND DISCUSSION

Table 1 presents the description of personal characteristics of farm household heads. A large proportion (43.4 %) of the farm household heads is aged between 31 – 50 years. The mean age of the household heads in the sample was 42. 8 years. This implies that most of the farmers are still in their active ages and thus expected to be productive for available resources. This is against the common reports (DFID, 2004; Okali, *et al.*, 2001) that there are aging rural farm population in Nigeria and that availability of off-farm livelihood options might be necessary to retain youths within the rural farm sector. Majority (70.6 %) of the households are headed by Males. This agreed with the tradition in the North Central part of Nigeria where Males are expected to be the head of the family.

Table 1: Distribution of sampled farm households by personal characteristics

Variables	Frequency	Percentage	Mean
Age of the Household Head (Yrs)			
≤30	78	21.7	
31 – 40	87	24.2	42. 8 years
41 – 50	69	19.2	
51 – 60	66	18.3	
> 60	60	16.6	
Sex of Household Head			
Male	254	70.6	
Female	106	29.4	
Marital status			
Single	87	24.2	
Married	231	64.2	
Divorced	12	3.3	
Widowed	30	8.3	
Educational level (Years)			
No formal education	66	18.3	4.2 years
Primary education	129	35.8	
Secondary education	96	26.7	
Tertiary education	69	19.2	
Primary occupation			
Farming	303	84.2	
Trading	24	6.7	
Civil Service	27	7.5	
Artisanship	6	1.6	
Farm Experience (years)			
1 – 10	75	20.8	
11 – 20	135	37.5	17.6 years
21 – 30	93	25.8	
>30	57	15.8	
Household Size			
1 – 4	90	25.0	
5 – 8	171	47.5	6.2 persons
9 – 12	63	17.5	
>12	36	10.0	

Source: Field Survey data, 2015

Majority (64.2 %) of the household heads are married with an average household size of six members. Spouse and children are important household family labour in traditional farming system. In terms of education, the mean education year of the household heads was 4.2 years with majority (87.7 %) of the sampled household heads having formal education. This finding implies that the rural households may be able to take full advantage of extension services, thus improving their income generation and poverty. Farming was the primary occupation of most (84.2 %) household heads with average farming experience of 17.6 years. This conforms with the claim that Nigeria is an agrarian nation as agriculture was once the main stay of the economy.

Pattern of Rural Farm Household Crop Diversification

Table 2 presents the pattern of rural farm households' crop diversification in the study area. Majority (51.7 %) of the respondents are highly diversified, 41. 7 % moderately diversified while 6.6 % did not diversify.

Table 2: Pattern of rural farm households' crop diversification.

Extent of Crop Diversification	Frequency	Percentage (%)
Not Diversified	24	6.6
Moderately diversified	150	41.7
Highly diversified	186	51.7
Total	**360**	**100.0**

Source: Field Survey data, 2015

Those that are highly diversified cultivate at least three crops, those that moderately diversified cultivate at least two crops, while those that did not diversify cultivate only one crop among the six crops studied. Most crop farmers in the study area did not depend on one crop because of risk associated with market price fluctuation, drought, excessive rainfall, fire, climate change etc. This strategy is adopted to ensure secured livelihood. In all, 93.4 % of the farm households diversified their cropping activities.

Determinants of Crop Diversification

Table 3 shows the results of the Tobit regression analysis of the determinants of crop diversification among the farm households with the sigma value and log likelihood function showing that the model is of good fit reasonably at $p < 0.01$. Table 3 revealed that age, household size, farming experience, gender, education, access to credit and membership of cooperative were the main albeit significant factors that determine crop diversification among farming households in the study area. Farming households' crop diversification level significantly increased with gender, farm size, access to credit, education and membership of cooperative; thus confirming that households' crop diversification was driven by larger farm size, higher level of education, and farmers participation in social group. An increase in farm hectarage, educational level of farmer, access to credit and being member of cooperative increase the crop diversification level of the household by 0.37, 0.81, 3.11 and 0.55 respectively. This implies that farmers involved in crop diversification for the following reasons; to ensure secured livelihood for the teeming household members, availability of farmland, awareness of the economic potentials of such practice (education) and easy access to loans.

Table 3: Estimated Tobit regression results on determinants of crop diversification.

Variable	Coefficient	Marginal Effect	T-value
Constant	-2.264	- 1.811	- 1.712
Age	-0.065*	- 0.062*	-1.748
Household size	-0.188**	-0.011	-1.034
Gender	2.449*	0.092*	1.723
Farming Experience	-0.045***	-0.002***	-2.087
Farm Size	0.365*	0.018*	1.354
Dependency ratio	-0.101	-0.096	-0.229
Membership of cooperative	0.553*	0.059*	-1.368
Average distance between land parcel	0.329	0.312	0.658
Access to credit	3.105*	0.117*	1.678
Nearness to market	-0.507	0.024	-1.917
Education	0.810***	0.029***	3.012
Availability of good road	0.352	0.334	0.938

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Sigma	0.1645***		
Log likelihood	-83.568		

Source: Field Survey data, 2015
* Statistically significant at 10 %
** Statistically significant at 5 %,
*** Statistically significant at 1 %,

The responses of the farmers' age, household size and farming experience to the level of crop diversification were significantly negative; thus signifying that farm households' crop diversification decreases as the farmers get older in age and farming experience by 0.065 and 0.045. Experience is a function of age. Thus, in many cases the aged farmers are more experienced in farming but less diversified because of old age. There was a significant negative relationship between household size and crop diversification because due to scarcity of land in the study area, an increase in household size tends to affect the farm size available for crop diversification. The marginal effects for significant variables showed that the crop diversification has decreasing effects of 0.062 and 0.002 as the farm household heads grow older in age and farming experience respectively while crop diversification has decreasing marginal effect of 0.011 as the household grows in size. Also, there were marginal increases in crop diversification by 0.092, 0.018, 0.059, 0.117 and 0.029 with an additional increase in gender, farm size, membership of cooperative, access to credit and farmers educational level respectively.

IV. CONCLUSION AND RECOMMENDATIONS

Based on the findings of the study, it could be concluded that most farm household heads in the study area are still in their active age. Thus, they are expected to be productive for available resources. Majority of the farmers had formal education and diversified their livelihood and economic activities. The Tobit results revealed that there were marginal increases in crop diversification with increase in farm size, membership of cooperative, access to credit, farmers educational level and gender but decrease with age, farming experience and household size. The policy implications and recommendations from this study include provision of enabling environment for the formation of cooperative societies and encouragement of farmers to join the existing cooperative societies. There is need for government to consider undertaking policies that will improve farmers' access to and control overland such as provision of ranches to avoid conflict between crop farmers and herdsmen. Government should work towards the expansion of infrastructures like road network, marketing and storage facilities which are important preconditions for the diversification of crops and are crucial in ensuring sustainable income and employment among farmers.

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The effect of sugarcane stillage on the yield of butternut squash (*Cucurbita moschata*) grown at Tambankulu Estates, a semi-arid region in the north eastern Lowveld of Eswatini.

Dlamini, M. V. [1*]; Manyatsi, A. M[1] and Simelane, S. B. [2]

[1]Dept. of Agricultural and Biosystems Engineering, University of Eswatini, Faculty of Agriculture, Private Bag, Luyengo, Swaziland.[2]Tambankulu Estates.
*Corresponding Author: musavd@uniswa.sz or musavd@gmail.com

Abstract— *An experiment was conducted to compare the effect of sugarcane distillery waste (stillage) and chemical fertilizer (N:P:K; 2:3:2 (22)) on the yield of butternut squash (Cucurbita moschata) grown under rain-fed conditions at Tambankulu Estates in the north eastern Lowveld of Eswatini. The experiment was run for a period of two years. Three levels of fertilizer, 43 kg, 86 kg and 129 kg and three levels of stillage 296 liters, 585 liters and 876 liters were applied to plots each measuring 450 m^2.Yield (fruit weight) and fruit size (fruit length and diameter) characteristics were measured at harvest. Data was subjected to the analysis of variance as per the design of the experiment. Yield and fruit length showed highly significant differences between the factors and levels whereas there were no significant differences in diameter. The highest yield of 960 g/fruit was recorded for stillage when applied at 585 liters followed by the highest concentration of 876 liters that yielded 950 g/fruit. The lowest level of fertilizer yielded the lowest yield of 721 g/fruit, with the two higher levels of fertilizer yielding the same 810 g/fruit. The control treatments with no fertilizer or stillage yielded the same lowest yield on average 550 g. The results of the experiment show that distillery waste (stillage) can be effectively used in the production of butternut squash in place of 2:3:2 (22) fertilizer.*

***Keywords*— Sugarcane stillage, fertilizer, butternut squash.**

I. INTRODUCTION

Butternut squash (*Cucurbit moschata*) is an important summer commercial crop grown by smallholder farmers in Southern Africa and is a type of winter squash (Department of Agriculture, Forestry and Fisheries, 2011). Butternut squashes are increasing in popularity because the opportunity of production and keeping of quality are good and sunburn is not a major problem. The harvested fruit is hardy and can be left on theland for a month or two. It has a sweet, nutty taste similar to that of a pumpkin. It has yellow skin and orangefleshy pulp. When ripe, it turns increasingly deep orange, and becomes sweeter and richer with time. It grows on a vine which a plus for farmers since local material could be used.It is the most commonly and regularly grown delicious vegetable among the cucurbits because it is a rich source of vitamin A, phosphorusand calcium (Yavuz, et al., 2015). It is also an excellent source of fibre, vitamin E, vitamin C, manganese, magnesium and potassium.The young and tender shoots make good vegetable salads.

The cultivation of this vegetable in Eswatini using sugarcane stillage as a source of fertilizer has not been investigated. Sugarcane stillage an organic waste, such as press mud or filter cake, is generated as a by-product of most sugarcane industries and characterized as a soft, spongy, amorphous, and dark brown to brownish material (Ghulam et al.,2012; Wynne and Meyer, 2002). It is generated during the purification of sugar by carbonation or sulphitation processes. Both the processes separate clear juice on top and mud at the bottom. It is considered as rejected waste material of sugarcane industries that cause problem of storage and pollution to the surrounding of sugar mills on its accumulation (Bhosale et al.,2012). It also supplies a good amount of organic manure (Bokhtiar et al.,2001) and can be an alternate source of plant nutrient (Rajagopal et al., 2014) and act as a soil ameliorates (Khan et al., 2016).

Sugarcane production is the biggest agricultural industry in Eswatini with over 60 000 hectares of land under irrigated sugarcane (SSA, 2014). There are three main

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sugarcane processing factories, Mhlume and Simunye sugar factories in the north eastern part of the country and Ubombo in the southern part. From these factories, stillage is produced as a by – product to be dumped in suitable areas like landfills. If improperly applied, the stillage can cause environmental problems, such as ground water pollution. This experiment was done to determine the effect of sugarcane stillage on the yield of butternut squash (*Cucurbita moschata*) grown under rain fed conditions. The results of are to be used to help local farmers in the proper disposal of stillage and the cultivation of vegetables.

II. MATERIALS AND METHODS

Location

The field experiment was conducted at Tambankulu Estates in the north eastern part of the Lowveld of Eswatini. This siteis located at a latitude of 26.13^{o}S, longitude 31.93^{o}E, and an altitude of 219 m above sea-level. The area receives an annual rainfall of about 600 mm. The soils are mostly the alluvial type which are deep red, well structured (medium to heavy clays) and free draining.

Experimental layout and crop management

The experiment was laid out as a split-plot, with stillage and fertilizer regimes as the main plots (factors) with each factor having three levels. The levels were split into three subplots namely; recommended, less than recommended and more than recommended. Stillage and fertilizer were not applied in the control plots. Butter nut seeds were manuallysown on the 9th of December, 2009, at a spacing of 75 cm between rows and 60 cm within rows, with one plant perstation (2.2 plants per m^2). Each plot was 450 m^2.

Fertiliser application and plant protection

Fertilizer and stillage were applied manually at the time of planting. Weeds were initially managed by herbicides and secondary weeds were manually uprooted using hoes. Bravoand Metafort 60SL were sprayed in a mixture at 800 ml per ha,in 500-1000 litres of water per hectare, every 14-21 days, inorder to control fungal diseases: powdery mildew (*Erysiphecichoracearum*, Jaczewski) and bacterial diseases.

Soil and stillage Properties

Soil samples were collected in each main block before planting and after planting just before harvesting using an auger in a zig-zag pattern within a depth of 0 to 20 cm and sent to Omnia Chemtech laboratory for analysis (Omnia, 2019). The samples were analysed for the following physico-chemical properties; bulk density, pH, sulphur, nitrogen, phosphorus, potassium, calcium, magnesium, sodium and electrical conductivity. Stillage samples were sent to Enviro Applied Products commercial laboratory (Enviro Applied Products, 2019)for chemical analysis.

Yield (growth) parameters of the butternut fruit

Yield (growth) parameters at harvest, fruit size, average fruit length (longitudinal) and equatorial diameter were measured using a digital scale and calliper.

III. RESULTS AND DISCUSSION

Stillage Analysis

The results of the chemical components of stillage are shown in Table 1. While most of the components have results with units of parts per million, nitrogen (N), Phosphorus (P), potassium (K) and chloride (Cl) have units of percentages since they were analysed following Kjeldahl’s method (Labconco, 2008).

Table.1: The physicochemical properties of the stillage applied in the butternut squash experiment

N	P	K	Cl	Fe	Cu	Mn	Zn	Ca	S	Sr	Bi	Sn	Ti
1.53 %	1.50 %	3.59 %	1.55 %	133 ppm	130 ppm	31.3 ppm	220 ppm	5944 ppm	4731 ppm	25.2 ppm	24 ppm	19 ppm	6 ppm

The result shows that stillage contains more potassium (K) and about equal amounts of nitrogen, phosphorus and chloride. It also contains a lot of calcium and sulphur when compared to zinc, copper, iron and manganese. In addition, the product contain trace amounts of titanium (Ti), tin (Sn), bismuth (Bi) and strontium (Sr) which all have alkali properties.

Soils analysis before and after planting

The results of the soil chemical analysis before planting and at harvesting of the butternut squash are shown in Table 2 and Table 3 respectively.

Table.2: The physicochemical properties of the soil before planting the butternut squash experiment

Bulk density (kg/m^3)	pH	S (ppm)	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	EC (s/m)
1251	5.56	10	52.7	8	79	1270	523	67	1.48

Calcium and magnesium contents of the soil were much higher than the other chemicals, with calcium the highest.

Table.3: The physicochemical properties of the soil after harvesting the butternut squash experiment

		Bulk Density (Kg/m^3)	pH	S (ppm)	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	EC (s/m)
	Control	1251	5.5	16	53	7	79	1270	523	67	1.48
Stillage	LC	1099	6.0	7	60	22	112	2020	878	198	1.40
	RC	1066	6.0	19	64	1	138	1990	819	234	1.48
	MC	976	6.8	4	67	7	226	4830	724	467	4.07
Fertilizer	LC	944	4.8	11	60	21	138	1420	707	47	1.23
	RC	1015	6.4	11	67	30	177	4000	1260	234	1.94
	MC	985	6.4	20	80	42	122	3210	1100	290	1.78

where; LC - low concentration, RC - recommended concentration, and MC - more than recommended concentration

Table 3 shows that the application of stillage and fertilizer in the soil had a reduction effect on the bulk density and the soil sulphur content. Fertilizer tended to have a slightly bigger reduction in bulk density than stillage and stillage had a bigger reduction in soil sulphur compared to the fertilizer treatments. Soil pH was slightly increased by both stillage and fertilizer. Both stillage and fertilizer resulted in increases in the availability of the other chemical elements, nitrogen, phosphorus, potassium, calcium, magnesium and sodium. Stillage did not affect the electrical conductivity of the soil except when applied at more than the recommended concentration which resulted in an increased soil EC. Increasing the concentration of fertilizer tended to increase the electrical conductivity of the soil.

Butternut squash yield (length, diameter and weight)

Butternut squash yield (fruit length (cm), fruit diameter (cm) and fruit weight (grams)) results are shown in Table 4 below.

Table.4: Butternut squash yield (fruit length (cm), fruit diameter (cm) and fruit weight (grams)) measured at harvest

		Butternut Squash Length (cm)	Butternut Squash Diameter (cm)	Butternut Squash weight (gm)
Control		17.1	30.7	547.7
Stillage	LC	18.5	30.3	770.1
	RC	20.0	31.7	960.3
	MC	19.9	31.3	952.0
	Mean	19.5	31.1	894.1
Fertilizer	LC	16.2	31.9	721.1
	RC	17.6	33.0	810.0
	MC	17.5	32.8	809.7
	Mean	17.1	32.5	780.3
	Significance	**	NS	**
	Interaction	NS	NS	**

*Values showing ** stand for significant differences at P < 0.01 probability level, whereas NS represents a non-significant value.*

Butternut squash fruit length was significantly increased by the application of stillage whereas fertilizer did not seem to improve the fruit length.

There were no significant differences in fruit diameter between the stillage and fertilizer treatments. The fertilizer treatments however showed a slightly bigger diameter compared to the stillage treatments.

There were highly significant differences in butternut fruit weight between the stillage and fertilizer treatments compared to the control. The mean weight for the stillage treatments was 894.1 g when compared to 780.3 g for the fertilizer treatment. Also, the weight for the fertilizer treatments was highly significantly ($P < 0.01$) greater than the control which was 547.7 g. This shows that the application of either stillage or fertilizer resulted in an

increased butternut fruit weight. These results are similar to those reported by Van Antwerpen, et al., (2003)
Figure 1 shows the effect of fertilizer and stillage concentration on the weight of butternut squash. Increasing the concentration beyond the recommended dosage seems to have no effect on the weight.

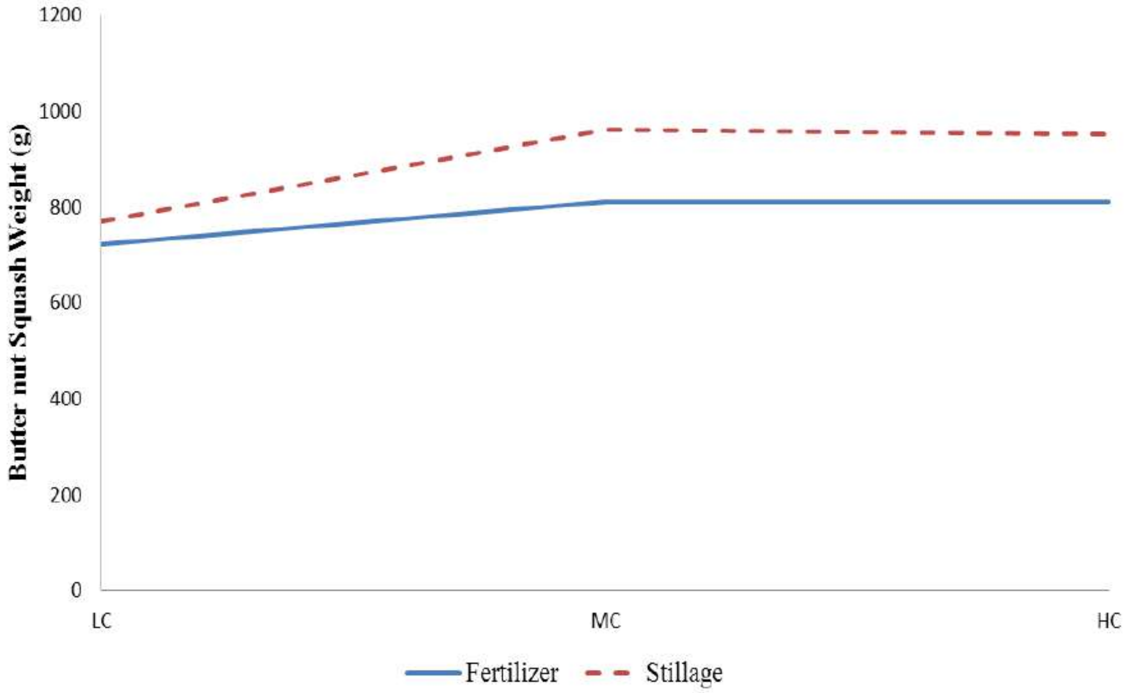

Fig 1.Showing the effect of the concentration of fertilizer and stillage on the weight of butternut squash.

IV. CONCLUSION

It can be concluded from the results of this experiment that the application of stillage result in improved soil chemical properties and increased butternut squash fruit yield (length and weight) when compared to fertilizer (N:P:K; 2:3:2 (22)). However, applying more than the recommended dose of stillage and or fertilizer seems to reduce yield.Depending on the economics, stillage is a better alternative to chemical fertilizer.

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Analysis of Water Quality in the Tabuk River Sub-Watershed, South Barito Regency

Nurul Fazriyanti[1], M.Ruslan[2], Erwin Rosadi[3], Agustiana[4]

[1]Master Program in Natural and Environmental Resource Management, Lambung Mangkurat University, Indonesia
[2]Faculty of Forestry, Lambung Mangkurat University, Indonesia
[3]Faculty of Fisheries and Maritime Affairs, Lambung Mangkurat University, Indonesia
[4]Faculty of Fisheries and Maritime Affairs, Lambung Mangkurat University, Indonesia

***Abstract*— *The Tabuk River Sub-Watershed is a tributary of the Barito River wich is Located in the Buntok City area of South Barito Regency. The River is Located in a residential area full of river flow Tabuk indicated has increased pollution caused by community activites on the riverbanks such as CWT activities (Cook, Wash, Toilet), Fisheries and tourism. This study aims to analyze the quality of Tabuk river water based on the status of water quality that refers to the decision of the state Minister of Environment No. 115 of 2003, and formulate strategies for controlling the pollution of the Tabuk river flow. The parameters analyzed are physics, chemistry and microbiology, the quality of river water is analyzed at three sampling points during high and low tide conditions. Analysis of river water quality status using the pollution index method. The result obtained are (1) the water quality of the Tabuk River Sub-Watershed for parameters that exceed the Class II water quality standards are TSS, BOD, COD, Total Coliform, Fecal colifirm during tidal or low tide conditions. TSS parameters 382 mg/L at low tide, and 157 mg/L at high tide. The BOD parameter is 61.5 mg/L during low tide, and 9.4 mg/L at high tide. The COD parameter is 83.7 mg/L during low tide, and 74 mg/L at high tide wich indicates a decline in water quality with moderate to mild water quality status. (2) efforts and strategies for the quality of tabuk river water to be in accordance with water quality standard criteria, namely (a) monitoring and maintaining Tabuk river water quality during low tide and tide conditions, (b) controlling domestic and non-domestic waste entering the river sub-watershed Tabuk, (c) establishes the concept of community participation as a reference for each agency that has an interest in carrying out river management activities.*
***Keywords*— Sub Watershed, Water Quality, Water Quality Status, Water Pollution Control.**

I. INTRODUCTION

The life of the people of the south Barito Regency in is inseparable from the existence of the river. With a population of 134.543 people and an are of 1829 km^2 (Statisticts Indonesia, 2018). Most people use river water as a source of clean water and some others use rainwater and surface water sources.

The river passes through 3 villages, namely Buntok Kota with a total of 16,621 people, which is a densely populated area. Downstream Sper with a population of 14,738 people, is a residential area, where the river is used by the community as a place of fishing in the river body, namely the existence of several fishponds around the river. Pamait with a population of 862 people, which is a small residential area and is used by the community as a place for bathing tourism.

Statistics from the South Dusun District, 2017 states that the number of houses living along the river is 217 houses in Buntok Kota, 79 houses in Hilir Sper, and 70 houses in Pamait. The average community that lives around the Tabuk riverbank has a place to stay facing the river, As a result, the potential of the community to dispose of garbage into the river is getting bigger, especially in gray water and black water waste as well as washing activities of BWT (Bathing, Washing, and Toilet) which can potentially produce liquid waste entering the river body.

Increased activity around the river can cause a decrease in the quality and quantity of the river. Water quality is a term to describe the suitability or suitability of water for certain uses, such as drinking water, fisheries, irrigation / irrigation, recreation and so on (Juanda, 2014). Besides the lack of knowledge, and research and public awareness of the condition of polluted rivers can cause the river does not meet quality standards or can not be used for certain needs, which will ultimately affect the surrounding community in using river water as daily necessities.

Maintaining or achieving water quality so that it can be used sustainably in accordance with the desired level of water quality, it is necessary to preserve and or

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control efforts. Preservation of water quality is an effort to maintain the function of water so that the quality remains at its natural condition. Water pollution control is carried out to guarantee water quality to conform to water quality standards through efforts to prevent and control water pollution and to restore water quality.

II. RESEARCH METHOD

The study was conducted using descriptive data analysis explaining the water quality and quality status of the Tabuk River Sub-Watershed. Water quality research was conducted at three monitoring points by dividing into two segments namely at low tide and tide, the following 3 (three) monitoring points for sampling the Tabuk River watershed in this study (Figure 1). As follow:

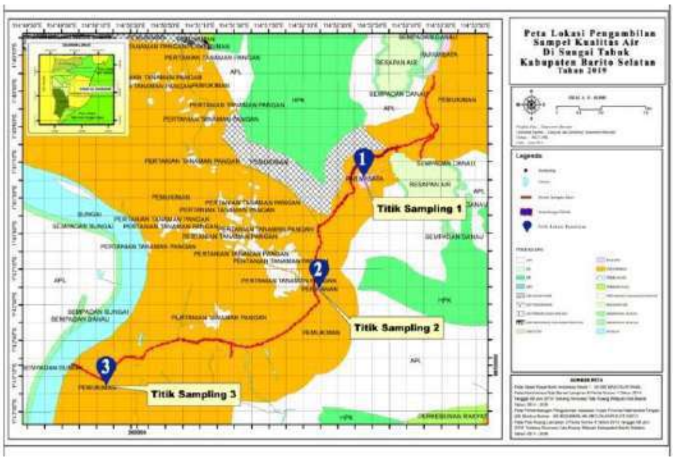

Fig.1: Sampling Location

The sampling method used is the Grab Sampling method, the method of taking samples for a moment that is directly taken from the body of water being monitored (Effendi, 2003). The sampling point is based on SNI procedures. 57. 6989. 2008 which can be seen in Table 1.

Table 1. Parameters and Methods for Testing Water Samples

	Parameter	*Metode*
1	*BOD (Biochemical Oxygen Demond)*	*SNI 6989.72:2009*
2	*COD (Chemical Oxygen Demand)*	*SNI 6989.73:2009*
3	*TSS (Total Suspended Solid)*	*SNI 6989.03:2004*
4	*Total Coliform*	*SNI 2897:2008*
5	*Fecal Coliform*	*SNI 2322:2006*

Analysis of water quality of the Tabuk River Sub-Watershed using Class II water quality criteria according to Government Regulation of the Republic of Indonesia Number 82 Year 2001 concerning Water Quality Management and Water Pollution Control. And the status of water quality is determined using the Pollution Index method in accordance with the Minister of Environment Decree No.115 of 2003. The Pollution Index calculation is performed by the calculation procedure using the equation:

$$PI_j = \sqrt{\frac{\left(\frac{Ci}{Lij}\right)^2_M - \left(\frac{Ci}{Lij}\right)^2_R}{2}}$$

where Lij: concentration of water quality parameters stated in Water Designation Standards (j) and Ci: concentration of water quality parameters (i), PIj is Pollution Index for designation (j), With (Ci / Lij) R: value, Ci / Lij average and (Ci / Lij) M: value, maximum Ci / Lij.

The data analysis method used in formulating a strategy for controlling pollution in the Tabuk River Sub-Watershed is a SWOT (Strength, Weakness, opportunity, Treat) analysis. SWOT analysis is the systematic identification of various factors to formulate a management plan (3).

III. RESULT AND DISCUSSION

1) Analysis of Water Quality in the Tabuk River Sub-Watershed

The results of water quality measurements on BOD, COD, TSS, total Coliform and fecal coliform at each monitoring point are compared with Class II river water quality criteria. As seen in Table 2 about the analysis of water quality at high tide and Table 3 at low tide conditions.

Table 2. Laboratory Analysis Results of the Tabuk River Sub-watershed during tidal conditions

No.	Parameter	Satuan	Sungai Tabuk			Baku Mutu
			Hulu	Tengah	Hilir	Kelas II
1	TSS**	mg/l	92^b	113^b	157^b	50
2	BOD**	mg/l	7^b	7.6^b	9.4^b	3
3	COD**	mg/l	40^b	68.7^b	74^b	25
4	Total *Coliform*	MPN/100ml	1700^a	2200^a	1300^a	5000
5	*Fecal Coliform*	MPN/100ml	330^a	1400^b	330	1000

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Table 3. Results of Laboratory Analysis of the Tabuk River Watershed during low tide

No.	Parameter	Satuan	Sungai Tabuk			Baku Mutu Kelas II
			Hulu	Tengah	Hilir	
1	TSS	mg/l	156[b]	241[b]	382[b]	50
2	BOD	mg/l	8.80[b]	14.3[b]	61.5[b]	3
3	COD	mg/l	43[b]	66.8[b]	83.7[b]	25
4	Total *Coliform*	MPN/l00ml	700[a]	1400[a]	24000[b]	5000
5	*Fecal Coliform*	MPN/100ml	170[a]	790[a]	13000[b]	1000

Description : [a] : still meet quality standards; [b] : Exceeds quality standards set; [c] : Minimum limit figure

Total Suspended Solid (TSS)

Analysis of the TSS parameters shows that the TSS concentration has increased to exceed the limits of the specified water quality standard. The increase in the value of TSS is due to the downstream so many land use changes become densely populated areas so that the community activities there in also increase which causes soil solids entering.

Habits of the community that function as a place for bathing, washing, toilet (BWT) and as fisheries and rubbish dumped directly into the river will pile upstream, causing the highest TSS value in the downstream of the Tabuk river.

Biochemical Oxygen Demand (*BOD*)

Tests on BOD parameters are known to increase from upstream to downstream and during low tide conditions it is known that BOD values are higher than BOD values at high tide. This indicates that the Tabuk river water has been polluted by waste disposal, both waste originating from tourism, fishery activities, and domestic waste generated by community activities along the river banks.

The results of the BOD test during low tide and tide conditions when compared with the class II water quality standards have passed the quality standard limit of 3 mg / l. the greater levels of BOD indicate that the indicated waters have been polluted especially in the downstream areas at low tide of 61.5 mg / l. the increase in BOD value at low tide can be caused by the disposal of organic waste originating from household waste where it is known that the downstream area is a densely populated area on the banks of the river and many of the houses make the kitchen enter the river, so that the direct household waste water goes directly to the river. In addition, the existence of aquaculture and leftover food derived from aquaculture also affects the high content of BOD. Organic wastes are generally in the form of waste that can rot or be degraded by microorganisms so that when discharged into water it will increase BOD (4).

***Chemical Oxygen Demand* (COD)**

COD concentration levels showed an increase from upstream to downstream both at low tide and at high tide. Increased levels of COD from upstream to downstream, can indicate that the Tabuk river water has been polluted due to waste disposal from upstream, where the upstream area is an area that contributes organic and non organic waste due to tourism activities. Then the flow of river water flows to the middle area where there is community activity in fish farming that can cause COD content to increase, because in these activities there is feeding which can cause the accumulation of leftover feed in the river which will eventually rot.

In the downstream areas there was a high increase of 74 mg / l during high tide conditions and 83 mg / l during low tide conditions this could be due to increased domestic waste disposal in downstream areas and is a densely populated area along the river. The high value of COD in waters is caused by the large amount of pollutants entering the waters, especially organic pollutants from household waste and aquaculture (5).

Total *Coliform*

The total coliform when tested was known when the tide conditions ranged from 1300-2200 MPN/100 ml from upstream to downstream. In these areas still meet the water quality standards for class II. So that for the tourism and fisheries areas it is still safe to be used as a cultivation and tourism activity. If it exceeds grade II water quality standards, it will cause disease both for tourism and fish farming. Because for fish farming must also pay attention to environmental health in order to be safe for consumption of fish. Success in fish farming is from maintaining environmental health from bacterial diseases (6). Communities that build latrines in rivers will cause rivers to be polluted with human excrement higher by indicating high total coliforms (7). The bacterial density indicates the suitability of water for drinking water, recreation, and fisheries.

Fecal Coliform

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The bacterial density indicates the suitability of water for drinking water, recreation, and fisheries. Where the results are compared with the class II water quality standard of 1000 MPN / 100ml it has exceeded the water quality standard set by Government Regulation No. 82 of 2001.

If it is found that the density of bacteria exceeds the quality standard threshold, then the waters are not suitable for cultivation activities because they can cause mass death of the seeds and a decrease in the quality of post-harvest biota (8). Bacterial content is influenced by the volume of water, when the volume of water is high it has a lower bacterial content compared to when the volume of water drops (9).

2). Quality Status Analysis of the Tabuk River Sub Watershed

The test results from the Tabuk river water samples, it is known that parameters that exceed the Class II water quality standards are TSS, BOD, COD, Total Coliform, Fecal coliform during tidal or low tide conditions.

The test results of the Tabuk river water samples at high tide and low tide the river pollution status using. Pollution Index calculation is the quality of tabuk river water when the tidal conditions are mild polluted, while the status of Tabuk river water quality at low tide conditions is mild polluted in the upstream and middle regions, however, in the downstream area the status is moderate, which can be seen in Table 4.

Table 4. Water Quality Status Value Value Based on Pollution Index

Sampling Point	Pollution Index	Category
Upstream When Tide	1,55	Light Pollution
Middel When Tide	2,51	Light Pollution
Downstrean When Tide	2,77	Light Pollution
Upstream At Low Tide	1,86	Light Pollution
Middle At Low Tide	2,99	Light Pollution
Downstream At Low Tide	5,30	Light Pollution

The level of pollution obtained based on calculations is known that the condition of the Tabuk river water at low tide is higher than during tidal conditions. This is due to the condition of the water when the tide has high water discharge and high rainfall can also cause dilution so that it can reduce the level of pollution. Whereas during low tide the water has a low flow of water and the flow of community waste goes directly to the river which will flow from upstream to downstream so that it is piled upstream downstream, this can cause pollution in the downstream area.

3). Formulation of River Water Pollution Control Strategies

Water Pollution Control is an effort to prevent and control water pollution and water recovery to ensure water quality in accordance with water quality standards (10). Based on the results of the water quality testing of the Tabuk River Sub-Watershed, interviews and literature studies, it can be described and presented in Table 5 as follows:

Table 5. Analysis of Efforts to Control Water Pollution in the Tabuk River Sub-Watershed

No (1)	River Water Pollution Control Aspects (2)	Indicator (3)
1.	Condition of the Tabuk River	1. In general, Tabuk river water quality class II water criteria on certain parameters when the tidal conditions have exceeded the water quality standard 2. The status of Tabuk river water quality is mildly or moderately polluted. 3. When the Tabuk river water conditions install some parameters such as COD, BOD, TSS have exceeded the pollution load capacity.
2.	Role of the Government	1. There is a related SKPD in charge of waste water management. 2. The existence of a complaint center for pollution cases at environmental agenciesi 3. Supporting Information and data relating to the Tabuk River watershed and it's pollution control are still incomplete condition of the Tabuk River

3.	Community Role	1. Lack of awareness from the the community in managing river cleanliness 2. People still throw garbage in the Tabuk river

Source: Primary and Secondary Data Analysis, 2019

The results of the strategies above, the policies to support the management of the Tabuk river water must involve the community and the government in paying attention to the sustainability of natural resources, such as:

1) Monitoring and maintaining water quality in the Tabuk River Sub-Watershed during low tide and tidal conditions at many community activities.
2) Control of domestic and non-domestic waste entering the Tabuk River Sub-WaterShed. Such as the socialization and construction of sewerage in residential areas that are covered by rivers.
3) Establish the concept of community participation as a reference for each agency that has an interest in carrying out river management activities. Further research can be done related to community behavior patterns that exist around the Tabuk River Sub-Watershed

IV. CONCLUSION AND SUGGESTION

CONCLUSION

1. The condition of water quality in the Tabuk River Sub-Watershed that the parameters that exceed grade II water quality standards are TSS, BOD, COD, Total Coliform, Fecal coliform during tidal or low tide conditions.
2. Pollution status in river Sub-Watershed at the upstream sampling point at high tide shows mild pollution, the middle sampling point at high tide indicates light pollution, the downstream sampling point at high tide shows mild contamination, the upstream sampling point at low tide indicates light polluted. the middle sampling point at low tide shows mild pollution, the downstream sampling point at low tide shows medium polluted.
3. The strategy for controlling the pollution of the Tabuk River Sub-Watershed is to monitor and maintain the water quality of the Tabuk River during low tide and tide conditions.
4. controlling domestic and non-domestic waste entering the Tabuk Sub-Watershed, establishes the concept of community participation.

SUGGESTION

Further research can be done related to community patterns that exist in the Tabuk river Sub watershed.

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Effect of Deficit Irrigation on Yield and Quality of Eggplant

Ransford Opoku Darko[1, 2*], Shouqi Yuan[1], Francis Kumi[2], Frederick Quaye[1]

[1]Research Centre of Fluid Machinery Engineering and Technology, Jiangsu University, Zhenjiang 212013, China.
[2]Department of Agricultural Engineering, University of Cape Coast, Central Region, Cape Coast, PMB Ghana.
*Corresponding author chiefrodark@yahoo.com

Abstract— *The aim of the study was to determine the effect of deficit irrigation on yield and quality of eggplant. Eggplant cultivated under different irrigation treatment. The treatment imposed included, treatment 1 (100ETc: full irrigation), treatment 2 (90ETc: 10% reduction of full irrigation), treatment 3 (80ETc: 20% reduction of full irrigation), treatment 4 (70ETc: 30% reduction of full irrigation). Eggplant cultivated under different irrigation treatment and were harvested and analyzed for total yield, fruit shape, pH of the fruit, moisture content, protein, carbohydrate, phosphorus. Deficit irrigation had significant effect on the protein content, carbohydrate content and fruit moisture. Results of the study showed that deficit irrigation had no significant effect on phosphorus and fruit shape. Also, deficit irrigation reduced disease incidence in eggplants while ensuring an improvement in fruit firmness and the overall marketable yield. The study concludes that a reduction of 10% ETc in the cultivation of eggplant would produce optimum and quality fruits thereby saving irrigation water.*
Keywords— ***Deficit irrigation, Water Use Efficiency, Yield, Eggplant.***

I. INTRODUCTION

Eggplant (*Solanum melongena L.)* is a short-lived perennial herb that belongs to the family Solanaceae. It is grown as an annual plant and is one of the consumed fruit vegetables in tropical Africa; probably the third after tomato and onion, and before okra (Grubben and Denton, 2004). Although excessive rainfall affects both vegetative growth and flower formation, the plant is well adapted to both wet and dry season cultivation. In West Africa the eggplant fruits are eaten raw, cooked or fried with spices in stews, or dried and pound as condiments (Fayemi, 1999). Eggplant is rich in essential vitamins and minerals. It contains 89.0g water, 1.4g protein, 1.0 fat, 8.0g carbohydrate, 1.5g cellulose, 105mg vitamin C and 1.6mg iron 130mg calcium (Ramain, 2001). In particular, eggplant is a good source of calcium, phosphorus and iron salts for bone and blood cell formation in the body (Schippers, 2000; Romain, 2001). According to Fereres and Soriano (2007), water scarcity has become a global problem. As cities grow and populations increases, the problem worsens since needs for water increase in households, industry and agriculture. This affects both the yield and quality of fruits and vegetables. Inability of farmers to determine the correct amount of water required by crops and adoption to the necessary irrigation practices during the growing season is one of the major challenges in vegetable production in Ghana. Studies have shown that eggplant can give a fruit yield of 0.5 kg to 8 kg per plant depending on the cultivar and growing conditions (Lester and Seck, 2004). Under rain fed, this translates to fruit yields varying from 5 to 8 ton/ha, while under optimal irrigation, potential fruit yield vary from 12 to 20 ton/ha. Currently, potential fruit of improved cultivars vary from 50 to 80 ton/ha (Lester and Seck, 2004). According to Norman (1992), the local egg plant can give an average yield of about 35-40 fruits per plant weighing between 0.9-1kg per plant. The aubergine types produce 5-10 fruits per plant depending on the cultivar and as the number of fruits produced increases, the size of the fruits decrease. (Tindall, 1992) also reported that 8-14 fruits per plant may be harvested with fruit size varying from 0.25-0.4kg per fruit with a yield of 2-5 t ha^{-1}. Without irrigation yield of about 5-8 t ha^{-1} can be obtained while with irrigation 12-20 t ha^{-1} can be obtained depending on the cultivar. Conventionally, irrigation is applied to avoid reduction in crop production due to water deficits (Fereres and Soriano, 2007). For commercial farmers, irrigation is applied to allow production of cultivated crops that will produce a satisfactory economic yield (Pereira *et al*. 2012). Crops are much supplied with sufficient water so that the crops can transpire and meet their full ET requirements throughout the growing seasons. Under conditions of water scarcity, the water available for farmers is normally below the maximum ET needs of the plants. Farmers are then forced to make decisions to concentrate on the limited water over a smaller land area or to irrigate the total area with levels below the full ET requirement. Irrigation application below the full ET requirement is termed as deficit irrigation. Deficit irrigation field studies normally derive production functions that can be used to predict yield depending on the amount of irrigation applied or the amount of water used by the crop (ETc) other than water, yield and quality of the crop is affected by several other factors some of which are unpredictable such as climate, incidences of pests and diseases and several agronomic factors (English and Roja, 1996). Therefore the production function will only be an estimate of the true relationships. Application of deficit irrigation in crop production is an approach to save water in areas of water shortage and longer drought during production period so as to maximize water productivity. Regulated deficit irrigation saves substantial amount of irrigation water and increase water use efficiency (Kirda, 2002). It is therefore important to use irrigation technique that suit to the local environmental condition and also with capability to improve yield and quality when complemented with good management practices, with the capacity to limit scarce resource wastage and require few inputs (Darko *et al*., 2016; Imtiyaz *et al*., 2000). Most of the agricultural production in Ghana is by smallholders who rely on seasonal rainfall that is unpredictable and sporadic. The onset of the climate change, insufficient rainfall and occasional uncontrollable floods results in frequent crop failures which are having a serious effect on the livelihood of the population. As a result, the population is extremely poor and food insecurity threatens every year. Inability of famers to determine the correct amount of water required by the crop and adoption to the necessary irrigation practices during the growing season is also another major challenge in vegetable production in Ghana. Thus application of deficit irrigation in eggplant production is an approach to save water in areas of water shortage and longer drought during the production period. By extension, this has left questions into the minds of many as to whether deficit irrigation has any effect on the yield and quality of crops. Hence, the study seeks to investigate the effect of deficit irrigation on the yield and quality of eggplant as well as water use efficiency.

II. MATERIAL & METHODS

Study area

The study was carried out at the School of Agriculture Teaching and Research Farm, University of Cape Coast. The study area experiences two rainy seasons namely the major season which starts from May and ends in July and a minor season that starts around September and ends around mid November to give the dry harmattan season that runs through to the end of March in the subsequent year. The area is characterized by an annual temperature range of 23.2-33.2 ºC with an annual mean of 27.6 °C and a relative humidity range of 81.3-84.4% (Owusu-Sekyere *et al*., 2011). The soil is described as sandy clayey loam of Benya series, a member of Edina Benya Udu compound association. Weeds were cleared to the ground level and allowed to dry. Pegging was done to demarcate the bed size. The hoe was used to loosen the soil to a maximum depth and with the peg and line; equal beds of 2.0m × 2.0m were prepared. The main cultural practices carried out included weeding, earthen up and stirring. These practices were carried out to ensure the optimal growth of the plant.

Experimental design and layout

The experimental design used in this study was Randomized Complete Block Design there were four (4) treatments with three (3) replications. The treatment were T1: 100%ETc; T2: 90%ETc; T3: 80%ETc and T4: 70%ETc. Egg plant seeds was nursed the healthy seedlings were transplanted unto prepared beds. All the plants comprising the treatment combination were given equal volume of water (500ml) for eight days to ensure uniformity among the seedlings before the various treatments were administered. A two day irrigation interval was employed. The volume of water applied to each treatment was obtained by the computation of crop evapotranspiration using the pan evaporation method. The amount of water applied represent 100%, 90%, 80% and 70%ETc. These water treatments were maintained for entire growing season of the eggplant crop. The stages are the developmental stage, the mid-season stage and the late season stage. The class A evaporation pan and a rain gauge installed at the teaching and research farm unit of the university of cape coast were used to record the amount of rainfall and the evaporative power of the atmosphere. The daily reduction in the pan water level with reference to the initial level noted the previous day was measured as the day's evaporation loss and then multiplied by the pan coefficient (kp) which is 0.7; the reference crop evaporation was obtained.

The reference crop evaporation was computed using the formulae

$ET_0 = K_p \times E_p$ (1)

Where;

ETo= reference crop evapotranspiration

Kp= pan coefficient

Ep= pan evaporation

Crop Evapotranspiration was computed using the formula

$ETc = ET_0 \times K_c$ (2)

Where

ETo = Reference evapotranspiration

Kc = Crop evapotranspiration

Data Collection

Data was collected on based on plant height, leaf area, average fruit weight, total yield, fruit shape, pH of the fruit, moisture content, protein, carbohydrate, phosphorus.

Plant Height

The plant height at the end of the initial stage, vegetative growth stage and final stage (fruiting) were measured using a tape measure. The data obtained were then summed up and their mean height was calculated for each treatment.

Leaf area

The longest length along the petiole line and the widest breath across the leaf of the eggplant were recorded by using a 30cm capacity roll up rule with a graduation of 10cm. A factor of 0.75 was multiplied by the product of the length and breadth to arrive at the leaf area according to Brown and Covey (1966).

Average fruit weight

Measurement of the mass of each treatment after harvesting was carried out using the electronic balance. Mean fruit mass was calculated for each treatment.

pH determination

The pH of the beverage was determined by the using a digital pH meter after calibrating with buffer solutions of pH 4.0 and 7.0 respectively. The beverage sample was then put in a 100 ml beaker, and thoroughly stirred. The electrode of pH meter was then immersed in and direct reading taken after the reading stabilized.

Determination of protein

The moisture content was determined using the oven drying method and Protein (%) was determined by first knowing the N(%) using Equation 3 below and computing it into Equation 4

$\text{N (\%)} = \frac{(\text{T}-\text{B}) \text{ x M x } 14.007 \text{ x } 100}{\text{Sample weight (mg)}}$ (3)

Where M = Molality of Acid; S = Sample titre value; B = Blank titre value

Protein = N (%) × 6.25 (4)

Determination of carbohydrate

$\text{Soluble carbohydrates (\%)} = \frac{\text{C (mg)} \times \text{extract volume (ml)}}{10 \times \text{aliquot (ml)} \times \text{sample wt (g)}}$ (5)

Where C = carbohydrate concentration from the calibration graph

Statistical analysis

Data collected was subjected to the analysis of variance (ANOVA) procedure using Genstat software statistical to investigate whether there were statistical differences in the parameters studied. Comparison of means will be done using Tukey Test at a probability level of 0.05.

III. RESULTS AND DISCUSSION

Eggplant growth and yield response to deficit application

Plant height

Analysis of variance on the effect of deficit irrigation on plant height (Figure 1) showed significant difference among the treatments, indicating that, at 24 days after transplanting, the height of plants which received 100% ETc (14.36 cm) was not significantly different from those that received 90% ETc (13.31 cm), but was significantly different from egg plants that receive 80% ETc (12.06 cm) and 70% ETc (10.29cm). A similar pattern was obtained for plant heights recorded at 41 DAT and 69 DAT. At 24 DAT, 41 DAT and 69 DAT there was no significant difference between egg plants that received 100% ETc and 90% ETc, a similar observation was made between egg plants that received 80% ETc and 70% ETc. This is in line with the findings of Owusu-Sekyere and Andoh (2010) who stated that after 69 DAT the highest plant height recorded from plant that received 100% ETc is not significantly different from the plant that received 90%ETc, while the lowest plant height recorded from plant that received treatment 70% ETc is not significantly different from the plant that received 80%ETc. Bilibio *et al*., (2013) reported that eggplant was more sensitive to water deficit and that plant height showed growth inversely proportional to soil water stress. This experiment has demonstrated that reducing a crops evapotranspiration beyond a certain reach would have a significant impact on the height of egg plants. The least plant height could be attributed to the non-availability of adequate moisture, which has a significant impact on the vegetative growth of egg plants. Water is a major component of plant cells and is the medium in which biological process such as photosynthesis occurs, without adequate moisture, photosynthetic rate of a plant is reduced. Reduced photosynthesis results in a retrogression of plant growth especially plant height. Since photosynthesis required for plant growth is not available or when available are present in smaller quantities. Also, plant under stress experiences difficulty in absorbing essential nutrients because transpiration which is linked with the roles of minerals salt absorbing, cooling and general effect on growth and development is negatively affected (Berrie and Berie, 1990).

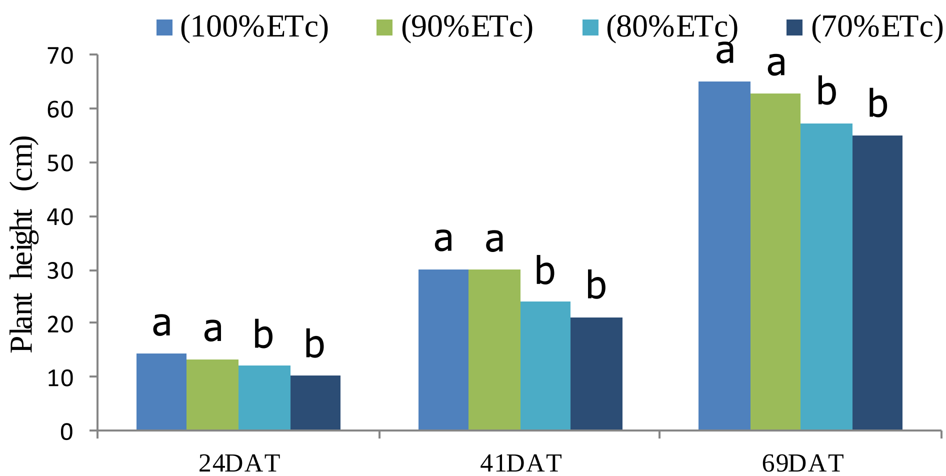

Fig.1: Effect of deficit irrigation on the plant height of eggplant

Leaf area

The effect of deficit irrigation on leaf area of eggplant is presented in (Figure 2). At 24 days after transplanting there was no significant difference between the mean leaf area of egg plants which received 100%ETc (29.67 cm^2) and 90% ETc (28.77 cm^2), a similar observation was made at 41 DAT and 69 DAT. However mean leaf area of egg plants that received 100% ETc (40.68 cm^2) and 90% ETc (39.74 cm^2) was significantly different from the mean leaf area of egg plants that received 80% ETc (37.37cm^2) and 70% ETc (35.82cm^2), a similar observation was made at 41 DAT and 69 DAT. Mean leaf area of egg plants that received 80% crop water requirement was significantly different from egg plants that received 70% crop evapotranspiration at 24 DAT, 41 DAT and 69DAT. After 69 DAT the lowest leaf area reordered was 44.42 cm^2, which was observed in plants that received 70% crop evapotranspiration. Owusu-Sekyere and Andoh, (2010) reported that they found the leaf area of egg plants reduced as the ETc of the plants was reduced. Egg plants that received the least ETc recorded the lowest leaf area. This could be attributed to the absence of adequate moisture, which has a major effect on the photosynthetic rate of the plants, hence it vegetative growth. Water alters a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation (Hu and Schmidhalter, 1998). Photosynthesis is the process in which plants combine water, carbon dioxide and light to produce carbohydrate for energy; chemical limitations due to a reduction in critical photosynthetic components such as water negatively have impact on plant growth. When these happen, leaf growth will be affected more since they are not able to compensate for moisture stress as compared to other parts of the plants such as the root.

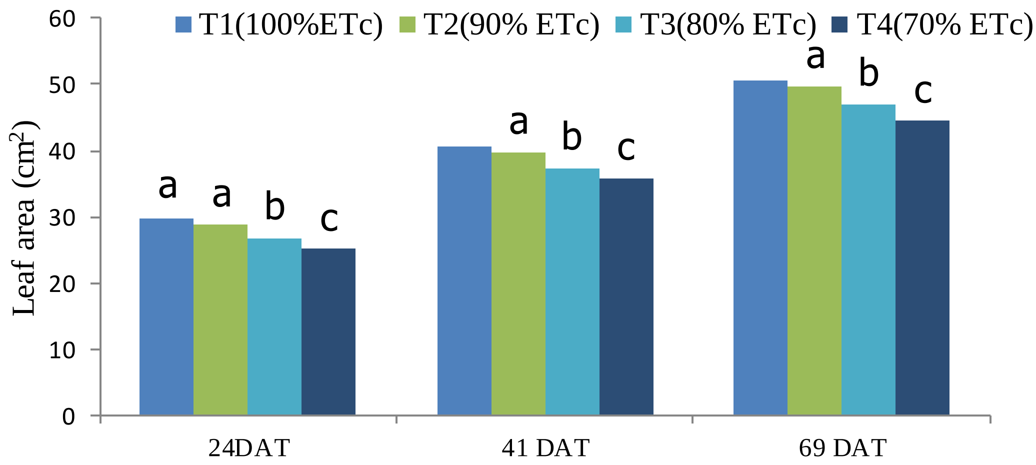

Fig.2: The effect of deficit irrigation on the leaf area of eggplant

Average fruit weight

Effect of deficit irrigation on the average fruit weight of eggplant is presented in Figure 3. Analysis of variance of the effect of different crop evapotranspiration on mean fruit weight showed significant difference among the mean fruit weight of egg plants which received 100%ETc (47.32 g), 90% ETc (45.93 g), 80% ETc (41.8 g) and 70% ETc (38.71 g). Research on eggplant also suggests that water stress limits fleshy fruit water accumulation but does not affect carbon partitioning to the fruit (Mitchell *et al*. 1991). Serhat (2017) reported that eggplant yield; length was significantly influenced by irrigation water level. The lowest average fruit weight was observed in eggplant exposed to the least irrigation amount that is 70% ETc. Diaz-Perez and Eaton (2015) reported that fruit yield of eggplant was lowest at 33% ETc and there were little yield differences among irrigation rates higher than 33% ETc. Dermirel *et al*., (2014) reported that yield reductions of 18.16 % and 27.13 % observed under low and moderate water stress. The highest average fruit weight was observed in eggplant exposed to full irrigation amount (100% ETc). This is in line with the findings of Kirnak *et al*., (2002). They reported that 100% ETc treatment had the highest yield as well as the largest and the heaviest fruit.

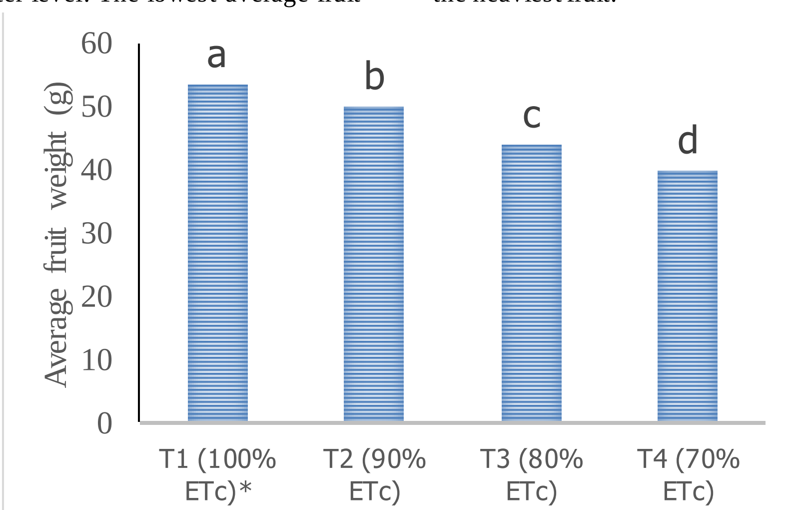

Fig.3: Effect of deficit irrigation on average fruit weight of eggplant.

Yield

Yield per tonnes of eggplant was significantly dependent on deficit irrigation (Figure 4). Analysis of variance of the effect of deficit irrigation on eggplant yield showed significant difference between the mean fruit weight of egg plants which received 100% ETc (4.0 MT), 90% ETc (3.8 MT) 80% ETc

(3.3 MT) and 70% ETc (3.0 MT). The highest yield was obtained from eggplant that received 100% ETc. This is in line with the findings of Serhat (2017), who reported that eggplant yield was significantly influenced by irrigation water level. The highest yield averaging 62 t ha^{-1} was obtained from eggplant that was given full irrigation amount. Although there was significant differences in the total yield of crops exposed to 90% ETc and 80% ETc was similar to that of 100% ETc. This is in tandem with earlier findings of Diaz-Perez and Eaton (2015) who suggested that eggplant may tolerate mild water stress, since plants irrigated at 20-30 % reduction of ETc produced fruit yields similar to those of plants irrigated at 100% ETc. Thus, there is a potential to save water by reducing current irrigation rates without negatively impacting fruit yields. Senyigit *et al.*, (2011) reported that the highest yield was obtained from full irrigation treatment and 10% reduction of full irrigation amount. The results of the study indicate that as the amount of irrigation water reduces total yield reduces. This could be attributed to the fact that water stress causes a reduction in the fruit number with decreasing soil water, this is further explained that lower soil moisture could result in pollen and stigma dehydration as well as unnecessary elongation of the flower's style which could result in up to 50% reduction in fruit setting and final fruit yield.

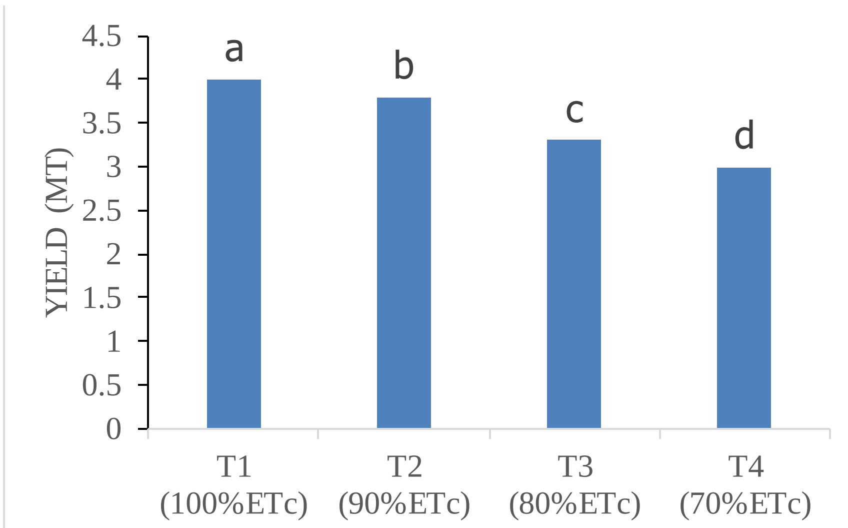

Fig.4: Effect of deficit irrigation on yield of eggplant

Effect of deficit irrigation on the quality of eggplant fruit.

Quality of eggplant fruits

Protein

The application of deficit irrigation instigated significant variation in the protein content of eggplant fruit (Table 1).

Table 1: Effect of deficit irrigation on the quality of eggplant fruit

Treatment	Protein (%)	Carbohydrate (%)	Phosphorus(ug/g)	pH	Fruit shape	Moisture (%)
100% ETc	17.4a	13.57a	3296a	5.5a	2286a	94.3a
90% Etc	19.3b	14.45a	3273a	5.5a	2777a	91.7b
80% Etc	15.6c	11.65b	3249a	5.6a	2759a	90.5c
70% Etc	15.6c	17.52c	3150a	5.6a	2571a	89.6d
L.S.D	0.40	0.967	208.2	0.02	625.6	0.92

The study portrayed that treatment 2 has the highest protein content of 19.34%, which was significantly different from the remaining treatments. Also treatment 4 had the least amount of protein content of 15.57% which was not significantly different from T3 with 15.63%. Present study indicate that eggplant fruit contains rich amount of vitamins and minerals of which protein is 1.4g according Romain (2001). This indicates that effect of water stress on eggplant had significant effect on the protein content of the fruit.

Carbohydrate and Phosphorous

The application of deficit irrigation instigated significant variation in the carbohydrate content of garden eggs as observed in Table 1. The study showed that treatment 4 (crops to which 70% ETc) has the highest carbohydrate content of 17.52%, which was significantly different from the other treatments. There was no significant difference between treatment1 (100% ETc) and treatment 2 (90%ETc) with 13.57% and 14.45% respectively.

The amount of phosphorus content in egg plant as a result of deficit irrigation is presented in (Table 1). There was high phosphorus content in treatment 1 (100%ETc) with 3296µg/g, followed by treatment 2 (90%ETc) with 3273µg/g, treatment 3 (80%ETc) with 3249µg/g, treatment 4 (70%ETc) with 3150µg/g in that order. The amount of phosphorus decreases with decrease in the ETc. There was no significant difference between the four treatments. The results of the study showed that, water stress of deficit irrigation had no significant effect with the phosphorus content of the eggplant. According to Fayemi *et al*., (1999), Phosphorus and Iron salts are used for bone and blood cell formation in the body, as well as a reasonable source of vitamin A(Carotene), Vitamin B-complex and vitamin C, all essential for good health and from the results of our study a decrease in ETc had no effect on the amount of phosphorus content in egg plant.

pH

The effect of deficit irrigation on the pH of eggplant fruits is presented in Table 1. pH measures the total acidity or alkalinity is an important factor in vegetable and fruit production as this relates to fruit quality.

The experiment discovered that, pH of eggplant fruit decreases with increasing water stress. Highest value of pH was as a result of high moisture content at the various treatments. This result is in line with the findings of Marouelli *et al*., (2007) who established that water supply restriction during either fruit development or maturation growth stages promoted a significant increase in fruit acidity. The results obtained as well confirm that of (Rouphael *et al*., 2008) who also illustrated that water stress can improve quality characteristics of fruits, pH inclusive. When crops are irrigated with less water, the plant regulate certain metabolic activities, such as osmotic adjustment in sink organs, to increase the sucrose and organic acid transformation rate and amount, consequently more assimilates shift to the fruits, thus improving the acid content. This implied that fruits from plants treated with less water had low pH values which would lead to an improvement in the flavour of the eggplant fruit.

Moisture Content

The results of moisture content of eggplant fruits with respect to deficit irrigation is presented in Table 1. Generally, it can be observed that as ETc reduces the moisture content of fruit decreases. Analysis of variance revealed that the variation in the moisture content of eggplant fruit after imposing deficit irrigation was statistically different. Bhattarai and Midmore (2002) noted that dry matter was highest for deficit irrigation treatments. Wahb-Allah *et al.* (2014) stated that water stress treatment significantly improve all fruit quality attributes in terms of fruit dry weight. The positive effect regarding water stress on eggplant fruit quality traits can be explained by a reduction in water accumulation in fruit. (Patanè *et al.*, 2011). The moisture content of eggplant fruit is inversely proportional to its firmness. Hence the lower the moisture content, the higher the firmness. Since the moisture content of the eggplant fruits decreases as the crop water requirement is reduced, it is obvious that the eggplants plants that received a lesser crop water requirement would produce fruits with the least moisture content.

Fruit shape

The effect of deficit irrigation on the pH of eggplant fruits is presented in Table 1. Fruit shape measures the total length and width as an important factor in vegetable and fruit production as this relates to fruit quality. The experiment discovered that, fruit shape of eggplant fruit remain the same even with increasing or decreasing water stress. When the crop water requirement of crops is reduced it makes the fruits hardy and increases firmness, thus reducing the susceptibility of the fruits to diseases.

Effect of deficit irrigation on water use efficiency of the eggplant.

Results of data on water use efficiency (WUE) of eggplant as a result of deficit irrigation is presented in Table 2.

Table 2: Effect of deficit irrigation on the water use efficiency of eggplant

Treatment	WUE (g/mm)
T1	2.012a
T2	2.517b
T3	3.237c
T4	3.372c
L.S.D	0.1600

It indicates that the highest water use efficiency was exhibited by crops that received 70% ETc (3.372 g/mm) and was significantly higher than crops that received 100% ETc (2.012 g/mm) and 90% ETc (2.517 g/mm), however it was not significantly different from crops that received 80% ETc (3.327 g/mm). This is in line with the findings of Serhat (2017) who reported that the highest WUE was observed in eggplants exposed to 75% ETc. It can be observed in Table 2 that the lowest water use efficiency (2.012 g/mm) was exhibited by crops that received 100% ETc. This agrees to earlier research done by Senyigit *et al.*, (2011). They reported that the lowest WUE values of eggplant were calculated in the treatment to which the highest irrigation water was applied. Their findings indicated that WUE decreased with the increasing irrigation water and ET. Generally, it can also be observed that as the eggplants were exposed to water stress, the water use efficiency of eggplants increased. The lowest amount of water applied (70% ETc) recorded the highest WUE value, whereas the highest amount of water applied (100% ETc) resulted in the lowest WUE values in that order. The phenomena where by water stressed plants gives the higher WUE indicates that as the crops are exposed to water stress there is high dry matter accumulation in the fruit. According to Birhanu and Tilahun (2010) this observation can be attributed to the fact that as total plant biomass decreases with water stress level, fruit dry matter increased, hence there is an increase in water use efficiency when water stress level increases.

IV. CONCLUSION

Production continue to increase and the prospects for increase in vegetable all year round will possibly come mostly from irrigation and amendment of the soil fertility status as rainfall is unpredictable and the soil is of low fertility. Research in irrigation water management for efficient use of water by crops will contribute to crop improvements. From the study, it can be concluded that, 90% crop water requirement or water-use efficiency is the best application for eggplant in a water scarce environment. Also, decreasing deficit irrigation level from T3 to T4 Water-Use Efficiency can yield to compensate marketable losses. The study showed that increasing or decreasing deficit irrigation level in eggplant production had no significant effect on the fruit pH, fruit shape and the phosphorus content of the fruit but brought about significant differences in carbohydrate content of garden eggs for the various treatments.

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Addition of Different Nitrogen Sources on the Cocoa Pod (*Theobroma Cacao*) fermented with *Pleurotus Ostreatus*

Junaidah[1], Nuraini[2], Ade djulardi[2]

[1]Department of Animal Science, Faculty of Animal Science, Andalas University, 25163, Indonesia
Email: junaidasyaf@gmail.com
[2]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, 25163, Indonesia

***Abstract*— *This research was aimed the composition of the substrate, inoculum dose and fermentation time suitable for the growth of Pleurotus ostreatus on cocoa pod substrate mixtures with different nitrogen sources (tofu waste, soy milk waste and rice bran) of the cellulase enzyme activity, laccase enzyme activity, crude fiber decreased, and crude fiber digestibility. This study used an experimental method with a completely randomized design (RAL) 3x3x3 factorial with 2 replications. Factor A is a substrate composition with a ratio of 80% cocoa pod and 20% for tofu waste, soy milk waste, and rice bran. Factor B is an inoculum dose of 6%, 8% and 10% with Factor C is the fermentation time of 7, 9 and 11 days. The results of the analysis showed that an interaction of the substrate composition and fermentation time with Pleurotus ostreatus which had a significant effect (P <0.05) of the activity of cellulase enzymes, laccase enzyme activity, crude fiber decreased and crude fiber digestion. The results of the study can be concluded that the selected treatment was found in the composition of 80% cocoa pod and 20% tofu waste with 9 days fermentation time (A1C2). In this condition cellulase enzyme activity was obtained of 2.70 U/ml, the laccase enzyme activity of 12.33 U/ml, crude fiber decreased of 44.01% and crude fiber digestibility of 53.78%.*

***Keywords*— Fermentation, Cocoa pod, Nitrogen source, Pleurotus osteratus**

I. INTRODUCTION

The problem of poultry feed is still a concern today because poultry is still one of the most developed livestock commodities. The price of poultry feed which is unstable tends to be expensive and the level of availability which continues to decrease simultaneously results in the decline of poultry business. The high price of poultry feed is caused by the constituents used which are still imported and compete with human needs. The price of feed tends to change at any time depending on the situation, natural conditions, and the market.

One effort to reduce the problem of poultry feed is to replace conventional feed that is commonly used to prepare poultry rations with non-conventional feed ingredients. Generally, this non-conventional feed material comes from agricultural, livestock and industrial wastes. Non-conventional feed ingredients must be available continuously, in large volumes, easily available and inexpensive. One of the non-conventional feed ingredients that can be used as animal feed is cocoa pods obtained from cocoa fruit processing.

Indonesia ranks third as the world cocoa producer country after Pantai Gading and Ghana. West Sumatra is one of the cocoa-producing provinces with a production of 52.2 thousand tons with an area of plantation reaching 158.9 thousand hectares in 2017 (BPS, 2018). Cacao consists of 74% pods cacao, 2% placenta and 24% seeds (Harsini and Susilowati, 2010) so it can be estimated that pod cacao production of 38,628 thousand tons in 2017.

Pod cocoa contains crude protein 11.75%, crude fat 11.75%, NFE 34.95%, crude fiber 32.12% (cellulose 22.11% and lignin 23.14%) (Nuraini *et al*, 2013), and tannins 0.11% (Nuraini *et al*, 2012). If viewed in terms of nutrient content and continuous availability, the cocoa pod is quite the potential to be used as animal feed. Research that has been done shows that cocoa pod can be used as a source of animal feed (Tequia *et al*., 2004).

However, with the high content of crude fiber and the presence of theobromin antinutrient compounds in the cocoa pod is one of the limiting factors in giving to poultry, due to the limited ability of poultry to digest crude fiber and feeding continuously containing theobromine can reduce growth (Tarka *et al*. 1998).

One effort to reduce the content of crude fiber and minimize the content of antinutrient compounds in the cocoa pod is fermentation by utilizing the role of microorganisms. Microorganisms that are effective in degrading crude fiber, especially lignin, are *Pleurotus ostreatus* from the *Basidiomycetes* group known as high-class mushrooms that produce extracellular ligninolytic enzymes such as laccase, lignin peroxidase and manganese peroxidase (Periasamy and Natarajan 2004; Mayer and Staples 2002). *Pleurotus Ostreatus* also produces cellulase enzymes (Sudiana and Rahmansyah, 2002) which can work synergistically to break down cellulose into glucose through a catalyst process (Santos *et al*., 2012).

Fermentation in principle is influenced by several factors including the composition of the substrate, the dose of the inoculum and fermentation time. Fermentation using *Pleurotus ostreatus* requires a substrate containing carbon, nitrogen and mineral sources to support the growth and development of mycelium maximally pod cocoa itself can be used as a carbon source (C) in the substrate fermentation, but must be supplemented with a nitrogen source (N) to get the balance C: N which is suitable for the growth and development of *Pleurotus ostreatus* mycelium. Sources of nitrogen (N) that can be used are tofu waste, soy milk waste, and rice bran.

Tofu waste is a waste of soybean processing industry that has nutrients namely crude protein at 28.36%, crude fat 5.52%, crude fiber 7.06% and NFE 45.44% (Nuraini *et al*., 2012). High crude protein content in tofu waste can be used as a source of N for the growth of mycelium. Muhfudz (2006) states that tofu waste contains lysine and methionine amino acids and calcium which is quite high.

Soy milk waste is a waste of the soybean processing industry such as tofu waste but different in processing. Soy milk waste has a high crude protein content of 27.62%, this content can be used as a source of N for mycelium growth. Other nutritional content of crude fat is 2.95%, NFE 52.66%, crude fiber 13.81% and ash 2.96%, Ca 0.09%, P 0.04% (Muis *et al*, 2009).

Rice bran is the biggest by-product of rice milling process which contains metabolic energy of 2980 kcal/ kg, crude protein 12.9%, crude fat 13%, crude fiber 11.4%, Ca 0.07%, phosphorus 0.22%, Mg 0.95% and 9% moisture content (Saputra, 2015). Rice bran is also rich in vitamin B complex and mineral components such as iron, aluminum, calcium, magnesium, manganese, phosphorus, and zinc (Astawan, 2010). It is hoped that cocoa pod fermented using Pleurotus ostreatus with different nitrogen sources can produce optimal cellulase and laccase enzymes to reduce fiber content and increase the digestibility of crude fiber.

II. MATERIALS AND METHODS

2.1. Materials

The ingredients used are cocoa pod, tofu waste, soy milk waste and rice bran. The fungus used is *Pleurotus ostreatus*. The equipment used is analytical scales, autoclaves, laminar airflow, ovens, a set of equipment for proximate analysis, cellulase enzyme activity and laccase enzyme activity.

2.2. Method

The design used is a Completely Randomized Design (CRD) Factorial 3x3x3 with 2 replications. Factor A is a substrate composition consisting of 3 (three) levels, namely:

A1 = 80% cocoa pod + 20% tofu waste
A2 = 80% cocoa pod + 20% soy milk waste
A3 = 80% cocoa pod + 20% rice bran

Factor B is the dose of inoculum which consists of 3 (three) levels, namely:

B1 = 6% dry matter of the amount of substrate
B2 = 8% dry matter out of the amount of substrate
B3 = 10% of dry matter from the amount of substrate

Factor C is the fermentation time consisting of 3 (three) levels, namely:

C1 = 7 days
C2 = 9 days
C3 = 11 days

2.3. Research Implementation

This research activity is fermenting cocoa pods with tofu waste, soy milk waste and rice bran. Cocoa pod is cleaned first then chopped 0.5-1 cm. Tofu and soy milk wastes are squeezed to reduce water content. Weigh the substrate by composition (80% cocoa pod and 20% for tofu waste, soy milk waste and rice bran). Homogenize the three substrates then add 7 ml of Mineral Brook, the substrate water content is ± 70%. Sterilize all substrates in the autoclave at 121^0 for 15 minutes. *Pleurotus ostreatus* inoculation according to treatment (6,8 and 10%). Incubation according to treatment (7, 9 and 11 days). Fermentation products will be analyzed for crude fiber content (AOAC method, 1990), crude fiber digestibility (Sibbald method, 1975), cellulase enzyme activity (Nelson method, 1944) and laccase enzyme activity (Buswell et al., 1995).

III. RESULT AND DISCUSSION

3.1. Cellulase enzyme activity

Statistical analysis showed that there was no interaction between the composition of the substrate, inoculum dose and fermentation time but the interaction was significantly different ($P<0.05$) found in the composition of the substrate with the fermentation time

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on the activity of cellulase enzymes (U / ml) cocoa pod fermented with *Pleurotus ostreatus* (Table 1).

DMRT (Duncan Multiple Range Test) showed that cellulase enzyme activity in A1C3 treatment (80% cocoa pod and 20% tofu waste with 11 days fermentation time) had no significant effect (P> 0.05) from A1C2 treatment (80% pod cacao and 20% tofu waste with 9 days fermentation time), A2C2 treatment (80% cocoa pod and 20% soy milk waste with 9 days fermentation time) and A2C3 treatment (80% cocoa pod and 20% soy milk waste with 11 days fermentation time); but significantly (P <0.05) higher than other treatments.

Table 1. The average cellulase enzyme activity (U/ml)

Substrate (A)	Inoculum dose (B)	Fermentation time (C)			Average
		C1	C2	C3	
A1	B1	2,02	2,52	2,69	**2,41**
	B2	2,18	2,77	2,87	**2,61**
	B3	2,37	2,80	3,03	**2,73**
Total		6,56	8,70	8,58	
Average		**2,19^b**	**2,70^a**	**2,86^a**	
A2	B1	1,99	2,40	2,52	**2,30**
	B2	2,13	2,62	2,68	**2,48**
	B3	2,22	2,76	2,85	**2,61**
Total		6,33	7,78	8,05	
Average		**2,11^b**	**2,59^a**	**2,68^a**	
A3	B1	1,94	2,05	2,18	**2,05**
	B2	2,02	2,12	2,24	**2,12**
	B3	2,11	2,28	2,33	**2,24**
Total		6,07	6,44	6,74	
Average		**2,02^b**	**2,15^b**	**2,25^b**	

The high activity of cellulase enzymes in A1C2, A1C3, A2C2, and A2C3 treatments is due to the presence of substrate composition and fermentation time which are suitable for the growth of *Pleurotus ostreatus*. On 80% cocoa pod and 20% tofu waste substrate and 80% cocoa pod and 20% soymilk waste substrate, it appears that the growth of *Pleurotus ostreatus* is fertile, white and evenly almost covered the entire substrate. This is supported by Gunam *et al*., (2011) that there is a correlation between protein content and enzyme activity produced when viewed from substrate concentration and fermentation time. In environmental conditions with high protein content produced, the enzyme activity is also high and otherwise if, in conditions where the protein content produced is low, it appears that the activity of the resulting enzyme is low.

Fermentation time allows mycelium to flourish to fill the substrate so that more cellulase enzymes are produced. According to Setyawan (2007) that the fermentation time is closely related to the time that can be used by microbes to grow and multiply so that the enzyme activity increases. The longer time of fermentation causes the fungus to flourish and the cellulase enzymes produced from hyphae will be more numerous, besides the conditions in the substrate also support the cellulase enzyme to move, so that the cellulase enzyme is more active in breaking down cellulose into glucose.

The selected treatment based on its efficiency was found in A1C2 (80% cocoa pod and 20% tofu waste with 9 days fermentation time) with cellulase enzyme activity 2,70 U/ml. This result is higher than Doharne (2015) where cacao pod fermented with *P.chrysosporium* and a dose of inoculum 7% with a fermentation period of 10 days and continued fermentation with *N.crassa* 9% with a fermentation period of 4 days has an enzyme activity of 0, 10 U/ml.

3.2. Laccase enzyme activity

Statistical analysis showed that there was no interaction between the composition of the substrate, inoculum dose and fermentation time but the interaction was significantly different (P <0.05) found in the composition of the substrate with the fermentation time on the activity of laccase enzymes (U / ml) cocoa pod fermented with *Pleurotus ostreatus* (Table 2).

Table 2. The average laccase enzyme activity (U/ml)

Substrate (A)	Inoculum dose (B)	Fermentation time (C)			Average
		C1	C2	C3	
A1	B1	9,78	11,75	12,09	**11,21**
	B2	10,59	12,27	12,56	**11,81**
	B3	11,11	12,96	13,02	**12,36**
Total		31,48	36,98	37,67	
Average		**10,49^c**	**12,33^a**	**12,56^a**	
A2	B1	9,32	11,23	11,40	**10,65**
	B2	10,47	11,98	12,09	**11,52**
	B3	10,53	12,44	12,91	**11,96**
Total		30,32	35,65	36,40	
Average		**10,11^c**	**11,88^a**	**12,13^a**	
A3	B1	8,45	9,66	10,65	**9,59**
	B2	9,78	10,36	11,00	**10,38**
	B3	10,13	11,05	11,98	**11,05**
Total		28,36	31,08	33,62	
Average		**9,45^d**	**10,36^c**	**11,21^b**	

DMRT (Duncan Multiple Range Test) showed that laccase enzyme activity in A1C3 treatment (80% cocoa pod and 20% tofu waste with 11 days fermentation time) had no significant effect (P> 0.05) from A1C2 treatment (80% cacao pod and 20% tofu waste with 9 days fermentation time), A2C2 treatment (80% cocoa pod and 20% soy milk waste with 9 days fermentation time) and

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A2C3 treatment (80% cocoa pod and 20% soy milk waste with 11 days fermentation time); but significantly(P <0.05) higher than other treatments.

The high activity of the laccase enzyme in A1C2, A1C3, A2C2, and A2C3 treatments due to the presence of a C: N ratio balance in the four treatments, can be seen from the fertile growth of mycelium compared to other treatments. The fertility of the mycelium is also affected by the fermentation time, where the fermentation time gives the mycelium opportunity to continue to grow. The C: N ratio of 80% cocoa pod and 20% tofu waste is 12.16: 1, and 80% cocoa pod and 20% soy milk waste is 12.47: 1. This is supported by the opinion of Gianfreda *et al*. (1999) that laccase production is strongly influenced by the concentration of nitrogen in the culture medium and the carbon source used (Galhaup *et al*., 2003). Furthermore, Nadeem *et al*. (2014) that carbon and nitrogen sources at the right ratio are needed for propagation and enzyme production. *Basidiomycetes* fungi including *Pleurotus ostreatus* have different responses to carbon sources and their concentration in the medium for growth. Significant laccase secretion occurs when the concentration of carbon sources in the growth medium reaches a low level. The best C: N ratio for the growth of *Pleurotus ostreatus* is 10 - 15: 1 and results in maximum laccase production, where an increase in C: N ratio will further reduce laccase production significantly.

Majeau *et al*. (2010) reported that laccase enzyme production was influenced by factors of nutrient availability such as carbon and nitrogen, concentration and C: N ratio as well as other factors such as characteristic and concentration of the inducer. The optimum concentration of organic carbon in growth media has an important role in laccase production. In the white-rot fungus group, the production of the enzyme laccase main role in ligninolytic activity (Bonnen *et al*., 1994). Furthermore, Ardon *et al*. (1998) show a strong indication that the activity of the laccase enzyme can increase the level of lignin degradation. Among the *Basidiomycetes* groups of white rot species dominant studied are *Pleurotus ostreatus* and *Trametes versicolor* because of their ability to mineralize lignin through the secretion of oxidative enzymes such as laccase (Halburgi, 2011) known as an efficient enzyme in degrading lignin (Bernardi *et al*., 2008).

The selected treatment based on its efficiency was found in A1C2 (80% cocoa pod and 20% tofu waste with 9 days fermentation time) with laccase enzyme activity of 12,33 U/ml. *Pleurotus ostreatus* also produces laccase enzymes in palm sludge fermentation obtained of 12.73 U/ml.

3.3. Reduction of crude fiber

Statistical analysis showed that there was no interaction between the composition of the substrate, inoculum dose and fermentation time but the interaction was significantly different (P<0.05) found in the composition of the substrate with the fermentation time on the reduction of crude fiber (%DM) cocoa pod fermented with *Pleurotus ostreatus* (Table 3).

DMRT (Duncan Multiple Range Test) showed that reduction crude fiber in A1C3 treatment (80% cocoa pod and 20% tofu waste with 11 days fermentation time) had no significant effect (P> 0.05) from A1C2 treatment (80% pod cacao and 20% tofu waste with 9 days fermentation time), A2C2 treatment (80% cocoa pod and 20% soy milk waste with 9 days fermentation time) and A2C3 treatment (80% cocoa pod and 20% soy milk waste with 11 days fermentation time); but significantly(P <0.05) lower than other treatments.

Table 3. The average reduction of crude fiber (%DM)

Substrate (A)	**Inoculum dose (B)**	**Fermentation time (C)**			**Average**
		C1	C2	C3	
A1	B1	33,02	39,95	46,57	**39,85**
	B2	32,09	43,95	46,78	**40,94**
	B3	35,31	48,14	49,40	**44,28**
Total		100,42	132,04	142,75	
Average		**33,47^{cd}**	**44,01^{ab}**	**47,58^{a}**	
A2	B1	30,19	39,71	38,48	**36,13**
	B2	32,34	41,83	44,46	**39,54**
	B3	33,40	46,36	47,67	**42,48**
Total		95,93	127,90	130,61	
Average		**31,98^{cd}**	**42,63^{ab}**	**43,54^{ab}**	
A3	B1	20,06	22,61	35,56	**26,08**
	B2	21,55	29,88	38,63	**30,02**
	B3	30,44	33,82	40,13	**34,80**
Total		72,06	86,31	114,33	
Average		**24,02^{e}**	**28,77^{de}**	**38,11^{bc}**	334,11

The decrease in crude fiber in A1C3, A1C2, A2C3, and A2C2 treatments was due to the activity of cellulase enzymes in the four treatments, which were respectively 2.86%, 2.70%, 2.68% and 2.59%. High cellulase enzyme activity is related to substrate composition (C: N balance) and fermentation time suitable for *Pleurotus ostreatus* to grow and flourish. *Pleurotus ostreatus* produces cellulase enzymes (Sudiana and Rahmansyah, 2002) which can work synergistically to break down cellulose into glucose through a catalyst process (Santos *et al*., 2012). Substrate composition that has a balanced C: N ratio can accelerate the growth of *Pleurotus ostreatus*, because fungi need carbon and nitrogen for its growth. Nadeem *et al*., (2014)

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stated that the best C: N ratio range for the growth of *Pleurotus ostreatus* is 10-15: 1.

Fermentation time allows the mycelium to grow more optimally and produce enzymes to degrade crude fiber components. This is supported by Musnandar (2004) where the longer the fermentation, the greater the opportunity for enzyme complex to degrade crude fiber components into simple sugars. This increase in simple sugars will increase the growth of fungal colonies, especially high doses of inoculum so that the production of enzymes increases which in turn increases the degradation of crude fiber on the substrate. The more fertile the mycelium, the higher the activity of the cellulase enzyme produced in degrading the fiber components of the fiber on the substrate.

The longer fermentation will cause the process of fungal metabolism to increase so that more energy is released by the fungus by degrading various energy sources in the substrate such as crude fiber. Furthermore, Perez *et al.* (2001) explained that each microfungus has a different ability to decompose the substrate. The longer the incubation period, the more complex the compounds that are broken down by microorganisms into simpler compounds that can accumulate into energy.

The selected treatment based on its efficiency was found in the A1C2 treatment (80% cocoa pod and 20% tofu waste with 9 days fermentation time) of 44,01%. This result is higher than Doharne (2015) where cocoa pods fermented with *P.chrysosporium* with 7% inoculum dose with 10 days fermentation time and continued fermentation with *N.crassa* 9% with 4 days fermentation time decreased fiber content of 25,47%.

3.4. Crude fiber digestibility

Statistical analysis showed that there was no interaction between the composition of the substrate, inoculum dose and fermentation time but the interaction was significantly different (P <0.05) found in the composition of the substrate with the fermentation time on the crude fiber digestibility (%DM) cocoa pod fermented with *Pleurotus ostreatus* (Table 4).

Table 4. The average crude fiber digestibility (%DM)

Substrate (A)	**Inoculum dose (B)**	**Fermentation time (C)**			**Average**
		C1	C2	C3	
A1	B1	43,77	48,60	49,30	**47,22**
	B2	47,29	54,87	56,82	**52,99**
	B3	49,53	57,86	58,76	**55,38**
Total		140,58	161,33	164,89	
Average		**46,86^{b}**	**53,78^{a}**	**54,96^{a}**	
A2	B1	42,31	46,33	48,01	**45,55**
	B2	45,75	49,79	53,29	**49,61**
	B3	49,30	51,31	55,28	**51,96**
Total		137,36	147,43	156,58	
Average		**45,79^{b}**	**49,14^{ab}**	**52,19^{a}**	
A3	B1	42,01	43,12	44,17	**43,10**
	B2	43,39	44,09	46,54	**44,67**
	B3	46,16	46,70	47,67	**46,84**
Total		131,56	133,91	138,38	
Average		**43,85^{b}**	**44,64^{b}**	**46,13^{b}**	

DMRT (Duncan Multiple Range Test) showed that laccase enzyme activity in A1C3 treatment (80% cocoa pod and 20% tofu waste with 11 days fermentation time) had no significant effect (P> 0.05) from A1C2 treatment (80% cacao pod and 20% tofu waste with 9 days fermentation time), A2C2 treatment (80% cocoa pod and 20% soy milk waste with 9 days fermentation time) and A2C3 treatment (80% cocoa pod and 20% soy milk waste with 11 days fermentation time); but significantly (P <0.05) higher than other treatments.

The high digestibility of crude fiber in the A1C3, A1C2, A2C3 and A2C2 treatments is due to the low crude fiber content in the four treatments due to the high activity of cellulase and lacase enzymes due to the C: N ratio in a balanced substrate composition and long fermentation time so high coarse fiber digestibility. Prawitasari *et al.* (2012) states that the lower crude fiber content in the ration causes the higher digestibility of crude fiber and otherwise. The content of crude fiber in the ration greatly affects the digestibility of crude fiber.

Maynard *et al*., (2005) stated that the digestibility of crude fiber is influenced by several factors including fiber content in feed, composition of crude fiber and microorganism activity. Poultry is difficult to digest high crude fiber because fiber-digesting microbes are only in the cecum and are few in number. Wahju (2004) states that crude fiber has bulky properties which consists of cellulose, hemicellulose and lignin which are mostly difficult to digest by poultry.

Van Soest (1985) states that the digestibility and digestion rate of hemicellulose is higher than that of cellulose, this is due to the constituent components and the abrasive components of cellulose, lignin, and silica cannot be digested by poultry, but the hemicellulose component can still be hydrolyzed by the acid content in proventiculus and gizzard.

Scott *et al*., (1982) reported that broiler can utilize energy from hemicellulose through the hydrolysis process that is in the proventiculus and gizzard or possibly digestion by microbes in the intestine to produce energy.

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The selected treatment based on its efficiency was found in the A1C2 treatment (80% cocoa pod and 20% tofu waste with 9 days fermentation time) of 53.78%.

IV. CONCLUSION

It can be concluded that the selected treatment was found in the composition of 80% cocoa pod and 20% tofu waste with 9 days fermentation time (A1C2). In this condition cellulase enzyme activity was obtained of 2.70 U / ml, lactase enzyme activity of 12.33 U / ml, crude fiber decreased of 44,01% and crude fiber digestibility of 53.78%.

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Soil Chemical Properties and Chilli (*Capsicum Frustescens L*) Yields Following Ameliorants derived from Taliwang Pond- Sidemen and Biocompost Application on the Dryland Farming System of Western Sumbawa, Indonesia

Erlina Azizah[1], Taufik Fauzi[2], Sukartono[2]

[1]Post Graduate Students of Mataram University, Mataram, Lombok, Indonesia;
[2]Faculty of Agriculture, Mataram University, Mataram, Lombok, Indonesia

***Abstract*— *A field study was conducted to evaluate the effects of Taliwang Pond sidemen (TPS) and biocompost application on the soil chemical properties, growth performance and yield of chilli on the dryland farming system in Belo village, Jereweh Sub-district, Western of Sumbawa, Indonesia from February to May 2019. The experiment was set up using a Completely Randomized Design (CRD) consisted of two factors namely sidement (0 t ha^{-1} as S0 and 40 t ha^{-1} as S1) and biocompost (0 t ha^{-1} (K1), 10 t ha^{-1}(K2), 20 t ha^{-1} (K3), 30 t ha^{-1}(K4) and 40 t ha^{-1} as K5) with three replications. The results of study showed that the application of both TPS and biocompost improved soil fertility status (C, P and K) and increased growth and yields of chilli. It was recorded that the application of both TPS and biocompost at rates of 40 t ha^{-1} resulted in the highest yields of chille as results soil fertility improvement. The highest yied was reach two times as compared to the control (plot with no soil organic ammendement).*

Keywords*— *Taliwang pond sedimen, biocompost, and Chilli yield, dryland.

I. INTRODUCTION

Cayenne pepper (Capsicum frustescens L) is a horticultural commodity that has high economic value in Indonesia. In West Sumbawa Regency (KSB), chilli production is still low so that chilli needs are still partially imported from outside the region. The low chilli production in the KSB is caused by one of the limiting factors related to the low status of soil fertility, including the low content of soil organic matter, unstable soil structure and nutrient poor soils such as N, P, and K. To overcome these problems, management efforts need to be made soil to improve and improve the quality of soil fertility through the addition of organic ameliorant.

Soil cleaners can be obtained from a variety of sources of ingredients containing organic matter, including from sedimentary materials / swamp sediment and compost swamp plant waste. Taliwang swamp sidemen has great potential to be used as soil amendment in the chilli planting system in dry land because it has a high C-organic content of 19.75%, other nutrient content such as total N-0.68%, P-available 8.26 ppm and K-swapped 4.51%, while compost from the Taliwang swamp plant contained 26.3% C-organic, N-Total 1.47%, P was available 0.90 ppm and K was swapped 1.86%. If both materials are added to the soil with low fertility status, it will naturally contribute to improving soil properties. Rosmarkam & Yuwono (2002) explains that sediments with organic matter content of ≥ 35% are classified as very high organic matter content.

The content of organic matter and other nutrients such as high phosphorus in the swamp sidemen is related to the substrate and organic materials that accumulate at the bottom of the water due to the decay of aquatic plants and the contribution of organic material derived from erosion upstream carried by the flow of rivers flowing into the swamps. Sediment is a good place to store phosphorus. Plants and animals that die will be broken down by decomposing bacteria which will then settle to the bottom of the water. Sinaga R. D., et al (2016), phosphorus-bound compounds in sediments can undergo decomposition with the help of bacteria or through the abiotic process to produce dissolved phosphorus compounds. Noor (2018) explained that plants that grow and develop in swamps have the potential to be used as a source of organic material that can improve physical fertility, chemistry and soil biology, including lotus (Nymphaea), water-nail plants (Salvinia natans), water hyacinth (water hyacinth) Eichornia crasipes), Kale (Ipomoea aquatica).

From the various potentials of Taliwang swamp sediments and plants as described above, an experiment has been carried out aimed to determine the effect of Taliwang swamp sedimentation and compost and the interaction of these two components on the chemical properties of the soil and the results of cayenne pepper.

II. MATERIALS AND METHODS

Field trials have been carried out on the dry land in Belo Village, Jereweh District, West Sumbawa Regency 8º51 'LS and 116o49' East with a height of 11 meters above sea level, during the rainy season from February to May 2019. Sidemen used were collected from the basic surface area swamp in the form of muddy solids at a depth of 1 m with a distance of ± 70 meters from the edge of the swamp. 250 kg of fresh sidemen were collected and dried until water levels were close to 14%. While composting uses biomass from aquatic plants (weeds) taken from swamps, finely chopped and dried and mixed with bran, husk and brown sugar with a 10% mixture amount of Taliwang swamp plants prepared. The material that has been mixed is watered with EM4 solution. Mixing is done slowly and evenly until the water content is ± 30-40%. The desired water content is tested by grasping the material, marked by not dripping water when the material is grasped and will bloom when the grip is released. Then the mixed material is put into a sack and the pile of material is covered with a tarp. Curing process lasts for 14 days. After the material becomes compost, the sack can be opened. This compost is characterized by its black, crumbly, non-hot, and odorless color. In such conditions, Taliwang swamp compost can be used as fertilizer. Furthermore, the Taliwang swamp sediment and compost were tested for their chemical content at the Soil Science Laboratory of the Faculty of Agriculture, University of Mataram.

The experiment was designed using a Completely Randomized Design (CRD) consisting of two factors. The first factor is the sidimen application which consists of 2 measurements, namely without sediment (S0) and sediment 40 t.ha-1 (S1). The second factor is the application of Taliwang swamp compost which consists of 5 levels, namely 0 t.ha-1 (K1), 10 t.ha-1 (K2), 20 t.ha-1 (K3), 30 t.ha-1 (K4) and 40 t.ha-1 (K5). The observed variables include variables of soil properties and agronomy. Soil properties observed both before and at the end of the experiment included pH (pH-meter), C-organic content (Walkley and Balack methods), N (Kjeldhal), available-P (Bray-1) and K-exchangeable (NH4OAc extractors) . Laboratory analysis of soil properties was carried out at the soil laboratory, Faculty of Agriculture, Unram. Agronomic measurements include the amount of flowers, fruit and dry weight of the fruit. Experimental data were analyzed by analysis of variance (Analysis of variance, ANOVA) at 5% significance level.

III. RESULTS AND DISCUSSION

1. Effects of Taliwang Swamp Plant Sediment and Compost on Land Chemical Properties

Analysis of soil properties before and after the experiment is shown in table 1. Data Table 1 shows that the administration of Taliwang swamp plant sediments and compost can improve soil properties especially the availability of P and K nutrients.

Table 1. Average soil properties before and after the application of Taliwang swamp sediment and compost

No	Treatment	Soil Chemical Properties				
		pH	C-org	Total-N	P (Bray-1)	K-dd
1	Control	6.60	0.91	0.07	92.17	3.37
2	Compost	7.64	0.82	0.11	101.61	3.98
3	Sediment	7.53	0.81	0.12	95.20	3.99
4	Sediment + Compost	7.53	1.07	0.13	116.06	4.10
5	Before the experiment	6.30	1.06	0.06	4.49	2.28

According to Handayani et, al., (2007) soil quality, cannot be separated from soil fertility status. Soil fertility is the effect of a combination of three main interacting components namely the chemical, physical and biological nature of the soil. Soil fertility in the narrow sense is the availability of plant nutrients at a certain time. The higher the availability of nutrients, the more fertile the soil will be, and vice versa.

Table 1. shows that the soil before the experiment had a low nutrient content, but after the application of sediment and compost there was an increase especially in the nutrient content of P and K.

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While at the end of the experiment the content of C and N slightly increased. In composted plots, the N content which was originally 0.06% increased to 0.11%. In the treatment sediment treatment compartment, the total N increased to 0.12% while in the plot receiving sediment material and compost, the total N reached 0.13%. The increase in N nutrients is not too high, it is suspected because N is mobile in the soil and has been absorbed by many vegetative plant formation. In accordance with what Munawar stated (2011) that the element N is needed in large amounts for the entire vegetative growth process.

The increase in available P reached 101.61 ppm on the use of compost from the initial soil which originally had a P-available content of 4.49 ppm. With the use of sediment increased to 95.20 ppm and with the combination of available sediment and compost P increased to 116.06 ppm.K-exchange increased by 3.98% in the use of compost from the initial soil which originally contained K exchanged by 2.28%, with the use of K sediment exchanged to 3.99% and by combining sediment and compost Taliwang swamp plant K-swapped to 4.10%.

Organic C decreased initially containing 1.06% by giving compost to 0.82%, by giving sediment to 0.81% and by giving sediment and compost to 1.07%. This is thought to be due to uptake by plants and activation of soil microorganisms in increasing nutrients and regulating substances grow. The effect of organic matter on soil biology is to increase microbial activity in the soil and from the results of this microbial activity will be released various growth regulators such as auxin and vitamins that will have a positive impact on plant growth (Sutanto, 2002). Despite a decline in organic C, the provision of organic matter in the soil must be carried out to maintain and increase land productivity. Nikmah (2010) states that continuous planting can deplete soil organic matter. Therefore, the addition of organic material must still be done every time planting to overcome soil degradation (decreased fertility) soil). The provision of organic fertilizer in addition to increasing soil physical fertility, can also increase the availability of nutrients (P and K) for plants. Sutanto (2002) revealed that the addition of organic matter to the soil can increase the availability of nutrients in the soil and be able to improve plant growth and increase soil moisture content.

The results of the diversity analysis showed that the sediment had a significant effect on organic C, total N, available P and K nutrients, compost had no significant effect on the supply of organic C but had a significant effect on the supply of nutrients N, P, K and interactions between sediments occurred and compost in P and K. nutrient elements Analysis of variance (anova) and further tests of Taliwang swamp sediment and compost treatment of organic C, N-total, P-available and K-exchanged C elements can be seen in table 2.

Table 2. Analysis of variance (ANOVA) and further tests of sediment and compost treatment of organic C, N-total, P-available and K-exchanged elements

Treatment	Observation variable C- organic (%)	N-Total (%)	P- available (ppm)	K- swapped (%)
S0	0.84b	0.10b	99.73b	3.89b
S1	1.02a	0.13a	111.89a	4.08a
BNJ	0.15	0.01	3.39	0.46
K0	0.86a	0.09b	93.68c	3.73c
K1	0.93a	0.12ab	105.77b	3.73c
K2	0.96a	0.11ab	106.84b	3.96b
K3	0.89a	0.12ab	109.12ab	4.21a
K4	0.99a	0.13a	114.99a	4.28a
BNJ	0.34	0.03	7.70	0.29

Note: Numbers followed by different letter notation in the same column and row are significantly different based on the tukey test at the 5% level.

Provision of 40 t/ha of sediment on land can increase organic C to 1.02% compared to without sediment only containing 0.84%. Provision of 40 t/ha of sediment gives a significant effect on increasing total N by 0.13% while without administration of total N sediment by 0.10%. Sediment has a significant effect on P. nutrient availability. By administering 40 t/ha the sediment will increase 111.89 ppm P nutrient higher than without the administration of sediment 99.73 ppm. Whereas the exchanged K increased to 4.11% compared without the provision of sediment which had an exchangeable K content of 3.89%.

Compost has a significant effect on N-total, P-available and K-exchanged nutrient content, but does not have a significantly different effect on organic C. N-total increased at 40 t/ha to 0.13% compost, available P increased to 114.99 ppm at 40 t/ha, while exchanged K increased by 4.28% at 40 t/ha Taliwang swamp compost.

2. Interaction of Sediment and Compost Giving of Taliwang Swamp Plant to Soil Nutrients (C, N, P and K)

The interaction of sediment and compost treatment occurs significantly in the availability of P and K nutrients for plants. Analysis of the interaction of sediment and composter treatment on the P-available and K-exchanged elements can be seen in Table 3.

Table 3. Interactions of Taliwang's swampy sediment and compost treatment on P-available elements

P available	Compost s (C)					
	K0	K1	K2	K3	K4	BNJ P-tersedia
Sediment (S)	(without compost)	(10 ton/ha)	(20 ton/ha)	(30 ton/ha)	(40 ton/ha)	
S0	92.17d	99.67cd	99.35d	104.18bcd	104.70bcd	12.88
S1	95.20d	110.56bc	114.33ab	114.66ab	125.29a	
K was switched	Compost s (C)					
	K0	K1	K2	K3	K4	BNJ
Sediment (S)	(tanpa kompos)	(10 ton/ha)	(20 ton/ha)	(30 ton/ha)	(40 ton/ha)	K-tertukar
S0	3.47d	3.62cd	3.83bcd	4.27a	4.24a	0.46
S1	3.99abc	3.99abcd	4.09bc	4.15cd	4.31a	

Note: Numbers followed by different letter notation in the same column and row are significantly different based on the tukey test at the 5% level.

The results of variance showed that the highest increase in P and K nutrients occurred in S1K4 treatment. P is available at 125.29 ppm S1K4 treatments, far higher than controls and other treatments. K exchange in S1K4 increased to 4.31% compared to control (S0K0) and other treatments. In general, P-available and exchanged K developed well when the administration of Taliwang swamp sediment and compost was increased

.

3. The effect of the provision of sediment and Taliwang Swamp Plant Compost on the Result of Chili

Interest Amount

Sutrisna and Yanto (2014) state that the presence of sufficient N, guarantees good growth, higher yields and fully developed fruit. The element P influences a lot on flowering and its development, fruit hardness, fruit color, vitamin content and accelerates fruit maturation. Element K influences the increase in sugar content, vitamin content, total acid content and increases the amount of fruit harvested

Table 4. Factors for the treatment of sediment and compost as well as further testing of the average number of cayenne pepper plants at the age of 56hst to 98 hst

Treatment	Amount of interest			
	56 hst	70 hst	84 hst	98 hst
S0	11.72b	14.09b	38.67b	45.73b
S1	28.88a	38.81a	90.67a	98.07a
BNJ	7.10	8.64	36.06	27.07
K0	11.93b	13.67b	48.26b	46.77d
K1	16.24ab	24.18ab	54.07b	60.35cd
K2	20.90ab	26.39ab	64.58b	71.86bc
K3	23.93ab	33.21ab	68.34ab	85.66ab
K4	28.50a	34.66a	88.80a	94.88a
BNJ	16.18	19.69	21.96	16.14

Note: Numbers followed by different letter notation in the same column and row are significantly different based on the tukey test at the 5% level.

Table 4 shows that the treatment of sediment and compost has a significant influence on the flowering of cayenne pepper plants. This can be seen in the results of the ANOVA variations at each observation interval. The best increase in the amount of flowers occurred in the provision of sediment 40 t/ha with an average number of flowers reaching 32.55 flowers at 56 days after planting, 39.23 flowers at 70 days after planting, 83 flowers at 84 days after planting and 104 flowers at 98 days after planting, whereas without sediment administration the average number of flowers at 56 days after birth is 7.06 flowers, 11.26 flowers at 70 days, 33.30 flowers at 84 days and at 98 days after only reaching 47.7 flowers.

In compost treatment, the highest number of flowers occurred in the provision of compost of 40 t/ha at the age of 98 days as many as 94.88 pieces, while without the provision of compost that was 46.77 fruit per planted flowers. This is presumably due to the role of P and K nutrients available through the provision of Taliwang swamp compost. The availability of these nutrients can increase the growth and productivity of chili plants (Alhrout, 2017). Between the two treatments there was no significant interaction seen on the number of flowers observed.

Number of Fruits

The number of fruits per plant increased with increasing doses of Taliwang swamp sediment and compost as shown in table 5.

Table 5. Factors for the treatment of sediment and compost as well as further testing of the number of cayenne pepper at the age of 56 hst to 98 hst

Treatment	Amount of fruit			
	56 hst	70 hst	84 hst	98 hst
S0	0.38b	4.90b	8.81b	30.08b
S1	0.52a	15.4a	25.47a	60.49a
BNJ	0.09	11.66	10.44	25.51
K0	0.170c	5.854b	7.54c	25.62c
K1	0.33bc	7.48b	12.69bc	36.88bc
K2	0.45b	10.67ab	15.71b	38.75bc
K3	0.53b	11.48ab	23.99a	57.83ab
K4	0.78a	15.33a	25.75a	67.36a
BNJ	0.09	6.98	6.25	25.51

Note: Numbers followed by different letter notation in the same column and row are significantly different based on the tukey test at the 5% level.

Table 5 shows that sediment has a significant effect on increasing the number of fruits at each observation interval. At the age of 98 days without the provision of sediment the number of fruits produced was only 30.08 fruit crops, but by giving sediment the average number of fruits could reach 60.49 fruit crops. As with compost sediment, it also affects the increase in the number of cayenne pepper. At the age of 98 days after giving 40 t / ha of cayenne compost can produce 67.36 fruits per crop, whereas without compost the number of fruits is only 25.62.

The addition of fruit is thought to be due to the sedimentation and compost of Taliwang swamp plant which is able to activate soil microorganisms in increasing N, P and K nutrients. These elements have their respective roles in sustaining plant growth. Nitrogen is a constituent of plants that determines the quality of plant organic matter. Nitrogen is present in a variety of plant protein compounds, nucleic acids, hormones, chlorophyll and a number of primary and secondary metabolite compounds. Nitrogen is also essential for cell division, cell enlargement, and for growth (Gardner et al., 1991). Phosphate compounds in plants act as energy dealers and energy storage needed for growth and reproductive processes (Salisbury and Ross, 1995). Potassium serves as a transportation medium that carries nutrients from the roots including P nutrients to the leaves and translocates assimilates from the leaves to the entire plant tissue. According to Lingga and Marsono (2001) the main function of potassium (K) is to help the formation of protein and carbohydrates. Potassium also plays a role in strengthening the body of the plant so that leaves and fruit do not fall easily. So that with the availability of nutrients N, P, and K plant growth for the better. Plant growth is good then the production will also be good.

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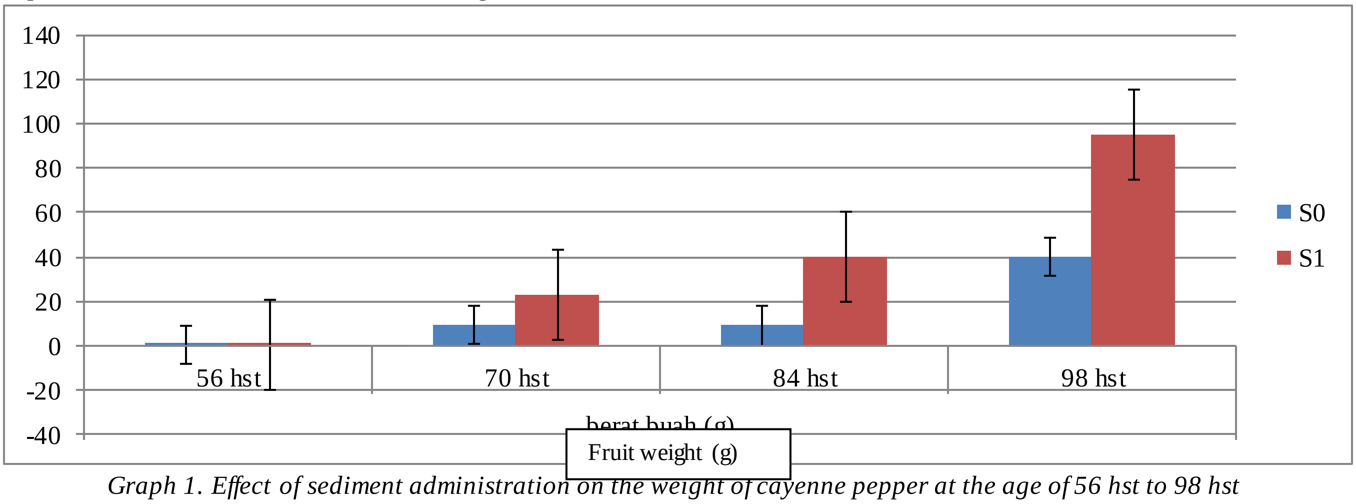
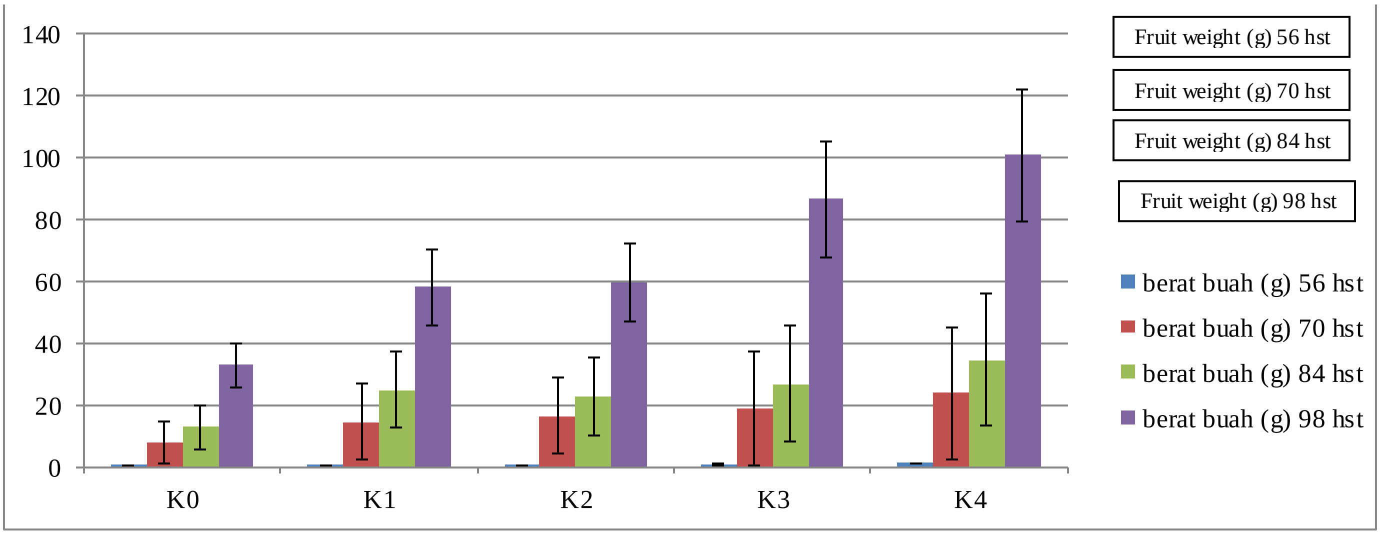
Fruit Weight

Treatment of sediment and compost and the interaction between the two treatments gave a significant effect on the weight of chillies at all observation intervals. The increase in the weight of cayenne is thought to be because sediment and compost are able to provide a large amount of P and K nutrients. These elements are very important for fruit formation and fruit weight. Primanto (1998) states that in the generative period plants need a lot of nutrients to produce energy for plants, namely phosphorus and potassium. Energy is needed by plants in forming flowers, fruit and other growth processes. The effect of sediment and compost on the weight of cayenne pepper at the age of 56 hst up to 98 hst can be seen in graphs 1 and 2



Graph 1. Effect of sediment administration on the weight of cayenne pepper at the age of 56 hst to 98 hst

Graph 1. shows that giving 40 t/ha of sediment can increase the average fruit weight of cayenne pepper. The effect of sediment is greater on the average fruit weight when cayenne peppers reach adulthood. At the age of 56 HST, the weight of cayenne pepper did not show a significant difference between sediment and those without sediment. Weight of fruit at the age of 56 hst 0.7 g in plants that received sediment and 0.46 g in plants that were not given sediment. Significant improvement began to be seen in the observation of 70 days to 98 days. At the age of 98 days the weight of the fruit without the administration of sediment was only 39.87 g, but with the administration of 40 t/ha of sediment, the average fruit weight could reach 94.93 g or 2 times heavier compared to plants without sediment administration.



Graph 2. The effect of compost on the weight of cayenne pepper at the age of 56 to 98 hst

Graph 2. Shows that compost treatment has a significant effect on average fruit weight at all observation intervals. The higher the amount of Taliwang swamp compost, the higher the weight of cayenne pepper.

From observations of 56 DAT to 98 DAT, 40 t / ha of compost had the highest fruit weight, namely 1 g at 56 DAT, 23.8 g at 70 DAT, 34.41 g at 84 DAT and 100.61 D at 98 DAT while plants without compost has the lowest weight at each observation interval which is 0.32 g at 56 days after planting, 7.89 g at 70 days after planting, 12.67 g at 84 days after planting and 32 g at 98 hst after planting.

An interaction between sediment and compost treatment occurred on the weight of cayenne pepper as shown in table 6.

Table 6. Interaction Factors of sediment and compost treatment on the weight of cayenne pepper

56 hst	Kompos (K)					
	K0	K1	K2	K3	K4	BNJ
Sediment (S)	(without compost)	(10 ton/ha)	(20 ton/ha)	(30 ton/ha)	(40 ton/ha)	
S0	15.09c	39.91bc	43.08bc	47.79bc	53.50bc	47.51
S1	50.29bc	75.85b	75.85b	124.97a	147.72a	

Note: Numbers followed by different letter notation in the same column and row are significantly different based on the tukey test at the 5% level.

Table 6 shows that sediments and compost are able to increase the weight of crop fruit. Provision of sediment and compost with increasing amounts helps plants produce fruit with a higher weight. This is thought to be due to the P and K nutrients produced through the provision of Taliwang swamp sediment and compost. P and K are very important for fruit formation in accordance with the report of Dewi (2016) which states that the presence of phosphate elements in plants can increase fruit yields. After the fruit is formed phosphate plays a role in the weight of the fruit to form proteins, minerals and carbohydrates in the fruit. Fruit weight is an indication of the results of photosynthesis stored in fruit flesh and other constituent parts of the fruit (Novizan, 2002). The percentage of flower to fruit is also influenced by nutrient Potassium. As stated by Lingga & Marsono (2001) that Potassium plays a role in strengthening the body of the plant so that leaves, flowers and fruit do not fall easily.

IV. CONCLUSION

Based on the results and discussion described above, the following conclusions can be drawn:

1) The application of Taliwang Sediment and compost for the Swamp plant does not have a significant effect on the supply of organic N and C, but it is very influential on increasing P and K nutrients.
2) Taliwang swamp sediment and compost have a significant effect on increasing the number of flowers, fruit and weight of cayenne pepper
3) Provision of 40 t / ha of sediment and 40 t / ha of Taliwang swamp compost can increase the number of flowers, fruit and weight of cayenne fruit up to 2 times compared without the provision of Taliwang swamp sediment and compost.

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Investigating local climatic factors that affected pineapple production, in Lampung Indonesia

Tumiar Katarina Manik[1*], Purba Sanjaya[1], Onny Chrisna Pandu Perdana[2], Dudy Arfian[3]

[1]Department of Agronomy, Faculty of Agriculture, Lampung University, Indonesia;
[2]Department of Seed Science, Lampung Polytechnique, Indonesia
[3]Great Giant Pineapple Company, Lampung, Indonesia
*Corresponding authors: katarina.manik@gmail.com

***Abstract*— In Indonesia, pineapple is one horticulture commodity which has high potential in international fruits trade, therefore the plantation should maintain its high yield and good fruit quality. One cause that determined pineapple yield was water availability even though pineapple could resist dry period. Irrigation is always needed in pineapple plantation, however, this is a costly treatment and high irrigation level has not always lead to significant increases in crop productivity. This study aimed to investigate the possible factors that determined pineapple productions in Indonesia biggest pineapple plantation in Indonesia using all climate factors available. Some statistics methods were used to utilize the available climate data to analyze the rainfall probability, rainfall frequency distribution, evapotranspiration estimation, water balance, water use efficiency and weather impacts on fruit qualities. The results showed that water from average annual rainfall should be adequate for the pineapple water needs, however there were months had water deficit and needed irrigation. Low evapotranspiration rate reflected dry soil which could be the results of high air and soil temperature. This high temperature also affected on fruit qualities. It is suggested to conduct researches on how pineapple productions and qualities that plants under shading trees.*

***Keywords*— pineapple production, rainfall probability, rainfall frequency, evapotranspiration, water use.**

I. INTRODUCTION

Pineapple [*Ananas comosus* (L.) Merr.] is the third most important tropical fruit by value after banana and citrus (Carr, 2012). The Pineapple are commercially grown in warm and humid climate, over a wide range of latitudes from 30° N in the Northern Hemisphere to 33°58′ S in the Southern Hemisphere. Pineapple grows well in tropical and subtropical climate ranging from mild coastal climate up to an altitude of about 1000 meters provided the area is free from frost. Pineapple is grown mainly for fresh, canned fruits and juice; also, it is the only source of bromelain and enzyme used in pharmaceuticals (Cahyono, Astuti and Rahmat, 2016).

Five leading pineapple producing countries are: Costa Rica, Brazil, Philippines, Thailand and Indonesia (Hossain, 2016) . World production of pineapple reached 19 million tonnes in 2008 with the industry dominated by Brazil followed by Thailand, the Philippines and Indonesia (Dhungel, Bhattarai and Midmore, 2012). One statistic report posted that leading countries in pineapple production worldwide in 2017 as in Figure 1. In Indonesia, pineapple is one horticulture commodity which has high potential in international fruits trade. Great Giant Agri-Group is an integrated plantation located in Lampung, Indonesia, operated in 32,000 Ha area and planted in large part with pineapple but also banana, cassava, guava and other fruits. This plantation company now is the third world largest pineapple producer with more than 600,000 MT fruit annually (Loekito, 2018).

Based on that good production, the climatic conditions of Indonesia must be favorable for pineapple crop. Pineapple most favourable temperature to grow is between 18 and 35°C and optimal development and better fruit quality is achieved with ambient temperature between 22 and 32°C, thermal amplitude between day and night of 8 to 14°C and relative humidity higher than 70% (Dhungel, Bhattarai and Midmore, 2012). Pineapple is generally produced under a wide rainfall range of 600 mm to more than 3500 mm annually (Zhang et al., 2016) which Indonesia rainfall could provide. However, the use of irrigation is essentially needed to guarantee and optimize the agricultural production due to its climatic restriction in terms of spatial and temporal variability of rainfall.

Indonesia receives significant rainfall year-round but experiences a wet season that peaks in January and a dry season that peaks in August. Dry season rainfall anomalies are spatially coherent, strongly correlated with local sea surface temperature, and tightly coupled to El Nino–Southern Oscillation (ENSO) variations in the Pacific basin (Hendon, 2003). Rainfall irregularity produces a delay in some phenological stages of the pineapple plants resulting in reduction of the fruits production, therefore, the actual crop water requirements needs to be investigated in more detail so that proper irrigation could be incorporated to its production system, especially since irrigation system for a big company is costly and high irrigation level has not always lead to significant increases in crop productivity (de Azevedo et al., 2007).

One of the main features of pineapple is its adaptation to areas of low rainfall. It differs from most other commercial crops in that it has a photosynthetic adaptation (crassulacean acid metabolism (CAM)) that facilitates the uptake of carbon dioxide (CO2) at night. This dramatically improves its water-use efficiency when it is grown under dry conditions. But in the Great Giant Agri-Group company, there has been a decrease in productivity and fruit quality, resulting in small fruits or large fruits with small-sized crowns or with a conical shape. This is disadvantageous because the crown is one of the main pineapple planting materials. It is always the goal to obtain the best fruit quality with vigorous crowns (Suwandi, Dewi and Cahyono, 2016).

One factor that strongly affected pineapple cultivation must be the local climate, for example, cultivation of pineapple in regions with high levels of solar radiation can burn the fruit, giving rise to a loss in yield or an increase in production costs (Custodio, 2016). *Penicillium funiculosum* infection is favoured by cool temperatures (16–20^{o}C) while *Chalara paradoxa* are produced under conditions of high humidity and can be dispersed by wind (Joy and Sindhu, 2012).

Based on the above reasons this study aimed to investigate some local climatic factors that affect the pineapple fruits production.

II. MATERIALS AND METHODS

The data came from the The Great Giant Agri-Group research station located in Terbangi Besar, Lampung, Indonesia (4◦ 49'15.5" S and 105◦ 15'27.4" E, 46 m asl). The texture of the soil is sandy clay with particle sizes of 52.4% sand, 2.6% silt and 45.0% clay.

2.1. Data availability:

1. Monthly rainfall 1981-2016
2. Daily maximum and minimum air temperature, humidity, wind speed and radiation 2007-2015 missing the 2013 for calculating daily evapotranspiration
3. Monthly water use from sprinkle irrigation in 2015
4. Fruit maturity (%) and plant diseases infection (scoring) and related weather record (maximum and minimum tempareture, radiation and rainfall) 2008 -2013
5. Soil temperature 2016

2.2. Data analysis

2.2.1. Rainfall analysis

2.2.1.1. The 75 % probability of exceedence

The first step in the frequency analysis is to rank all the rainfall data. After the rainfall data are ranked, a serial rank number (r) ranging from 1 to n (number of observations) was assigned. Subsequently the probability have to be determined that should be assigned to each of the rainfall depths. If the data are ranked in descending order, the highest value first and the lowest value last, the probability is an estimate of the probability that the corresponding rainfall depth will be exceeded. When data are ranked from the lowest to the highest value, the probability refers to the probability of non-exceedance. Hence the probabilities are estimates of cumulative probabilities. They were formed by summing the probabilities of occurrence of all events greater then (probability of exceedance) or less than (probability of non-exceedance) some given rainfall depth. Finally, the probabilities of exceedance have to be estimated by a chosen method which was $\left(\frac{r}{n} * 100\right)$ and in this study the 75% probability was chosen (Dirk, 2013). The results was presented in Table 1.

2.2.1.2. Normal Rainfall

Normal rainfall is an average of the precipitation values over a 30-year period. Rainfall may very often be either well above or well below the seasonal average, or "normal.".

2.2.1.3. Rainfall Frequency Distribution

A frequency distribution is an overview of all distinct values in some variable and the number of times they occur. This method was used to analyze number of certain rainfall range occured in the study area (Dirk, 2013). The frequency distribution and the histogram could be done using Excel (Table 2).

2.2.2 Evapotranspiration estimation

The CROPWAT model was developed by the Department of Land and Water Resources of FAO. CROPWAT 8.0 for Windows is a computer program for

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the calculation of crop water demand/requirements and irrigation demand/requirements based on soil, climate and crop data and using Penman-Monteith equation to estimate evapotranspiration. The input data used for calculating potential evapotranspiration are: minimum temperature (°C), maximum temperature (°C), sunshine hours (hrs), wind speed (km/day), relative humidity (%) and latitude, longitude and altitude of the study area (Clarke, 1998) (Table 3).

2.2.3. Water balance

The water balance was conducted following Thornthwaite (1957) method; and the components and steps were as below:

P = Rainfal counting with 70% probability of excedence; PET = Evapotranspirastion calculated using CROPWAT; P-PET= Difference between P and PET; APWL= Accumulation Potential Water Loss = 0, if P > PET and if P < PET (negative) then APWL on the previous month was added with the next month negative P-PET ; SWC = Soil water content= PWP + [{ (1.00041-(1.07381/(FC-PWP)}^|(APWL)|*(FC-PWP)}; AWC = Accumulation water content= SWC of a month subtracted to SWC of precious month ;AET = Actual evapotranspiration; if P> PET then AET=PET; if P < PET then AET= P + |AWC|; Finally, water surplus = P- AET-AWC and Water deficiency = AET-PET

The field capacity and welting point of the study area were measured at the soil laboratory and the results were: field capacity was 90.2 mm and permanent welting point was 67.7 mm. The result was shown in Table 4.

2.2.4. Irrigation amount in general and water use eficiency

Irrigation was applied in turn on different block area (ha); to estimate the irrigation amount in general for each month, the average irrigation and the frequent irrigation amount that applied per day on vertain month was calculated; the results was shown in Table 5.

2.2.5 The main weather factor for fruit quality

Multiple regression and correlation were statistic method used to investigate weather impact on each of the fruit qualities. This calculation is available on Excel spreadsheet.

III. RESULTS AND DISCUSSION

3.1 Rainfall analysis

The rainfall 75% probability of exceedence in Table 1 showed that from May to October rainfall that could be expected with 75% probability was under 100 mm. Pineapples can grow well under a wide range of rainfall from mean annual precipitation (MAP) 600 - 1 200 mm. However, irrigation is necessary when MAP is < 500 mm or when consecutive months of low rainfall occur (Schulze and Maharaj, 2017). Mean annual precipitation in the study area was 2021.9 mm; should be enough for pineapples growth, however, the rain did not fall evenly while pineapples is planted all year. From that rainfall data it could be predicted that irrigation would be needed from May to October.

Rainfall frequency distribution and the histogram were presented in Table 2 and Figure 2.

Roughly, 84% of rainfall felt under 400 mm/month; and the most frequent monthly rainfall was between 50 to 100 mm. In general, eventhough the total amount of rainfall was adequate for supporting pineapples growth, irrigation is still needed in this plantation.

Table.1: Rainfall 75% probability of exceedence and normal value from rainfall data 1981-2016

Months	75 % probability (mm)	Normal (mm)	Months	75 % probability (mm)	Normal (mm)
January	256.0	378.9	July	35.0	85.0
February	219.5	333.3	August	2.0	67.1
March	298.0	388.4	September	6.5	72.5
April	150.0	211.4	October	45.0	104.9
May	87.5	143.3	November	143.5	195.0
June	40.0	82.9	December	236.0	347.6

Table 2. Monthly rainfall frequency distribution (1982-2016)

Rainfall Bin (mm)	Frequency	Rainfall Bin (mm)	Frequency	Rainfall Bin (mm)	Frequency	Rainfall Bin (mm)	Frequency
0	13	250	46	450	12	700	1
50	49	300	35	500	15	750	1
100	51	350	31	550	8	800	0
150	48	400	28	600	5	More	0
200	46			650	7		

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3.2 Evapotranspiration estimation

The estimated daily evapotranspirastion for each month in the year 2008-2015 was presented in Table 3.

Table 3. Daily evapotranspiration for each month on period 2007-2015.

Months	Year							
	2007	2008	2009	2010	2011	2012	2014	2015
January	3.48	3.53	3.51	3.53	3.18	3.63	3.54	4.10
February	2.97	2.73	2.86	3.58	3.00	3.72	3.79	3.88
March	3.79	3.23	3.81	4.05	3.26	4.05	4.06	4.42
April	3.46	3.41	3.67	3.91	3.30	4.34	4.34	4.04
May	3.60	3.35	3.63	3.13	3.40	4.16	3.85	4.16
June	3.07	3.18	3.29	2.65	3.12	3.90	3.51	4.11
July	3.24	3.72	2.83	3.20	3.35	4.08	3.70	4.41
August	3.86	3.53	3.81	3.15	4.10	4.47	4.45	4.70
September	3.92	3.79	4.11	3.16	4.12	4.90	4.86	
October	4.02	3.88	3.64	3.08	3.48	4.28	4.54	
November	3.60	3.17	3.29	3.12	3.48	3.60	3.98	
December	3.17	2.97	3.49	3.17	3.19	3.22	3.37	

Crop water requirements (ETm) for high pineapple production are very different from those of most other crops. Because of crassulacean acid metabolism (CAM), pineapple is adapted to dry environments by suspended transpiration during the day (Dhungel, Bhattarai and Midmore (2012) and Carr (2012). As a result, maximum evapotranspiration is low and varies between 700 and 1000 mm per year. Data above showed that daily mean reference evapotranspiration was about 3.42 mm or about 1249 mm/year, while from the data above mean annual precipitation in the study area was 2,446.5 mm. The results was lower that that in Brazil which crop evapotranspiration was (ETc = 4.6 ± 0.5mm day-1) and reference evapotranspiration was (ETo = 5.1 ± 0.4mm day-1) (de Azevedo et al., 2007)

Carr (2012) also mentioned that over the monitored 341-day period in Brazil the potential
evapotranspiration rate (ETc) was relatively constant 4.1 ± 0.6 mm per day and ETc totalled 1420 mm and ETo 1615 mm. For the crop coefficient Kc (=ETc/ETo), in the FAO crop evapotranspiration manual, Allen et al [16] specified the following Kc values for pineapple: the initial stage, Kc = 0.50; mid-season, Kc = 0.30; end-season, Kc = 0.30 (all values assume that 50% of the ground surface is covered with black plastic mulch, as practised in Hawaii). However, for well-watered pineapple crops Kc has maximum values of 0.8–0.9. Indeed, (Souza and Reinhardt, 2007) even suggested that, for a crop with 100% ground cover, Kc = 1.0–1.2, which would appear to be excessive. Assumed this study using the Kc suggested by Carr (2012) then the crop water requirement would be about 1124 mm/year which was supplied adequately from the rainfall (2,446.5 mm/year). Again, this area should not lack of water for the crops growth if it distributed evenly through the year and if 100% of the rainfall would be utilized by crops.

The root system of pineapple is shallow and sparse. In deep soils, maximum root depth may extend up to 1m but roots are generally concentrated in the first 0.3 to 0.6 m, from which normally 100 percent of the water is extracted (D = 0.3-0.6 m). Under conditions when assumed the maximum evapotranspiration was reached (5 to 6 mm/day), water uptake started to be reduced when about 50 percent of the available soil water has been depleted
(p = 0.5) (Steduto et al., 2012).

3.3 Water Balance

Based on water balance analysis (Table 4), months with surplus water were January to April and November to Desember; and months with deficit water were May to October except July. Cahyono, Astuti and Rahmat (2016) calculated the water balance at the same location using data from 30 years found similar results that deficit moths were June to October. Related to those results, it was obvious that irrigation was still a need if crops were planted on those deficit months.

Pineapple can survive long dry periods through its ability to retain water in the leaves which is used during these periods. However, the crop is sensitive to water deficit, especially during the vegetative growth period, when the size and fruiting characteristics are determined. Water deficits retard growth, flowering and fruiting. Water

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supply during this period should meet full water requirements of the crop (Dhungel, Bhattarai and Midmore, 2012).

Water deficit at flowering has a less serious effect and may even hasten fruiting and result in uniform ripening. An ample water supply at flowering will lead to vigorous stem growth and a large core which is disadvantageous when the fruit is used for canning. Frequent irrigation or rain at the time of harvest may cause deterioration of the quality of the fruit and make the crop susceptible to the fungus causing heart rot. In addition, waterlogging affects fruit quality. Where water supply is limited, mulching is practised to reduce soil evaporation and soil temperature. Dew has been found to contribute to meeting the wafer requirements of the crop (de Azevedo et al., 2007).

3. 4 Irrigation amount in general during deficit months and water use eficiency

From the standard evapotranspiration it could be assumed that crop water requirement was about 3.42 mm/day. Table 5 showed that the irrigation application was satisfied the crop water requirement. Total area irrigated from May to November 2015 was 42,103.7 ha and total irrigation was 4,235.8 m^3/ha. On one study in Queensland, Australia irrigation input during the crop period for the oxydation and control treatments was 2,524 and 2,405 m^3/ha respectively (Dhungel, Bhattarai and Midmore, 2012) ; the irrigation in the study area was higher than that at the Queensland area, could be because of lower precipitation (mean annual precipitation in the study area was 2,021.9 mm) compared to 4,250 mm in Queensland. A study of water consumption for some crops including pineapple in Thailand gave the results as follow mean annual rainfall was 832 mm and irrigation was 5,402 m^3/ha (Gheewala, 2014).

Table 5. Average Irrigation and amount of irrigation frequently applied

Month	Total area (ha)	Average irrigation (mm/day)	Most frequent irrigation (mm/day)	Month	Total area (ha)	Average irrigation (mm/day)	Most frequent irrigation (mm/day)
May	1316.95	4.36	2.95	September	8032.24	19.47	13.80
June	5489.50	15.52	11.92	October	7531.39	25.03	15.98
July	6666.81	221.53	169.16	November	5282.02	116.85	48.80
August	7784.79	20.82	11.67				

Average yield in 2015 was 62.44 ton/ha and total irrigation on that period was 4,235.8 m^3/ha; then it can be concluded that irrigation water requirement was 67.83 m^3/ton. The result was in good comparison with the irrigation water requirement for pineapple plantation in Thailand 135-326 m^3/ton in dry season and 6-67 m^3/ton in wet season (Cahyono, Astuti and Rahmat, 2016).

3.5 Efect of weather factors to fruit quality

Besides water other weather/climate factors were also important in crops production especialy the fruit quality. Statistic correlations between fruit maturity and some weather factors were shown in Table 6; and for diseases infections were in Table 7.

Table 6. Statistic correlations between fruit maturity and weather factors

	Column 1	*Column 2*	*Column 3*	*Column 4*	*Column 5*
Column 1	1				
Column 2	0.292475	1			
Column 3	-0.17027	-0.10144	1		
Column 4	0.147971	0.526875	-0.30591	1	
Column 5	-0.24557	-0.61235	0.168391	-0.55934	1

Column 1: fruit maturity; column 2: maximum air temperature; column 3: minimum air temperature; column 4: radiation intesity and column 5: rainfall.

Table 7. Statistic correlations between diseases attack and weather factors

	Column 1	*Column 2*	*Column 3*	*Column 4*	*Column 5*
Column 1	1				
Column 2	0.337658	1			
Column 3	0.123746	-0.10144	1		
Column 4	-0.18676	0.526875	-0.30591	1	
Column 5	-0.15571	-0.61235	0.168391	-0.55934	1

Column 1: diseases attack; column 2: maximum air temperature; column 3: minimum air temperature; column 4: radiation intesity and column 5: rainfall.

For the fruit maturity, the multiple regression was 0.34 and regression was (r^2) = 0.11

The equation was Y= 49.55+ 1.240 tmax – 1.44 tmin - 0.03 radiation- 0.004 rainfall; while for diseases infections the multiple regression was 0.55488 and regression was (r^2) = 0.307891. The equation was Y= -35.88 +1.271 tmax +0.168 tmin -0.082 radiation-0.002 rainfall

In both quality factors air temperature especially the maximum temperature played important roles. In general this study site had maximum temperature 31.89 °C and minimum temperature 23.57°C; good fruit quality for pineapple is attributed to growing sites having a combination of relatively cool night temperatures, sunny days and day temperatures ranging from 21 to 29.5°C but not exceeding 32°C (Hossain, 2016). Similar to that, it is noted that optimal development and better fruit quality is achieved with ambient temperature between 22 and 32°C, thermal amplitude between day and night of 8 to 14°C and relative humidity higher than 70% (Dhungel, Bhattarai and Midmore, 2012).

Key issue concerning the cultivation of pineapple in regions with high levels of solar radiation is it could burn the fruit, giving rise to a loss in yield or an increase in production costs (by up to 11.7%) since the crop has to be protected through the provision of artificial shade. Under shade conditions, the growth and survival of a plant is closely associated with its ability to intercept light efficiently. Some species exhibit phenotypic plasticity, in that they could modify their shape and structure in response to changes in luminosity to improve photosynthetic efficiency (da Silva et al., 2017).

High temperature in the study area was also reflected on the soil temperature as presented in Table 8 (data from the year 2016), As stated above, pineapple roots are generally concentrated in the first 0.3 to 0.6 m, from which normally 100 percent of the water is extracted, on this depth the soil temperature could reach a range of 33.9 to 37.3°C. In contrast, Table 2 showed that daily mean reference evapotranspiration was about 3.42 mm much lower than that in Brazil which the reference evapotranspiration was (ETo = 5.1 ± 0.4mm day $^{-1}$) (Allen, 1998). Lower evapotranspiration indicated that the soil was dry -water was not available to be evaporated. It could also indicated that even water from rainfall and irrigation was not adequate to prevent the soil from being dry by sun radiation. Therefore, to maintain good yield and good fruit quality, from agroclimate views, irrigation should be intensified especially during months with water deficit. Regarding global warming, high air and soil temperature and probably decreasing rainfall trend could be negatively impacted pineapple production in the future. Farmers in Nsawam Adoagyiri district, Ghana considered supplementary irrigation especially during the dry months of the growing periods where rainfall is generally low (Williams et al., 2017)

Table 8. Soil temperature (°C) for morning, noon and afternoon in different depth.

Months	07.00				13.00				17.00			
Depth (cm)	0	5	50	100	0	5	50	100	0	5	50	100
January	27.7	27.3	30.3	30.1	34.5	33.5	30.2	30.1	31.8	31.9	30.3	30.2
February	27.2	27.1	29.7	29.8	33.3	32.6	29.8	29.8	31.8	31.6	29.7	29.8
March	27.9	27.9	30.2	30.1	33.5	33.8	30.2	30.1	31.1	31.5	30.1	30.1
April	27.2	27.2	29.9	30.1	34.2	34.0	30.0	30.1	31.6	32.1	30.0	30.1
May	27.4	27.3	30.2	30.1	33.7	34.1	30.2	30.2	31.6	32.3	30.2	30.1
June	26.5	26.4	29.4	29.5	33.3	33.4	29.4	29.5	31.2	31.9	29.4	29.5
July	26.4	26.5	29.2	29.3	34.5	33.8	29.2	29.3	31.9	31.8	29.2	29.3
August	26.8	26.5	29.3	29.2	37.1	34.9	29.3	29.2	33.9	33.5	29.3	29.2
September	27.1	27.1	29.8	29.7	37.3	35.2	29.8	29.7	33.6	33.7	29.8	29.7
October	27.0	26.9	29.2	29.3	32.7	31.9	29.2	29.3	30.8	30.8	29.2	29.3
November	27.4	27.3	29.4	29.4	33.3	31.8	29.4	29.4	31.3	31.0	29.4	29.4
December	27.7	27.6	29.8	29.4	33.7	32.7	29.7	29.4	32.2	31.7	29.7	29.4
Maximum	**27.9**	**27.9**	**30.3**	**30.1**	**37.3**	**35.2**	**30.2**	**30.2**	**33.9**	**33.7**	**30.3**	**30.2**
Minimum	**26.4**	**26.4**	**29.2**	**29.2**	**32.7**	**31.8**	**29.2**	**29.2**	**30.8**	**30.8**	**29.2**	**29.2**

Lampung Province produces about 32.77% from all pineapple production in Indonesia and 99.71% is planted in Central Lampung. Most of Central Lampung is a flat lowland area (25 -75 m asl) and pineapple plantation is a large open area without any trees; this could be the reason of hot and dry environment. Some smaller pineapple plantations in Indonesia is located in hills area and with shading trees, however, most common is in an open area of lowland (Pusat data dan sistim informasi pertanian, 2016). An experiment of planting pineapple under shade conditions in Brazil resulted that under normal light conditions, the rate of synthesis and degradation of chlorophylls of plants under direct sunlight and under shading was the same, but degradation can be accelerated by excess solar. The thicker aquiferous hypodermis detected in nonshaded plants might function as a filter to protect chlorophyllous tissue from intense radiation, thereby reducing the level of chlorophyll degradation. Moreover, no significant differences regarding yields or mean masses of pineapple fruits were detected between treatments. However, the percentages of sunburned fruits were significantly higher under direct sunlight treatment (da Silva et al., 2017).

IV. CONCLUSION

This study did not aim to solve all problems in this pineapple plantation since other factors: soil and cultivation management factors that important in pineapple cultivation were not included. High air and then soil temperature seemed to be the critical factor that needed to manage. Researches on how pineapple productions and qualities under shading trees should be considered in the future especially in general it is predicted that air temperature might increase.

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Table 4. Water balance based on Thornthwaite and Matter

No	Code (mm)	Months												Anual
		Jan	Feb	March	April	May	June	July	August	Sept	Oct	Nov	Dec	
1	Precipitation	314.8	357.0	388.3	222.0	80.0	83.3	154.3	70.5	71.0	105.8	182.3	222.0	2251.0
2	PET	103.4	90.8	108.8	106.5	102.7	91.9	98.0	110.7	114.6	108.6	100.0	96.0	1231.9
3	P-PET	211.4	266.2	279.4	115.5	-22.7	-8.6	56.2	-40.2	-43.6	-2.8	82.3	126.0	1019.1
4	APWL	0.0	0.0	0.0	0.0	-22.7	-31.3	0.0	-40.2	-83.9	-86.7	0.0	0.0	
5	SWC	90.2	90.2	90.2	90.2	133.0	165.6	90.2	216.8	1228.1	1392.2	90.2	90.2	
6	AWC	0.0	0.0	0.0	0.0	42.8	32.6	-75.4	126.6	1011.3	164.1	-1302	0.0	
7	AET	103.4	90.8	108.8	106.5	122.8	115.8	98.0	197.1	1082.3	269.9	100.0	96.0	2491.4
8	Water Surplus	**211.4**	**266.2**	**279.4**	**115.5**	0.0	0.0	**131.6**	0.0	0.0	0.0	**1384.3**	**126.0**	2514.4
9	Water Deficiency	0.0	0.0	0.0	0.0	*-20.1*	*-24.0*	0.0	*-86.4*	*-967.6*	*-161.3*	0.0	0.0	- 1259.4

Influence of different stages of corpus luteum on ovary size, oocytes grades and follicular population in Indian buffaloes

Eias Elzein, I Osman[1]; Sharma. RK[2] ; Majdi .E. Badawi[3]; Dahiya, S[2].; Prem, Y[2].; Pholia, A[2].; Dharmendra, K[2].; Selokar, N[2].; Jerome, A[2].; Pradeep, K[2].; Saini, M[2].; Nidhi, R[2].; Wakil, A[2]. and Rasika, R[2]

[1]Department of clinical studies, Faculty of Veterinary Science, University of Nyala, Nyala- Sudan.
[2]Physiology and Reproduction Division, Central Institute for Research on Buffaloes, Hisar-125001, India.
[3]Department of medicine and animal surgery, College of Veterinary Medicine, Sudan University of Sciences and Technology- Khartoum.
***Corresponding author:** eiasalzain66@yahoo.com

Abstract— *The aim of this study was to evaluate the effect of different stages of corpus luteum on ovary size, oocytes grades and follicular population. A total of 109 buffalo ovaries were collected from the slaughterhouse and transported to the laboratory for determination of ovaries weight, length, width, thickness, follicular population and oocytes grades. The results obtained revealed that the effect of types of CL on ovary weight and size showed a significant difference in the type of CL groups with ovary weight and ovary size. Ovaries having CL in late-stage showed the highest mean of ovary weight, length, width and thickness over the CL in the early and middle stage. Moreover, the results showed no significantly different in the type of CL groups with follicular population and oocytes grades.*

Keywords— ***Buffalo, Ovary, Corpus luteum, Follicular population, oocytes grades.***

I. INTRODUCTION

Corpus luteum (CL) is an endocrine gland formed after ovulation of graffian follicle and contributes to regulate estrous cycle and maintenance of pregnancy (Schams and Berisha, 2004). In different stages of estrus cycle and pregnancy, corpus luteum has several stages in size and structure (fields and fields, 1996). Corpus luteum synthesizes and secretes hormones such as progesterone, estrogen, relaxin, oxytocin, vasopressin and inhibin (Fields, 1991). Progesterone is essential steroid hormone necessary for establishing of pregnancy in domestic animals (Tomac *et al*., 2011). Moreover, blood progesterone has useful tool to determine an appropriate time of insemination, monitoring of cyclicity and pregnancy diagnosis in buffaloes (Batra and Pandey, 1983) .The aim of the current study was to evaluate the effect of different stages of corpus luteum on ovary size, oocytes grades and follicular population.

II. MATERIALS AND METHODS

2.1 Study area:

The present research was conducted in year 2018 – 2019 at Central Institute for Research on Buffalo, Hisar Haryana, India, located between Latitude: 29°09′14″ N Longitude: 75°43′22″ E and Elevation above sea level: 216 m.

2.2 Experiment design

One hundred nine buffalo ovaries having CL were collected immediately after slaughtering from Delhi slaughterhouse and transported to the laboratory in an insulated container containing normal saline with antibiotics. In laboratory, all tissues attached to ovaries were removed and all ovaries were washed twice in saline solution containing antibiotics (Dharmendra *et al*., 2011). After wash all ovaries were classified into three groups: Group having CL in early stage, middle stage and late stage.

2.2.1 Determination of ovaries weights:

Ovaries were weighed by using an electronic scale balance and expressed in gram (Kouamo *et al*., 2014).

2.2.2 Determination of ovaries lengths:

Ovaries lengths were measured using electronic Vernier calipers as the distance from anterior pole to posterior pole and expressed in cm (Samad and Raza, 1999).

2.2.3 Determination of ovaries widths:

Ovaries widths were measured using electronic Vernier calipers as the greater distance from the medial to the lateral surfaces and expressed in cm (Bukar *et al*., 2006).

2.2.4 Determination of ovaries thicknesses:

Ovaries thicknesses were measured using electronic Vernier calipers as greatest distance along an axis vertical to the longitudinal axis and expressed in cm (Razzaque *et al*., 2008).

2.2.5 Determination of follicular population:

For each ovary, visible follicles were counted and follicle size was measured with electronic Vernier calipers. Follicles were classified into 3 categories: small (<3 mm), medium (3 to 8 mm) and large (> 8 mm) (Baki Acar *et al*., 2013).

2.2.6 Oocytes collection:

Oocytes were collected by aspiration of surface follicles (2–8 mm diameter) using 18-gauge disposable needle attached to a 10 ml syringe in aspiration medium. The follicular fluid was collected in tube and kept for 15 minutes. The sediment was collected in 60 mm Petri dish and oocytes were searched under stereo zoom microscope.

2.2.7 Grading of Oocytes:

Oocytes were graded as: A, B, C and D according to homogenous of ooplasm and cumulus cells layer.

III. STATISTICAL ANALYSIS

Data were analyzed using SPSS (Statistical Package for Social Sciences) Version 18. The analysis of variance and Duncan's test statistics were used to analyze appropriate data sets. Differences were significant at $P < 0.05$.

IV. RESULTS AND DISCUSSION

The effect of types of CL on ovary weight and size is presented in table 1. The results showed significantly difference ($P<0.05$) in type of CL groups with ovary weight and ovary size. Ovaries having CL in late stage showed highest mean of ovary weight (5.03±0.17), length (2.35±0.05), width (1.74±0.05) and thickness (1.48±0.03) over the CL in early and middle stage. The mean weight, length, width, thickness were significantly higher in ovaries having CL in late stage as compared with ovaries having CL in early and middle stages. This result may occur due to hyperplasty of fibroblast of the connective tissue and vascularity contributes to an increase in size of the CL (Jablonka-Shariff *et al*., 1993).

The effect of types of CL on follicular population is presented in table 2. The results showed no significant difference between type of CL and follicular population. May be due to progesterone mechanism which inhibits follicular growth through suppression of LH which is critical for continued growth to large follicles (Bartlewski *et al*., 2001). Campbell *et al* (1991) reported that the CL secreted inhibin hormone into ovarian venous blood which has widely affect on ovarian follicular growth. These results were different than that found by (Mervat and Marwa, 2019) in cow. This difference might be due to animal and environment.

The effect of type of CL on oocyte grades is presented in table 3. The results showed no significant difference between type of CL and oocyte grades.

Table -1 Means (± SE) values of early, middle and late stage of CL:

Factors		No. of ovary	Ovary size			
			Weight	**Length**	**Width**	**Thickness**
	Early CL	26	$3.38±0.24^c$	$2.09±0.09^c$	$1.41±0.06^c$	$1.20±0.02^c$
	Middle CL	23	$4.03±0.24^b$	$2.14±0.09^b$	$1.54±0.06^b$	$1.47±0.04^b$
	Late CL	60	$5.03±0.17^a$	$2.35±0.05^a$	$1.74±0.05^a$	$1.48±0.03^a$
CL	**P < 0.05**	-	**.000**	**.015**	**.000**	**.000**

a,b,c In each column different letters (a, b) indicated significant difference between group (p<0.05 No =number SE = Standard Error CL = corpus leutum

Table -2 Means (± SE) values of early, middle and late stage of CL:

Factors		No. of ovary	Number of follicles			
			Small	**Medium**	**Large**	**Average No**
	Early CL	26	1.46±0.30	0.46±0.16	0.31±0.11	2.23±0.29
	Middle CL	23	1.13±0.29	0.61±0.18	0.26±0.14	2.00±0.35
	Late CL	60	1.22±0.18	0.52±0.13	0.28±0.06	2.02±0.20
CL	**P < 0.05**	-	**.690**	**.858**	**.957**	**.821**

No =number SE = Standard Error CL = corpus leutum

Table -3 Means (± SE) values of early, middle and late stage of CL

			Oocytes grades				
Factors		No. of ovary	I	II	III	IV	Selected oocytes for IVEP. I and II
	Early CL	26	0.23±0.20	0.46±0.34	0.50±0.46	0.12±0.12	0.69±0.53
	Middle CL	23	0.39±0.27	0.52±0.38	0.35±0.27	0.09±0.09	0.91±0.64
	Late CL	60	0.20±0.15	0.35±0.25	0.78±0.65	0.0	0.55±0.39
CL	**P < 0.05**	-	**.791**	**.920**	**.890**	**.295**	**.880**

No=number SE = Standard Error CL = corpus leutum

V. CONCLUSION

From the present study, it is concluded that the highest ovary weight and size in ovaries with CL in late stage than others stages. So, the corpus luteum has a great effect on ovarian morphology without having effect on oocyte grades and follicular population in buffaloes.

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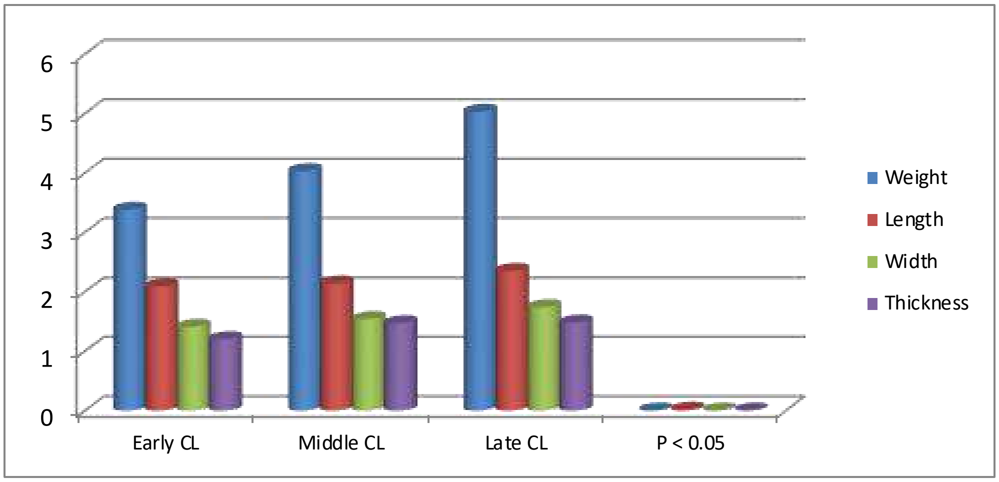
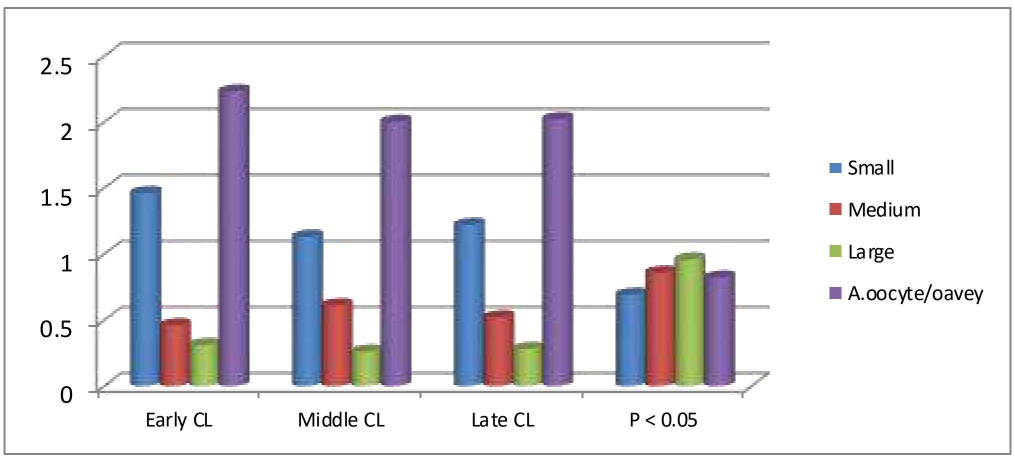
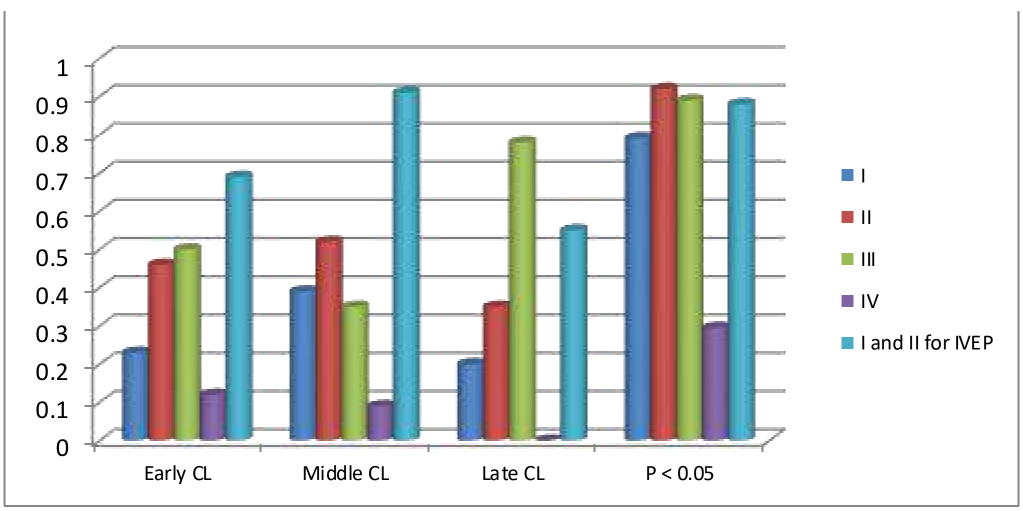


Relationship between types of CL, ovary weight and ovary size



Relationship between types of CL and follicular population



Relationship between types of CL and oocytes grades

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Identification of adequate Sahel climate prediction models based on CMIP5: Mali case

Indentification des modèles adéquats de prévision du climat sahélien sur la base du CMIP5: Cas du Mali

Hamidou Diawara[1], Vladimir A. Lobanov [2]

[1]Département de Géographie, Faculté d'Histoire et de Géographie, Université des Sciences Sociales et de Gestion de Bamako (USSGB), Mali, diawarahamidou@yahoo.fr

[2] Department of climatology, Russian State Hydrometeorological University (RSHU), Saint-Petersburg, Russian Federation

***Abstract*— *In this work, a multi-model of evaluation of future normals of temperature and rainfall for Mali was built. For this, the normals of temperature and precipitations of twenty (20) climate models out of the forty-two (42) in the CMIP 5 were compared individually with those of seventeen (17) stations for the temperature and thirty (30) stations for precipitations. The objective function used is the standard deviation, which is even smaller than the model is more efficient. The standard deviation of normals for all models with observations exceeds 10% of the average of precipitations over the territory, however, for the temperature 85.0% of the models (17 out of 20) give standard deviations representing less than 10% of the territorial average of the latter. This which allowed classifying the models, their intrinsic performance for each element of the considered climate. The analysis has shown that most models occupy very different range from one classification to another. Thus, a general classification was made which has shown that the most efficient multi-model for temperature and rainfall are different but the one giving the best approximation for the whole of the two elements (with a minimum of standard deviation) is composed of the models of the Institute Max-Planck of the German meteorology, this is MPI-ESM-LR and MPI-ESM-MR. The performance of this multimodel, compared to that of each of the two models composing it, second rank for temperature and first for precipitations..*

***Keywords*— Model; Multimodel; Standard deviation; Scenario; RCP.**

I. INTRODUCTION

Il existe plusieurs méthodes d'estimation du climat futur dont celles basées sur les scenarios d'évolution de la concentration des gaz à effet de serre dans l'atmosphère (SRES) ou sur l'évolution du bilan radiatif de la terre ou "forçage radiatif" (RCP), qui jouent un rôle de premier plan dans les variations du climat global. Pour l'amélioration des modèles du climat et l'identification des modèles les plus efficaces parmi tous les autres, le projet international d'inter-comparaison des modèles AMIP (Atmospheric Model Intercomparison Project) a vu le jour en 1990. C'est dans ce cadre que s'effectue la comparaison des modèles de la circulation globale de l'atmosphère AGGMs (Global Atmospheric General Circulation Models), développés par différents groupes de chercheurs à travers le monde (Gates, 1992), aussi bien entre eux qu'avec les données d'observations (Alves et *al.*, 2016). Ainsi, en 2005-2006, la troisième phase CMIP3 (Coupled Model Intercomparison Project phase 3) du projet AMIP, sous l'autorité du Groupe d'experts intergouvernemental sur l'évolution du climat (GIEC), a constitué une archive des résultats de la modélisation du climat passé, présent et futur. Dans cette archive on dénombre quatre (04) familles de scenarios d'évolution du climat (A1, A2, B1 et B2) dans le SRES (Special Report on Emissions Scenarios) et 26 modèles climatiques (Meehl et *al.*, 2007). Ce qui a constitué un bon moyen d'estimation de la dynamique du climat dans le futur dans différentes localités afin d'évaluer les possibles conséquences de ce phénomène et de pouvoir développer des stratégies d'adaptation. Pourtant ces modèles climatiques donnent des estimations, parfois très différentes, des paramètres du climat suivant les zones climatiques connues (suivant les critères choisis, l'ordre

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de mérite des modèles diffère. Certains modèles reproduisent bien le climat européen, d'autres sont plus performants pour représenter les systèmes de moussons tropicales)[1]. Ainsi, la prédétermination des caractéristiques futures fiables du climat dépend de l'efficacité des modèles utilisés. Afin d'aider aux prises de décisions dans la lutte contre les changements climatiques et leurs conséquences à court, moyen et long terme, le choix des modèles pour un milieu concret s'est imposé comme une priorité (Planton, 2003 ; Foamouhoue et Buscarlet, 2006). L'évaluation de la performance des modèles du climat consiste à confronter les résultats de leurs simulations aux données d'observations disponibles.

Depuis 2012, dans la cinquième phase (CMIP5) du projet AMIP, le nombre et/ou la nature des scenarios et de modèles climatiques a considérablement évolué. Le nombre de scenarios est resté a quatre (RCP2.6, RCP4.5, RCP6 et RCP8.5), mais désormais basés sur l'évolution du bilan radiatif de la terre (Representative Concentration Pathways, ou RCP). Par contre le nombre de modèles a passé à 42 (Taylor et *al.*, 2012), rendant encore plus difficile l'identification du/ou des modèles appropriés pour une région. En plus nous avons assisté à l'apparition de nouveaux modèles dans le CMIP5, souvent au profil de la disparition de certains anciens du CMIP3.

La tâche de la sélection des modèles climatiques performants pour une région donnée est un exercice courant dans les pratiques hydrométéorologies. Par exemple Perez et *al.* (2014), dans leur évaluation de la performance des modèles climatiques globaux pour la région nord-est Atlantic, recommandaient, pour des estimations futures du climat régional, les modèles UKMO-HadGEM2, ECHAM5/MPI-OM et MIROC3.2 (hires) pour les scenarios du CMIP3 et ACCESS1.0, ECEARTH, HadGEM2-CC, HadGEM2-ES et CMCC-CM pour ceux du CMIP5. Quant à Jena et al. (2015), ils estimaient que quatre (04) modèles du CMIP5, à savoir CCSM4, CESM1-CAM5, GFDL-CM3 et GFDL-ESM2G, donnaient la meilleure approximation des précipitations de la mousson indienne après comparaison avec les données des observations de 1871 à 2005. Les modèles recommandés dans ces exemples précédents sont tous différents, ce qui montre clairement la complexité spatio-temporelle des conditions climatiques et la nécessité de l'identification des modèles les plus efficaces pour chaque région du monde.

En 2006, la simulation du climat de l'Afrique de l'Ouest tropicale sur la période 1961-1990 a été réalisée par Foamouhoue et Buscarlet (2006). Pour cela, ces chercheurs on utilisé le modèle climatique régional HadRM3 développé par le Met Office Hadley Centre. Ils sont arrivés à la conclusion que ce modèle donnait une assez bonne simulation de la température et des précipitations, malgré quelques écarts aux observations mis en évidence, notamment pour les précipitations. Ce modèle, utilisé dans le troisième rapport d'évaluation (Assessment Report 3 – AR3) du GIEC en 2001, a depuis disparu de la liste des modèles climatiques dans suivants rapports.

La plupart des études de comparaison des données, obtenues des modèles climatiques, d'avec les données d'observations se limitent à la comparaison individuelle des modèles. Or il s'avère que généralement l'utilisation d'un multimodèle (ensemble de modèles) apporte moins d'erreurs, comparativement à l'utilisation d'un seul modèle (Malenfant, 2016). Cela est dû au fait que les biais s'annulent dans le multimodèle, permettant d'obtenir des erreurs absolues plus petites que pour les modèles individuels.

L'approche multimodèle est couramment utilisée en modélisations hydrologiques et météorologiques depuis le début des années 1980. Pratiquement tous les auteurs (Shamseldin et *al.*, 1997 ; Fritsch et *al.*, 2000) qui s'y sont essayés sont arrivés à la même conclusion, qui est la plus grande précision du multimodèle comparé aux modèles individuels.

Mais la performance d'un multimodèle n'est pas forcement proportionnelle au nombre de modèles individuels qui le composent (Ajami et *al.*, 2006). Un multimodèle composé d'un nombre restreint de modèles peut être beaucoup plus performant qu'un multimodèle plus complexe.

Ainsi, l'objectif principal de cette étude est l'identification des modèles climatiques efficaces, issus du CMIP5, pour la construction d'un multimodèle performant d'évaluation des conditions climatiques futures de la température et des précipitations au Mali.

Les objectifs spécifiques qui en découlent sont :

- Evaluation de la performance intrinsèque de chaque modèle climatique utilisé en comparaison des données d'observations de la température et des précipitations.
- Classification des modèles selon leurs performances individuelles et le choix des meilleurs pour la construction du multimodèle de la température et des précipitations au Mali.
- Comparaison de la performance du multimodèle avec celle de chacun des modèles individuels le composant.

[1] Climat. Modéliser pour comprendre et anticiper : http://www.insu.cnrs.fr/files/plaquette_missterre.pdf

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II. CADRE GEOGRAPHIQUE DE L'ETUDE

L'étude a été réalisée sur l'ensemble du territoire du Mali (Figure1). Le pays, situé au cœur de l'Afrique de l'Ouest, est caractérisé par un climat chaud avec l'alternance d'une saison sèche et d'une saison des pluies. La durée de ces deux saisons varie inversement du sud au nord. La première est courte au sud et longue au nord. Le cumul pluviométrique annuel sur le territoire varie de moins de 200 mm à plus de 1000 mm. Selon la quantité de pluie reçue par an et la durée de la saison des pluies, le territoire du Mali est habituellement divisé en quatre zones agroclimatiques (DNM, 2007); il s'agit, du sud au nord, de:

- la zone Soudano-guinéenne (ou pré-guinéenne) avec une saison des pluies qui dure plus de 6 mois et une pluviométrie annuelle depassant 1000 mm;
- la zone Soudanienne dont la saison des pluies dure environ 5 mois et le cumul annuel des pluies se situe entre 600 mm et 1000 mm;
- la zonne Sahélienne avec une pluviometrie de 200 mm à 600 mm en 3-4 mois;
- la zone Saharienne dont la pluviométrie annuelle est inférieure à 200 mm et la saison des pluies ne dure que 2 mois.

III. MATÉRIELS ET MÉTHODES

3.1 Matériels

Dans cette étude nous avons utilisé les températures moyennes annuelles et les précipitations annuelles, sur trente ans (1951-1980), de de dix-sept (17) stations pour la température et de trente (30) stations pour les précipitations, réparties sur tout le territoire du Mali. Nous nous sommes limités aux données d'observations de la période climatique de 1951 à 1980 pour deux raisons fondamentales: 1. la faible qualité des données observées des années plus récentes; 2. cette période est considérée comme la dernière de la stabilité du climat. Pour ces stations et sur la même période, une base de données de types identiques a été constituée pour vingt (20) modèles climatiques retenus. Les détails sur ces derniers sont présentés dans le tableau suivant:

Tableau 1: Informations générales sur les modèles climatiques retenus

№	Modèle	Institution	Pays
1	CSIRO-Mk3.6.0	CSIRO-QCCCE	Australia
2	CanESM2	Canadian Centre for Climate Modelling and Analysis	Canada
3	BCC-CSM1.1	Beijing Climate Center	China
4	BNU-ESM	College of Global Change and Earth System Science	China
5	CNRM-CM5	Centre National de Recherches Météorologiques	France
6	IPSL-CM5A-LR	Institut Pierre-Simon Laplace	France
7	MPI-ESM-LR	Max-Planck-Institut für Meteorologie	Germany
8	MPI-ESM-MR	Max-Planck-Institut für Meteorologie	Germany
9	CMCC-CM	Centro Euro-Mediterraneo per I Cambiamenti Climatici	Italy
10	MIROC5	MIROC	Japan
11	MIROC-ESM	MIROC	Japan
12	NorESM1-M	Norwegian Climate Centre	Norway
13	INMCM4.0	Institute for Numerical Mathematics	Russia
14	HadGEM2-AO	Met Office Hadley Centre	UK
15	HadGEM2-ES	Met Office Hadley Centre	UK
16	HadCM3	Met Office Hadley Centre	UK
17	GISS-E2-R	NASA Goddard Institute for Space Studies	USA
18	CCSM4 (Journal)	National Center for Atmospheric Research	USA
19	GFDL-CM3	NOAA Geophysical Fluid Dynamics Laboratory	USA
20	EC-EARTH	EC-EARTH consortium	Various

Les données d'observations ont été fournies par le service météorologique du Mali. Quant aux données des modèles, elles ont été recueillies de la plateforme canadienne « Données maillées du CMIP5 »[2], sous forme de maillage et en format « NetCDF ». Elles ont subi de multiples prétraitements tels que leur extraction en format « xlsx » et la constitution des séries pour chaque station par interpolation linéaire double ou bilinéaire (Thiery, 1986).

3.2 Méthodes

Pour l'atteinte des objectifs de l'étude, nous avons eu recours à différentes procédures d'analyse des données, chaque objectif étant spécifique. Les données de la température de l'air et des précipitations sont analysées séparément.

3.2.1 Evaluation de la performance intrinsèque de chaque modèle climatique

La performance d'un modèle climatique est évaluée en fonction de la comparaison des données dont il génère avec les données réelles mesurées sur le terrain. Pour ce travail, dans un premier temps, les normales de la température et des précipitations de 1951 à 1980 ont été calculées pour chacune des stations météorologiques,

[2] Données maillées du CMIP5, http://scenarios-climatiques.canada.ca/?page=gridded-data

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avec les données d'observations historiques et les données de chaque modèle climatique. Ensuite ces normales climatiques, d'observations et de chaque modèle, sont comparées. Le critère (la fonction-objectif) choisi pour l'évaluation de la qualité d'approximation des observations par le modèle est l'écart-type:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(X_{obs.} - X_{mod.})^2}{n}}, \qquad (1)$$

où $X_{obs.}$ est la normale des observations ; $X_{mod.}$ est la normale du modèle et n est le nombre de stations (ici n égal à pour 17 la température et 30 pour les précipitations).

L'interprétation de cette fonction-objectif est: « le modèle est d'autant plus performant que l'écart-type est petit ».

3.2.2 Classification des modèles selon leurs performances individuelles et la construction du multimodèle

La performance du modèle est inversement proportionnelle à l'écart-type obtenu de sa comparaison avec les observations. Pour chaque élément du climat de l'étude (température et précipitations), les modèles ont été rangés par ordre décroissant de la performance, du plus performant (ayant le plus petit écart-type) au moins performant (ayant le plus grand écart-type).

Pour la définition du multimodèle pour chaque élément du climat, à partir du modèle de rang 1 (le plus performant) les moyennes pour les stations ont été calculées, en intégrant progressivement les modèles de rangs suivants, et comparées aux observations selon la fonction-objectif précédemment définie jusqu'à l'obtention du maximum de vraisemblance. Mais le multimodèle définitif pour l'ensemble de la température et des précipitations doit comporter les modèles classés parmi les meilleurs pour chacun d'eux.

3.2.3 Comparaison du multimodèle avec chacun des modèles le composant

L'étape précédente permet de déterminer la composition du multimodèle pour la prédétermination des conditions climatiques futures sur la base des scenarios climatiques. Afin de vérifier l'hypothèse qui stipule la performance du multimodèle par rapport à chacun des modèles qui le composent, une classification de cet ensemble est réalisée dont le multimodèle fait partie.

IV. RÉSULTATS ET DISCUSSION

Les résultats obtenus des différents traitements statistiques auxquels ont été soumises les données d'observations et des modèles climatiques sont présentés dans l'ordre des objectifs spécifiques que s'était fixée cette étude.

4.1.1 Evaluation de la performance intrinsèque de chaque modèle climatique

Les premiers résultats de l'étude portent sur l'évaluation de la performance de chacun des modèles utilisés, sur la base des écart-types calculés. La distribution des écart-types selon le modèle pour la température et les précipitations est présentée sur la Figure 2. Pour faciliter la représentation graphique avec ceux de la température, les écart-types pour les précipitations (qui sont d'ordre très élevé) ont été divisés par cent (100). Et pour faciliter l'analyse de la figure, des limites de 10% de la moyenne sur le territoire des normales de la température et des précipitations ont été matérialisées sur cette dernière par deux traits verticaux.

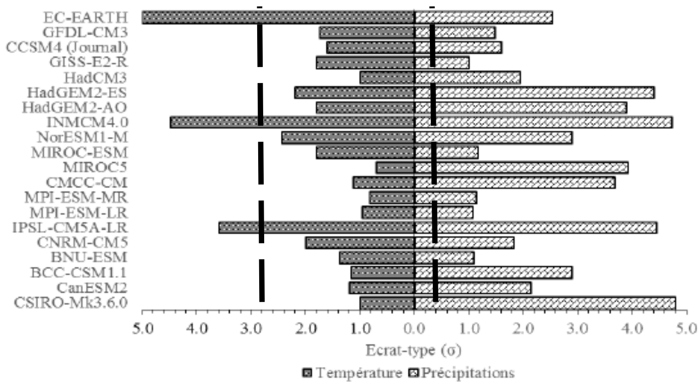

Fig.2 : Distribution des écart-types selon le modèle pour la température et les précipitations.

N.B.: Les traits hachurés verticaux représentent 10% de la moyenne des normales de l'ensemble des stations.

L'analyse de la figure montre une différente remarquable entre les performances de la plupart des modèles pour la température et les précipitations. On remarque que l'écart-type de tous les modèles dépasse 10% de la moyenne des précipitations sur le territoire. Il y a cinq (05) modèles (GISS-E2-R, MPI-ESM-LR, BNU-ESM, MPI-ESM-MR et MIROC-ESM) sur les vingt (20) qui donnent des écart-types entre 10% et 20%.

Pour la température, les modèles donnant moins de 10% de la moyenne sont beaucoup plus nombreux, ils représentent 85,0% (soit 17 modèles sur 20 que compte l'ensemble). Cela montre que les modèles donnent une meilleure approximation de la température que des précipitations, ce qu'avec pu remarquer Foamouhoue et Buscarlet (2006). Parmi ces modèles, les cinq (05) premiers par ordre décroissant sont : MIROC5, MPI-ESM-MR, MPI-ESM-LR, CSIRO-Mk3.6.0 et HadCM3.

Aucun des modèles classés ici parmi les meilleurs n'a été retenu par Perez et *al.* (2014), dans leur évaluation de la performance des modèles climatiques globaux pour la

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région nord-est Atlantic. Le même constat est valable par rapport aux conclusions de l'étude menée par Jena et *al.* (2015) sur la qualité des modèles dans l'approximation des précipitations de la mousson indienne. Parmi les modèles recommandés par ces auteurs, il y'en a cinq (05), soit CMCC-CM, HadGEM2-ES, EC-EARTH (pour Perez et *al.*, 2014) et CCSM4 (Journal), GFDL-CM3 (pour Jena et *al.*, 2015) qui ont été analysés dans cette étude et qui se sont avérés beaucoup moins performants pour les conditions climatiques du Mali. Cette analyse nous permet de comprendre que la performance des modèles climatiques est très variables dans l'espace d'où la nécessité de leur vérification et validation avant toute application de ceux-ci pour une zone climatique donnée.

4.1.2 Classification des modèles selon leurs performances individuelles et la construction du multimodèle

Les modèles sont rangés selon leurs performances, celui de rang 1 étant le plus performant de tous. La classification des modèles est effectuée aussi bien pour chaque élément du climat utilisé dans l'étude (température et précipitations) que pour les deux. Cette dernières s'est basée sur la moyenne des rangs des modèles pour les deux éléments. Ces classifications, en fonction des écart-types des approximations des observations par des modèles, est donnée le tableau suivant :

Tableau 2 : Classification des modèles climatiques selon leurs performances

Modèle	Ecart-type (σ) [Rang]		Rang général
	Température, ºC	Précipitations, mm	
MPI-ESM-LR	0.95 [3*]	106.84 [2**]	1
MPI-ESM-MR	0.82 [2*]	114.00 [4**]	2
BNU-ESM	1.37 [9]	108.44 [3**]	3
HadCM3	0.99 [4*]	194.26 [9]	4
GISS-E2-R	1.79 [14]	99.15 [1**]	5
MIROC5	0.69 [1*]	392.46 [16]	6
CCSM4 (Journal)	1.59 [10]	159.95 [7]	7
GFDL-CM3	1.74 [11]	147.28 [6]	8
CanESM2	1.20 [8]	214.19 [10]	9
MIROC-ESM	1.79 [13]	116.20 [5**]	10
BCC-CSM1.1	1.16 [7]	290.30 [13]	11
CMCC-CM	1.12 [6]	367.41 [14]	12
CNRM-CM5	1.99 [15]	182.99 [8]	13
CSIRO-Mk3.6.0	0.99 [5*]	479.86 [20]	14
HadGEM2-AO	1.79 [12]	389.05 [15]	15
NorESM1-M	2.42 [17]	290.24 [12]	16
EC-EARTH	5.00 [20]	252.86 [11]	17
HadGEM2-ES	2.18 [16]	440.61 [17]	18
IPSL-CM5A-LR	3.59 [18]	444.26 [18]	19
INMCM4.0	4.47 [19]	472.94 [19]	20

N.B.: * indique les 5 meilleurs modèles pour la température et ** indique meilleurs modèles pour les précipitations.

Ce tableau nous permet de constater que les rangs, dans la hiérarchie des modèles, sont très différents pour la plupart des modèles en fonction de l'élément climatique (température ou précipitations). Par exemples les modèles GISS-E2-R et MIROC5 occupent respectivement les rangs 14 et 1 pour la température et les rangs 16 et 1 pour les précipitations. Seuls deux (02) modèles occupent le même rang dans les deux classifications. Ces modèles sont IPSL-CM5A-LR (rang 18) et INMCM4.0 (rand 19). Il est aussi à noter que seuls deux (02) modèles (les modèles MPI-ESM-LR et MPI-ESM-MR de Max-Planck-Institut für Meteorologie d'Allemagne) sont présents dans le groupe des cinq (05) plus performants modèles à la fois de la température et des précipitations. Les trois autres modèles du groupe des cinq (05) meilleurs selon le classement général sont BNU-ESM (College of Global Change and Earth System Science, China), HadCM3 (Met Office Hadley Centre, UK) et GISS-E2-R (NASA Goddard Institute for Space Studies, USA).

La comparaison des performances des multimodèles formés pour la température est illustrée sur la Figure 3. Sur cette figure la fourchette de rangs indiquée signifie la composition du multimodèle. Par exemple le Multimodèle de rang 1 à 2 est composé des deux (02) premiers modèles selon leur classification dans le tableau 2 et le Multimodèle de rang 1 à 10 est composé des dix (10) premiers et ainsi de suite.

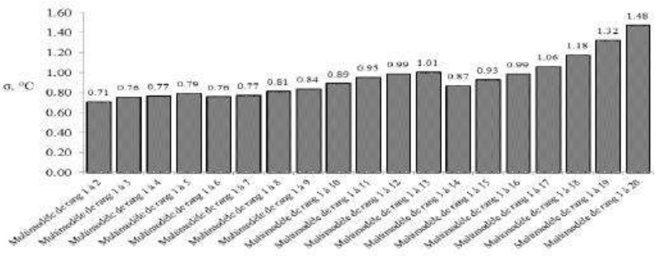

Fig.3 : Illustration de la comparaison des performances des multimodèles pour la température, composés de 2 jusqu'à 20 modèles climatiques.

La figure montre que le multimodèle le plus performant pour la température est celui formés des deux (02) premiers modèles uniquement (MIROC5 et MPI-ESM-

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MR). Après on constate que, globalement, la performance du multimodèle est plus faible d'autant plus que le nombre de modèles est élevé. Ce qui conforte la thèse de Ajami et *al*. (2006)[3].

Quant aux multimodèles pour les précipitations, ils sont présentés sur la figure suivante :

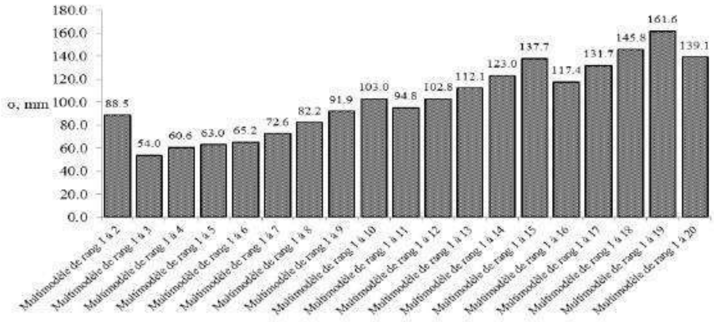

Fig.4 : Illustration de la comparaison des performances des multimodèles pour les précipitations, composés de 2 jusqu'à 20 modèles climatiques.

Ici, le multimodèle le plus performant est celui composé des trois (03) premiers modèles, à savoir GISS-E2-R, MPI-ESM-LR et BNU-ESM. Le même constat que pour la Figure 3, par rapport à la diminution de la performance du multimodèle avec l'augmentation du nombre de modèles le composant, est aussi valable ici.

Ainsi, compte tenu du fait susmentionné que les modèles MPI-ESM-LR et MPI-ESM-MR sont les seuls ayant un rang confortable (parmi les 5 premiers) dans la classification de ceux-ci aussi bien pour la température que pour les précipitations (voir tableau 2), ils sont recommandés pour la composition du multimodèle unique d'évaluation des normales de la température et des précipitations au Mali.

4.1.3 Comparaison du multimodèle avec chacun des modèles le composant

Enfin cette dernière tâche a été réalisée suite à la comparaison de la performance du multimodèle MPI-ESM-LR/MPI-ESM-MR avec celle de chacun des deux (02) modèles le composant. Ces comparaisons sont présentées sur la Figure 5 (pour la température) et la Figure 6 (pour les précipitations).

[3] La performance d'un multimodèle n'est pas forcement proportionnelle au nombre de modèles individuels le composant.

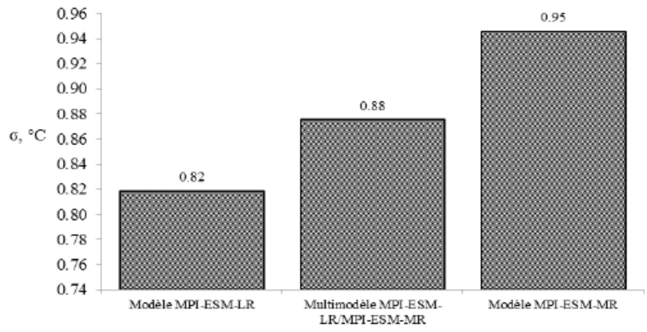

Fig.5 : Comparaison de la performance du multimodèle avec celle de chacun des modèles le composant, cas de la température.

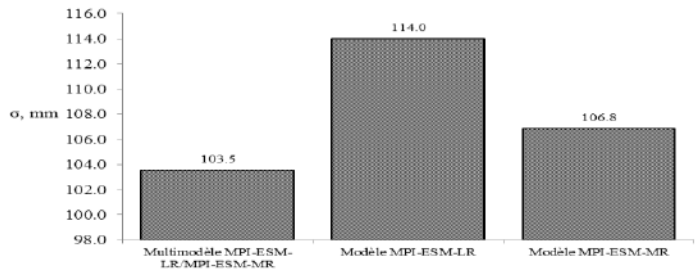

Fig.6 : Comparaison de la performance du multimodèle avec celle de chacun des modèles le composant, cas des précipitations.

L'analyse comparative des deux (02) figures permet d'affirmer que le multimodèle MPI-ESM-LR/MPI-ESM-MR, pour la température, donne un meilleur résultat que le modèle MPI-ESM-MR, mais de moins bon que le modèle MPI-ESM-LR. Au même moment, le multimodèle s'est avéré plus efficace que chacun des modèles le composant pour les précipitations.

Ainsi, pour l'évaluation des normales de la température et des précipitations au Mali, sur la base des vingt (20) modèles climatiques utilisés dans cette étude, l'hypothèse de la meilleur performance du multimodèle par rapport à celle des modèles individuels qui le composent (Shamseldin et *al*., 1997 ; Fritsch et *al*., 2000) est vérifiée pour le cas de la température mais ne l'est pas pour le cas des précipitations.

V. CONCLUSION

Cette étude nous a permis de construire un multimodèle d'estimation des normales de la température et des précipitations au Mali. Nous avons constaté que la performance de la plupart des modèles pour la température et les précipitations, en comparaison avec les observations, est très faible. L'écart-type des normales pour tous les modèles avec les observations dépasse 10% de la moyenne des précipitations sur le territoire. Les meilleurs modèles, avec un écart-type variant entre 10% et 20% de la moyenne, pour l'approximation des précipitations, sont GISS-E2-R, MPI-ESM-LR, BNU-ESM, MPI-ESM-MR et MIROC-ESM. Par contre pour la température 85,0% des modèles (17 sur 20) donnent des écart-types représentant moins de 10% de la moyenne territoriale de celle-ci. Ici les modèles MIROC5, MPI-ESM-MR, MPI-ESM-LR, CSIRO-Mk3.6.0 et HadCM3 se sont révélés meilleurs aux autres.

La classification des modèles selon leurs performances intrinsèques pour la température et les précipitations a montré une différence notable de rangs de certains modèles en fonction de l'élément climatique approché. Par exemple les modèles GISS-E2-R et MIROC5 occupent respectivement les rangs 14 et 1 pour la température et les rangs 16 et 1 pour les précipitations. Seuls deux (02) modèles sont présents à la fois dans le groupe des cinq (05) plus performants modèles de la température et des précipitations, ce sont les modèles MPI-ESM-LR et MPI-ESM-MR de Max-Planck-Institut für Meteorologie d'Allemagne. C'est pourquoi l'étude recommande ces modèles pour la composition du multimodèle unique d'évaluation des normales de la température et des précipitations au Mali.

Ce multimodèle, comparé à chacun des modèles le composant, s'est avéré plus efficace que chacun de ces composants pour les précipitations alors qu'il est moins efficace que le modèle MPI-ESM-LR pour la température.

On peut donc recommander le modèle MPI-ESM-LR l'estimation séparée de la normale de la température dans les scenarios climatiques. Mais pour les études faisant intervenir à la fois la température et les précipitations, le multimodèle MPI-ESM-LR/MPI-ESM-MR de Max-Planck-Institut für Meteorologie d'Allemagne plus efficace.

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The Study of the Rate of Infiltration and Soil Permeability on Different Land Cover in Watershed Maluka Province of South Kalimantan

Badaruddin[1*], Syarifuddin Kadir[1], Ahmad Yamani[1], Nurlina[2]

[1]University of Lambung Mangkurat, Faculty of Forestry, South Borneo, Indonesia
E-mail: badaruddin.hamdie@ulm.ac.id; odeng1987@yahoo.com;a.yamani@ulm.ac.id
[2] University of Lambung Mangkurat, Faculty of Mathematics and Natural Science, South Borneo, Indonesia E-mail: nurlina_abdullah@ulm.ac.id

Abstract*—Infiltration is part of the hydrologic cycle, namely the process of entering water from the surface into the soil. Infiltration is affected by vegetation, slope and soil type. This study aims to analyze the amount of capacity and volume of infiltration in open land, shrubs and rubber plantations in the watershed Maluka. This research method using the formula Horton with point observation using a purposive sampling by observing the various land closure. The results showed that the highest infiltration capacity value on the secondary forest land cover of 5,945 mm/hour and the lowest is the reeds with a value of 0,687 mm/hour. The value of the highest volume of infiltration in the secondary forest of 3,249 mm³ whereas the lowest on the Palm of 0,153 mm³.*
***Keywords*— Infiltration, Soil Permeability, Watershed of Maluka.**

I. INTRODUCTION

Infiltration is the process or percolating water ingress into the ground through the ground surface which is a thick amount of water can seep into the ground in a unit of time. Infiltration is the main source of the existence of groundwater, in the absence of infiltration of rainwater into the ground then limited water in the ground. Next Indarto suggests that the rate of infiltration is the amount of water that goes into the ground for a certain period[1]. Some of the factors that affect infiltration are: (a) soil texture; (b) the closing of land vegetation and land surface characteristics; (c) levels of permeability land; (d) morphology of the land; (e) the slope of the land, (f) the type and pattern of rainfall; and (g) the intensity of the rain[2,3,4].

Infiltration is a part of the hydrological cycle i.e. the process of water from the surface into the soil. A region if infiltrations is interrupted, it will affect the region in the hydrological cycle, so that the natural balance is not met. Hydrological cycle is the movement of water into the air then fall to the floor the Earth as rain[5].

Patterns of land use is a major factor that affects the soil infiltration. Thus, improving the soil infiltration and reduces runoff-the surface is very important for the conservation of soil and water, it can help farmers choose a rational cropping pattern[6]. Measurement of infiltration on a surface of land or under the closure of land usually consist of Infiltration capacity and infiltration rate, expressed in the thick water per unit of time (inches per hour or centimeters per hour). Rainfall exceeding infiltration capacity can lead to the occurrence of surface flow to lower and ended in rivers, creeks or other shelters in a basin. Some of the factors that affect the rate of infiltration are: (1) the ability of the land to clear water on the surface and infiltrated into the soil; (2) water content in the soil profile, (3) the amount of water available in the soil surface; and (4) the characteristics of the land surface[7].

The study of the rate of infiltration is intended to find out how the speed and magnitude of the influx or his pervasive water vertically to the body ground. By observing or testing of this nature is expected to give an overview of the needs of irrigation water that is required for a type of soil for certain kinds of plants at one time. Infiltration rate data can also be used to infer when a run-off will occur when a type of land has received a number of specific water through rainfall or irrigation water of ground level. Infiltration rate measurement results data of this kind can also be used for the purpose of planning the management of irrigation water and soil and water conservation[8].

Sosrodarsono suggests that the vegetation factors affect the variation rate of infiltration, because vegetation in addition to hardening the surface reduces role, it can also improve infiltration[9]. According to Lee *et al.*, the capacity of infiltration on soil vegetation higher than land does not bervegetasi, and the type of vegetation is largely determining the infiltration capacity[10]. Related to problems relationship this infiltration, vegetation and Widianto *et al.* suggests that deforestation or trees simultaneously and tripe exhausted has been disrupting the function of forest hydrology, because such logging damages the soil surface in the form of a decline in organic matter, the amount of pore space, and rate infiltration of rain water[11]. Efforts improve the physical properties of the soil and hydrological functions of forest damaged can be charged at the coffee plants, but there needs to be other efforts such as the granting of extra organic matter, closing down plants, making hole sink in, making the terrace and drains.

II. RESEARCH METHODS

The research was implemented on watershed Maluka. Implementation of research, starting from the month of February 2018 until finished. The object examined was infiltration in secondary forest, Scrub, gardens of rubber, palm oil, dry land Farming, open land, Garden blend, and reeds. The equipment used in this research is the Jerry cans to hold water, double ring infiltrometer to measure the rate of infiltration, the stopwatch to measure time, clinometer to measure sloping, a ruler to measure the height of the face of the water, hammer to insert the infiltrometer into the ground, the camera for documentation during the study, the calculator to calculate data, stationery. The materials needed in this study is water.

2.1. Research Procedure

Layout of data retrieval or infiltration rate measurements done on a purposive sampling, double ring infiltrometer tools laying on the area or area that are considered to be representative of the entire area are examined according to the provisions.

Procedures of Data collection and Retrieval Research layout of data retrieval or infiltration rate measurements done on a purposive sampling, double ring infiltrometer tools laying on the area or area that are considered to be representative of the entire area of the examined according to the provisions. Efforts are being made to collect the necessary data in this research in the form of primary data obtained by observing it directly in the field (observation), which consists of the data retrieval rate of infiltration in secondary forest, Shrubs, Rubber, Palm Groves, dry land Farming, open land, Garden blend, reeds. Secondary data retrieval is performed to complete the study, the data collected in the form of data about an overview of the location of the research obtained from the relevant agencies, the rainfall data representing the region of watershed Maluka retrieved from BMKG Station Banjarbaru climatology, as well as a map of the watershed, land cover Maps, sloping maps and maps of soil type.

2.2 Data Analysis

Infiltration Measurement Data analysis conducted in secondary forest, Scrub, gardens of rubber, palm oil, dry land Farming, open land, Garden blend, the reeds so that the retrieved data and volume of infiltration capacity. On the calculation of data research results using the formula Horton infiltration. Model Horton is one of the well-known infiltration m... ...wledged that infiltration ca... ...to approach the value of ... be expressed mathematical... [12]:

$$f = f_c + (f_0 - f_c)\, e^{-kt}$$

$$V = f_c t + \frac{f_0 - f_c}{K}(1 - e^{-kt})$$

Description

f_c	: constant Infiltration (mm/h)
f_0	: infiltration of the beginning (mm/h)
f	: infiltration Capacity (mm/h)
v	: Volume of infiltration (mm ³)
t	: time
k	: Constants
e	: 2.718

Follows details the level of classification of the infiltration which can be seen on the Table 1.

Table 1. Classification of soil infiltration

Description	Infiltration (mm/h)
Very slow	< 1
slow	1 – < 5
Being-slow	5 – < 20
Being	20 – < 65
Medium fast	65 – < 125
fast	125 – < 250
Very fast	> 250

Source: Lee, 1988

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III. RESULTS AND DISCUSSION

3.1 Analysis of Infiltration

Analysis of Infiltration Measurement tool using the Double Ring Infiltrometer outer diameter 50 cm and the inside diameter of 30 cm and a height of 30 cm above the ground surface. The outer ring of water function is to maintain the flow of the water ring the inside so that it moves vertically to the bottom so it doesn't spread. Infiltration measurement approaches are conducted every 5 minutes, this is in accordance with the Madrid *et al.* that the infiltration measurement using a circular metal ring with five-minute intervals[13]. Infiltration measurement is done on a variety of land such as the closing of secondary forest, Scrub, gardens of rubber, palm oil, dry land Farming, open land, Garden blend, and reeds.

3.2 Permeability of Soils

Based on the results of laboratory analysis of soil permeability values obtained Agricultural land as in table 2.

Table 2. The Results of the Analysis of Permeability

No.	Land Cover	permeability	Texture Class	Description
1	Secondary forest	2.21	Sandy clay	Medium
2	Scrub	9.02	Days clay	Rather fast
3	Rubber Gardens	4.95	Days clay t	Medium
4	Palm	1.28	Sandy clay	Rather slow
5	Dry Land Agriculture	1.49	Sandy clay	Rather slow
6	Open Land	8.87	Sandy clay	Rather fast
7	Garden Mix	1.59	clay	Rather slow
8	the tares	7.24	Sandy clay	Rather fast

Source: Primary Data Field

The data obtained can be seen the highest permeability in bushland 9.02 cm/sec and lowest in plantations of 1.28 cm/sec. This shows that the permeability on the area can be said to be rather slow up rather quickly because affected by factors of soil types sandy clay and clay namely clay according to Rachim that the permeability of the land has the increasingly rough texture then the permeability of the faster, whereas soil texture on the areas of research, namely clay, sandy clay and dusty clay which is smooth, slick and somewhat very closely so that permeabilitas low[14]. Permeability is closely related to the land if the pore size large movements of water and air in the soil will be free so that his infiltration will be high.

Texture soil from the results of research are most clays clay and clays. The soil clays and clay higher in water saving compared to the sandy soil it is in accordance with the opinion of Buckman and Brady; Islami and Utomo that store water for power ground sandy have the binding power against relatively low land permeability, because the surface of contact between the surface of the soil with water on soil texture and soil sandy this dominated by macro-pores, therefore water that falls to the ground sandy will soon be experiencing percolation and capillary water will easily be separated because of evaporating[15,16].

3.3. Average Capacity and Volume of Infiltration

Based on the results of the calculation of the capacity curve of infiltration on the various land closure (Figure 1), the situation analysis conducted further to find out the average capacity and volume of infiltration. The results of the analysis of capacity and volume of infiltration in watershed Maluka presented at table 3. infiltration capacity analysis results and the volume of infiltration in the closure of land and sloping in watershed Maluka.

Table 3. The results of the analysis of capacity and volume of infiltration on the various closures of land and sloping in Watershed Maluka

No.	Land Cover	fo (mm/h)	fc (mm/h)	f (mm/h)	v (mm³)
1	Secondary forest	11	5,0	5,696	3,134
2	Scrub	8	3,0	3,217	1,493
3	Rubber Gardens	5	2,0	2,141	0,734
4	Palm	1	0,6	2,201	0,249
5	Dry Land Agriculture	1,3	0,7	0,750	0,304
6	Open Land	0,3	0,2	0,824	0,160
7	Garden Mix	8	4,0	4,303	3,151

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8	the tares	0,4	0,2	0,234	0,123

Source: Primary Data Field

Description

fo = When the initial infiltration capacity (mm/h)

fc = infiltasi time constant capacity (mm/h)

f = infiltration capacity or maximum rate of water into the soil (mm/h)

v = volume of infiltration (mm ³)

The results of the analysis of infiltration capacity that the highest obtained at the close of the secondary Forest land with a value of 3.134 mm/hour while infiltrating the lowest land on the tares with a value of 0.123 mm/hour. This is due to the magnitude of infiltration in secondary forest affected by the density of the heading. The closure of the heading which more meetings will increase the organic matter of the resulting litter. Palm land vegetation cover soil is dominated by short grass that rooting, so infiltration is low. This is in accordance with the statement of Yanrilla where the rainwater that falls on the soil surface is not direct but halted by the vegetation in the form of the heading and the lower plants so that the resulting infiltration will be high[17]. This is supported by the statement that the existence of trees then the rooting will increase in the absorption of water so it will enlarge infiltration[18].

Based on soil vegetation in addition to rooting activity that helps to form aggregates of soil is also able to protect surfaces from rain resistant so as inhibit the flow of the surface. Vegetation can enhance infiltration because of the rooting that is able to absorb the water goes into the ground. As for the land that is not vegetation have a low infiltration because there is no root that can absorb water so high surface flow and can cause the onset of erosion. Change and conversion of land use and forests usually have an impact on the reduction of the rate of infiltration of rainwater and capacity, as well as an increase in surface runoff and soil erosion[19,20,21,22,23,24,25].

Table 3 shows that the highest volume of infiltration on the closure of secondary forest land of 3.249 mm ³, while the lowest volume of infiltration in the land of Palm 0.153 mm ³. It is influenced by soil conditions, permeability and organic ingredients. Research on soil contains clay obtained a high volume of low flow and infiltration of the surface height. In addition to organic materials and permeability infiltration also affected by the abundance of rain water which fell (rainfall) and the amount of water that is absorbed by the soil (infiltration) and left on the ground and on the surface of the leaves and stem[26,27].

Different soil causes the air permeated with different pace. Every land has different absorption, on the type of sandy soils tend to high infiltration rate and conversely clay tend to rate his infiltration low. Note that the pore on the clay in size small so that the movement of water and air in the soil will not free then his infiltration is low and if rain occurs on the area although with fairly low rainfall will cause a flow of surface. On one of the same soil type with a different density, then the rate of his infiltration is also different, if the more dense the soil the more small rate of his infiltration. Ground with grain that is too coarse (sand) could not resist water and nutrient elements, thus the plants that grow on the soil of this type is prone to drought and nutrient deficiencies[28].

3.4.Land cover against Infiltration

Based on conditions in the field of land cover factors affecting the rate of infiltration. If the more dense the soil then the surface flow is high and low his infiltration. The magnitude of the surface flow will cause the high removal of topsoil and there is no chance of water that goes into the soil (infiltration). Land cover data are based on the rate of infiltration can be seen in table 4

Table 4. Land Cover Data Against The Infiltration Rate

No.	Land Cover	f (mm/jam)	Description field
1	Secondary forest	5,696	medium- slow
2	Scrub	3,217	Slow
3	Rubber Gardens	2,141	Slow
4	Palm	2,201	slow
5	Dry Land Agriculture	0,750	very slow
6	Open Land	0,824	very slow
7	Garden Mix	4,303	Slow
8	the tares	0,234	very slow

Source: Primary Data Field

Based on data obtained in field on his infiltration rate of secondary forest at the time of observation, the situation that is being slow and more slowly. This can be affected by the State of the soil and the vegetation. On all land cover only the rate of secondary forest his infiltration is slow and more slow, this is because there is the vegetation in the form of the trees roots can absorb water into the ground but also influenced by the circumstances the soil in the form of clay so that the rate of his infiltration slow.

While the rate of land cover on all his infiltration slow is due to the State of the vegetation then the surface flow is high and low his infiltration as well as on the influence by the circumstances of a clay soil. Soil texture is the comparison of the content of the particles in the form of clay faction, dust and sand. Soil particles have different shapes and sizes. Coarse-textured soils have a high infiltration capacity while the fine-textured soil his infiltration capacity is small. Undertook to determine soil texture in ground water that is in the form of infiltration speed and ability of the binding of water by land.

Soils containing high amounts of clay can be suspended by a grain of rain that fell on him and the pores of the surface layer will be clogged by grains, this can lead to the occurrence of surface flow and erosion which is quite height. From a wide range of research on infiltration capacity is indeed a sand fraction greater than the fraction of clay, the clay fraction on induced rich smooth pore but poor will pore large, otherwise the poor will sand smooth and rich pore large.

Based on the above discussion of known capacity and volume of infiltration on watershed of Maluka is influenced by soil type and vegetation. But not only vegetation and soil type that can affect the rate of infiltration but also a high rainfall, which will cause the ground became saturated in the absorption of the water be not optimal, thus causing a high surface flow and triggered erosion and can be concluded that the area of watershed Maluka is going to flood-prone areas.

A very important part of infiltration in the hydrological cycle, with the process of infiltration so can reduce the occurrence of floods, reduce the occurrence of soil erosion as well as meet the needs of crops or vegetation will provide water and river water on When the dry season.

IV. CONCLUSIONS AND SUGGESTIONS

Based on the results of research that has been carried out in watershed Maluka retrieved Data research results obtained as a result of infiltration the highest on secondary forest of 5.696 mm/h and low infiltration on the Tares of 0.234 mm/hour. And the highest Volume of infiltration in the secondary forest of 3.134 mm³, while the lowest volume of infiltration on the tares of 0.123 mm ³. Research on soil contains clay and sandy clay high so obtained a low infiltration volume and flow of the surface height.

The research of infiltration in watershed Maluka can be said that in the process of his infiltration is low. This is due to the condition of the soil will be less vegetation so that the organic material content a bit. So should the area of watershed Maluka is need for conservation action so that the soil conditions are getting better and the danger of erosion which will cause catastrophic flooding can be resolved.

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Effects of Water Deficiency on Phosiological Traits, Grain Nutrition Quality and Yield of three Maize (Zea mays L) Genotypes

Salifu Mahama[1], Fulop Lajos Doka[2]

[1]PhD Student, Kerpely Kálmán Doctoral School, 4032 Debrecen, Böszörményiút 138, Hungary
Salifumahama@agr.unideb.hu/ Salifu_mahama@yahoo.com
[2]Lecturer, University of Debrecen, Hungary Institute of Plant Sciences, Hungary
doka@agr.unideb.hu

***Abstract*— Water deficit or drought has an adverse effect on crop production, and it is one of the limiting factors of world food security. A field experiment was conducted on different maize genotypes in University of Debrecen to assess the impact of water deficit on the physiology, grain nutrition and yield of three maize genotypes. The studied treatments were full irrigation and non-irrigation. The irrigated treatment did not significantly affect grain yield and starch content. Grain protein, moisture content SPAD and leaf area index(LAI) were significantly affected by irrigation. There was significant yield difference between the hybrids and grain nutrition quality was also affected by hybrids difference at LSD 1%. For good nutritional quality grain from the studied genotypes, the researchers propose (P 9911) Genotype under similar environmental condition.*

***Keywords*— Maize, Genotype, water deficiency, Physiology, Nutrient, and Yield.**

I. INTRODUCTION

Soil water deficit is one of the biggest challenges in maize plant growth Wu et al., (2011a). The effect of the global changes resulting in varying rainfall patterns makes drought more severe and frequent Easterling et al., (200)), andmodels predict a 25% decrease in soil moisture content between 2000 and 2040 Sabate et al., (2002), which will bring big challenge to crop production because about 66% of total cereal crop production is by rain-fed agriculture Lobell et al., (2014).

The sensitivity of drought stress to plants depends on varied degree of the drought stress, plant variety, and plant developmental stages Demirevska et al., (2009). Several studies have shown a reduction in plant growth because of reduction in chemical signals being the cause of water deficit in the soil. (Ismail and Davies, 1998; Hurley and Rowarth, 1999; Ismail et al.,1994). Fresh and dry biomass reduction are the common adverse effect of drought on maize plants as reported by Zhao et al., (2006). The effect of water deficit on the height, stem diameter photosynthesis reduction caused because of decreases in maize leaf expansion which impaired photosynthetic activities has been reported (Zhao et al.,Demirevska et al.,2009; Wahid et al.,2005). Photosynthetic pigments and reduction in relative water content has been noted in a wide variety of plants Anjum et al., (2003) and Nayyar and Gupta, (2006).

Drought is a protracted period of water deficient precipitation resulting in extensive crops damage and yield loss. Drought stress or water deficit is an inevitable and recurring feature of global agriculture. Kramer (1980) reported that about one-third of the world’s potentially arable land suffers due to water shortage, and most of the crops production is often reduced by drought. Water being integral part of plants plays a pivotal role in the initiation of growth, and subsequent maintenance of developmental process throughout the plant’s life. The maize crops may experience reductions of grain yields when subjected to water deficit during the critical period of crop cycle from tasseling stage to initiation of grain filling.

Plants productivity growth is fundamental to meeting the increasing demands of the ever-increasing population, but projection indicates that the future growth in cereal yields will decline across nearly all regions. Water deficits are responsible for a significant amount of this decline in developing countries, where food security is will continue to be a problem.

Irrigation plays a particularly large role in agricultural production in developing countries, where it is projected to account for 57% of the growth in cereal production between 1995 and 2025, and 80% of the growth in global irrigated cereal production between 1995 and 2025 (Rosegrant*et al.* 2002). Nevertheless, added constraints will continue to be placed on irrigation water supplies as non-agricultural demand for water rises at much faster rates than water demand for irrigation — though total water consumption for non-irrigation uses will still be much lower than consumption for irrigation. Several other factors will also affect the availability of water for agricultural production, including unsustainable groundwater use, environmental demands for water and water quality problems.

Maize (*Zea mays* L.) is a major cereal crop world over, serving as a major staple for both human consumption and animal feed. It has also become a major resource for industrial applications and bioenergy production. Maize grain is widely used for the preparation of corn starch, corn syrup, corn oil dextrose, corn flakes, gluten, grain cake, lactic acid and acetone which are used by various industries such as textile, foundry, fermentation and food industries. The consumption of maize as feed has also increased tremendously with the development of poultry and livestock industry. Maize is one of the grain crops in the world which is the most versatile. It is used in the human diet in both fresh and processed forms. The value added has been an important economic driver in the corn markets (Hallauer and Miranda, 1988).

II. MATERIALS AND METHODS

This is a long-term experimental set up at Latokep the experimental field of university of Debrecen, Hungary in the 2018 cropping year. Latokep experimental field is about 15km away from the city centre with(Latitude $47^0 33^{'}$N and Longitude $21^0 27^{'}$E).The area of Latokep Crop Production Experiment Site lies along road 33 and has an independent water extraction facility which provides water necessary for irrigation from the water reservoir. The soil for the experimental site calcareous chernozem formed on the sag loess ridge. The climate and weather condition are continental and often extreme. The 30-year – average value of precipitation 565.3mm, whiles the precipitation for the planting to harvesting time was 290.4mm.

In this experiment set up, three maize genotypes(DKC5943, P9903, and P9911) were planted on a plot of land which has been used for the past 30 years. The plot was divided into fully-irrigated and non-irrigated plots. Fertilization of NPK(500kg ha^{-1}) and a plant density of 72,500/ha^{-1}were used for all genotypes

In the irrigation treatment, crops were irrigated three times at different dates as follows in the 2018 cropping season;

24^{th} June – 50mm water

5^{th} July – 25mm water

30^{th} August-25mm water.

The leaf area index (LAI) was measured using SS1-SunScan Canopy Analysis System (Delta-T Devices, UK) at four growing stages. The chlorophyll content of the leaves was recorded using SPAD-502 plus (Konica Minolta, japan). The Normalized Difference Vegetative Index (NDVI) was measured using GreenSeeker Hand-held NDVI Sensor, whiles plant height was measured by a long ruler just before harvest. The grain quality (protein, and starch) was recorded using a grain analyzer granolyzer after harvest.

The aim of this experiment was to determine the effect of water deficits on physiological traits, grain quality and yield of three maize genotypes. Statistical analysis of data was done using SPSS version 19 software to compare the means.

III. RESULTS AND DISCUSSION

In this study, we examined the effect of irrigation on different genotypes with regards to photosynthetic parameters, grain nutrient quality and crop yield on the selected hybrids. The evaluation of the data collected was done using SPSS 19 for Windows statistical program.

From table1 (A) below, there is no significant difference between the three Hybrids of maize from the analyzed data of SPAD and LAI. NDVI had a significant difference at LDS 5% between the hybrids.

Grain nutrients quality and grain yield in this experiment were significantly affected at LDS 1% between the different maize hybrids.

Table 1: Effects of water deficit on physiological trait, grain quality and yield on different hybrids.

HYBRID (A)	SPAD	LAI	NDVI	PROTEIN	MOISTURE	STARCH	YIELD(t/ha)
DKC 4943	61.23	2.44	0.75	8.68	14.85	74.38	15648.33
P 9903	61.15	2.56	0.74	9.23	15.25	73.60	15624.12
P 9911	59.58	2.55	0.77	9.61	16.09	74.48	16584.54
SZD 5%	3.38	0.77	0.02	0.22	0.25	0.52	749.04
SIG	ns	ns	*	**	**	**	**
IRRIGATION(B)							
NONIRRIGATED	62.90	2.45	0.74	9.43	15.08	74.05	15025.16
IRRIGATED	59.61	2.34	0.76	9.74	15.35	73.86	15200.01
SZD 5%	1.95	0.44	0.01	0.13	0.14	0.30	432.46
SIG	**	ns	**	**	**	ns	ns

**=1%, *= 5% and ns= not significant

This study find out that, SPAD, NDVI, Protein and Moisture content were all significantly affected irrigation at LSD 5% while Leaf area index, starch and yield were not affected by irrigation as shown in table 1(B) above.

The non-significant difference of maize grain yield noted from this study could be attributed to the good precipitation in the cropping season resulting to unsevered or harsh deficit conditions for the non-irrigated crops. This study did not coincide with (Hall et al.,1980) who revealed that, water deficit reduces leaf area and delays silking and thereby reducing grain yield components, particularly grain number.Stegman (1982) reported that yield of maize was not significantly affected when it was exposed to water deficit that induces a 30-40% depletion of available water between irrigation. The review made by Kirda (2000) indicated that similar works on many other crops like sugar beet, sunflower, wheat, and potato have demonstrated the possibility of achieving optimum crop yields under deficit irrigation practices by allowing a certain level of yield loss from a given crop with higher returns gained from diverting the saved water for irrigating other crops. McPherson and Boyer (1977) on their work indicated that water deficit during grain filling stage on sorghum caused only a small drop in yield. The average grain yield of the genotype P9911 (15200.01 t/ha) under irrigation condition.

Protein content was significantly affected by the level of irrigation and varied considerably between the genotypes ss shown in Table 1A.The protein content of maize grain was significantly lower in treatments under non-irrigated condition. The highest protein content (9.61% on average) was noted in the genotype P9911 under irrigation treatment. This showed that the protein content decreased if the quantity of irrigation was above or below a certain level and that shows that, proper water supply to maize was beneficial to protein formation of maize grain. The high protein content under irrigation treatment could be attributed to the increase in the activities of glutamate synthase and glutamine synthetase, which are involved in nitrogen metabolism by promoting nitrogen accumulation and increasing the protein content of maize grains.This study agrees with , (Aydinsakir et al. 2013)who determined that in the ecological conditions in Antalya, Turkey, the protein content of maize grain was the highest with full irrigation and significantly lower with deficit irrigation and under rainfed conditions. Contrary to this present findings, Ertek and Kara 2013 studied the effects of different irrigation levels on the yield and quality of sweet corn in Turkey and reported the results that, increasing irrigation decreased the protein content of maize grain. Since protein is an important aspect of maize grain production, the irrigation level for maize in the agro-ecological environment of Hungary should be maintain.

From Table 1(A) above, the irrigation treatments had a significant effect on the starch content in all three studied genotypes meaning that the rate of grain filling differed between genotypes. In the present research, the average values of starch content percentage gradually increased with increasing irrigation water, indicating that the grain filling rate was different between the rainfed non-irrigated treatment and irrigated treatments. The increase was not large but statistically significant at LDS 5%. The reason for these changes could be alterations in starch biosynthetic enzyme activity and the accumulation of starch in grain as water availability enhance enzyme activities. The conclusion to this phenomenon is that irrigation might result in qualitative and quantitative differences in the rate of grain carbohydrate metabolism, as well as endosperm changes that enhances the rate of starch synthesis. (Zhao et al.,2009)however reported that, mild drought increased and that a large water deficit decreased the starch content. Research conducted by Lu et al.,2015 in China showed that water deficit had no effect on the starch content of fresh waxy maize. Liu et al.,2013 also reported that the starch content of the grain samples of maize grown with less irrigation was 3.0% smaller than with high irrigation levels.

IV. CONCLUSION

In summary, maize growth and yield responses were related to adequate supply of water and careful selection of cultivars in any given ecological zone. In this study, there was a significant difference between different Maize hybrids in their grains nutrition quality thus; protein, moisture and starch content as well as maize grain yield were all significantly affected at LSD 1% ($P < 0.01$). Photosynthetic parameters were not affected expect NDVI which was significantly affected at LSD 5%. Irrigation treatment significantly affected photosynthetic parameters of SPAD and NDVI, and Maize grain protein and moisture content at LSD 1%. Maize grain yield was not affected although the irrigated treatment recorded a higher average value of 15200.01t/ha, it was not statistically significant.

Water deficit regulated the activities of antioxidative defence system in all the maize hybrids thus, reducing the severity of the drought. Again, all maize hybrids in this study showed a variable response to water deficit stress resulting in the production and accumulation of different osmolytes in all hybrids. This study needs to carry out again for one year of this experiment is enough to give a definite general conclusion.

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Analysis of the Profitability of Okra production among Small holder Okra farmers in Akinyele Local Government Area, Oyo State, Nigeria

Osalusi, C.S.[1]; Akanni-John, Rachael[1]; Okeke E.N[2] and Ogunsola, J.O[1]

[1]Federal College of Forestry, P.M.B 5087, Ibadan, Nigeria.
[2]Federal College of Forestry and Mechanization, Afaka, Kadunna, Nigeria.
Corresponding email: uwanosalu@yahoo.com

***Abstract*— *The study was carried out to analyse the profitability of okra production among small holder okra farmers in Akinyele local government area, Oyo state. Multi-stage and purposive sampling technique was used in data collection. A total of 75 respondents were sampled. Statistical tools such as frequency distribution, production function and budgetary analysis were used in analyzing the data for this study. The result shows that most of the respondents were male (90.7 %) and married (73.3%). The major problems faced by the respondents in the study area include, inadequate transport facilities, weather condition and high cost of input. The estimated costs and return of Okro producers realized per okra farmer per annum were ₦161,137.00 and ₦ 77,317.76 respectively. Okra farming is lucrative in the study area with profitability index of 56.38 and a rate of return on investment of 117.50% and operating cost ratio of 38.59%.*
***Keywords*— Okro, Production, Gross margin, Budgetary.**

I. INTRODUCTION

Okra (*Abelmoschus esculentus*) (L) Moench) is an annual fruit vegetable crop belonging to the *Malvaceae family. Abelmoschus* has several cultivated species of economic importance, two of which was identified as *Abelmoschus esculentus* and *Abelmoschus caillei* (Siemonsma, (Siemonsma, 1982). Varieties vary by plant height, size of fruit, colour, early or late maturity etc. and they are namely; white velvet, green velvet, long pod, lady finger, dwarf green pods (Udoh et al., 2005. Vegetables are among the stable food component whose production has continued to increase in most countries of the world. (Udoh and Akpan 2007). They are important protective food for the maintenance of health and prevention of diseases. They contain valuable food ingredients, which can be successfully utilized to build up and repair the body (Bakhru, 2003; Edet and Etim, 2007). The level of vegetable consumption in Nigeria is rising annually owing to the greater appreciation of their food value. (Haruna, 2003). According to Kebede and Gan, (1999), the source of farm income for small and limited resources farmers are basically arable crop production, Vegetable and non-vegetable crops. Its importance has been long recognized all over the world (Ndaeyov et al, 2007). In Nigeria, okra is grown basically in all the states of the federation both as rain fed and irrigated crop because of the highlighted values. Also, it serves as a source of income to its producers, labourers and marketers (Alimi, 2004). Production of these vegetables, especially okra as a small scale enterprise can financially empower the farmers especially those with little capital, limited access to land and working under labor constraints. (Lewis 1997). The cash they provide contributes significantly to the food security at the house hold level and enables farmers to attain a degree of independence within the family budget. This study examined how profitable okra production is among small holder okra farmers Akinyele local government area.

II. MATERIALS AND METHODS

Study area

The study was carried in Akinyele local government area, Oyo state, Nigeria. Akinyele Local Government area was created in 1976 with the administrative headquarters located in Moniya. The Local Government shares the same boundaries with Afijo local government to the north, Lagelu local government area to the east, Ido local government area to the west and Ibadan north local government area to the south. The area lies between the latitude 7.5309°N and longitude 3.9110°E, it occupies a land area of 464.892 square kilometers with a population density of 516 persons per square kilometers. Using 3.2% growth rate from 2006 census figures, the 2010 estimated population for the local government is 239,745. It is dominated by the Yoruba's among other resident tribes such as Ibo, Tiv, Hausa, Nupe, Fulani etc. The residents are of Christianity, Islamic and traditional religion. The people of the local government are predominantly farmers who mostly involve in crop production such as cassava, pepper, yam, maize and vegetables, while some are youths who are involved in trading and other businesses. Akinyele Local Government is highly heterogeneous and metropolitan in nature especially in areas like Ojoo, Orogun, Shasha, Moniya and Akinyele where Nigerians from different tribes and foreign Nationals resides.

Sampling Techniques

Multi stage and purposive sampling technique was employed for this research. The first stage involves the selection of the study area, Akinyele local government area was purposively selected based on a prior knowledge that it is an okra producing area and contains a considerable amount of okra farmers. The first stage involved stratified sampling which was used in grouping the various villages in Akinyele Local Government Area. The second stage involves the simple random selection of 50% of the total number of the afore mentioned wards in the study area i.e. 6 wards out of the 12 wards which are ward1-Ikereku, ward4 -Olode/ Amosun/ Onidundu, ward5 -Oje-emo / Moniya, ward7: Iwokoto/ Talonta / Idi-oro, ward11: Olorisa-oko/ Okegbemi/ Mele, ward12: Iroko. The third stage involved random selection of three communities in each town making five (5) communities. A list of such farmers was collected from the respective community's council of baales and Oyo State A.D.P (Agricultural Development programme). A total of seventy-five (75) respondents was used for the study.

III. METHOD OF DATA ANALYSIS

Statistical tools such as frequency distribution, Gross Margin and Net income analysis. Afolami (2002) defined gross margin analysis as the difference between the gross farm income (GFI) and the total variable production cost (TVC); while the Net farm income (NFI) was defined as the difference between gross margin and total fixed production cost.

Budgetary Model

GM =TR - TVC

Where

GM = Gross margin (₦/respondent/annum)

TR =Total revenue (₦/respondent/annum)

TVC =Total variable costs (₦/respondent/annum)

NFI =GM –TFC (₦/respondent/annum)

Where

NFI =Net farm income (₦/respondent/annum)

TFC =Total fixed cost (₦/respondent/annum)

In addition, various profitability indices were computed. They are:

Profitability Index (PI) = NI/TR X100

Rate of Return on Investment (RRI) = NI/TC X 100%

Operating Cost Ratio (OCR) = TVC/TR X 100%

PI was used to determine the extent to which investment in okra farming in the study are is profitable; RRI was used to measure the percentage of profit derived from an investment outfit; while OCR was used to estimate the relative expenditure structure in the okra farming business.If, PI is greater than 1, the business is profitable; RRI is greater than 1, the investment is profitable; OCR greater than 1, it is profitable to invest on the project.

IV. RESULTS

Table 1 shows the Socio-Economic Characters of Okro farmers in the study area. The table shows that 90.7% of the Okro farmers were male. This means that males dominated okra farming in the study area which might be due to the fact that okra farming might be too tedious for females especially the process of land preparation. It is observed from the study that the highest percentage (36.0%) of respondents were at age bracket of 21-30 years. This supports proposition of Madur (2000) who said that older people of older ages are more into farming than younger people in Africa. The result also shows that majority of the okra farmers in the study area (58.7%) had first school leaving certificate while 40.0% had no formal education. This means that majority of the okra farmers in the area can read and write. This is line with Swanson (2008) education enables farmers to make informal decision regarding production and marketing of their produce. Findings also shows that 90% of the plantain farmers had a household size of less than 4 people, the large household size showed available labor for okra production in the study area. Enete and Okon, (2013) reported a large household size in the area serves as cheap source of family labour.

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Table 1: Socio-Economic Characteristics of Respondents

Variables	Frequency	Percentage	Mode	Std Deviation
Gender				
Male	68	90.7	Male	0.293
Female	7	9.3		
Age (years)				
21-30	27	36.0	21-30	0.838
31-40	23	30.7		
41-50	25	33.3		
Marital status				
Single	3	4.0		
Married	55	73.3	Married	0.835
Divorced	3	4.0		
Widowwidower	14	18.7		
Household size				
1-3	29	38.7		
4-6	31	41.3	4-6	0.833
7-9	12	16.0		
Above 9	3	4.0		
Level of Education				
No formal education	30	40.0	Primary school holder	0.517
Primary school holder	44	58.7		
Secondary education	1	1.3		

Source: Field survey, 2019

Budgetary Analysis Results

Table 2 presents the average costs and returns of okra production in the study area. Total revenue of ₦161,137.00 was realized per okra farmer per annum. The total cost of ₦ 77,317.76 was incurred. Of this, variable cost constituted about 62,188.88. The breakdown of the variable costs is shown in Table 2 below. A financial and Net Income of ₦ 90,848.12 was realized per okra farmer in a year. This is agreement with Oladimeji and Abdulsalam (2014) who found out that dry season irrigated farming in Asa River, Kwara State, Nigeria, has a net margin per hectare of ₦62,501.10 meaning a profitable venture. This indicated that okra production is profitable in the study area. Okra farming is lucrative in the study area with profitability index of 56.38 and a rate of return on investment of 117.50% and operating cost ratio of 38.59%. The okra farmers are primarily interested in selling their outputs to raise income and probably satisfied the household's food need or subsistence. Thus, the okra farmers like any other entrepreneur may have a profit motive.

Table.2: Costs and Revenue Per Okra Farmer Per Annum

VARIABLES	**₦**
TOTAL REVENUE	161,137.00
VARIABLE COST	
Seeds	4,172.06
Fertilizer	9,562.50
Herbicide	2,891.81
Labour	39,375.00
Transportation	6,187.50
Total variable cost	62,188.88
Fixed cost items	
Cutlass	1,950.00
Hoe	1,800.00
Basket	350.00
Land charges	3,000.00
Knife	1,000.00
Total Fixed Cost	8,100.00
SUB-TOTAL	70,288.88
Contingency (10%)	7,028.89
Total Cost	77,317.76
Gross Margin	98,948.13
Net Income (NI)	90,848.12
Profitability Index (%)	56.38
Rate of Return on Investment (%)	117.50
Operating Cost Ratio (OCR) (%)	38.59

Source: Computed from field survey data, 2019

Constraints Associated With Okro Production

The result of analysis of constraints encountered by okra farmers in the study area ranked from most critical to the least showed that pest and diseases took the lead indicated by 17.3%. This was followed by the inadequate transportation facilities (16.2%), weather condition (16%) and high cost of input (14.7%) It is interesting to note that these three constraints identified as most important constraints sum up to over half (58.2%) of the problems of vegetable farmers in the study area. It may be concluded that if these four constraints are looked into, other impediments such as 5th, 6th and 7th constraints may cease to exist or reduce to minimum in the study area.

Table.3: Constraints faced by Okro farmers in the study area

S/N	Constraints*	Frequency	Percentage	Rank
1	Lack of credit facilities	10	13.3	5th
2	Pest and diseases	13	17.3	1st
3	Poor storage facilities	8	10.3	7th
4	Weather condition	12	16	3rd
5	Inadequate capital	9	12.2	6th
6	High cost of input	11	14.7	4th
7	Inadequate transportation facilities	12	16.2	2nd
	Total	75	100.0	

*Multiple responses
Source: Field survey, 2019

V. CONCLUSION AND RECOMMENDATIONS

The study was carried out to investigate the profitability of okro production among small holder farmers in Akinyele Local Government Oyo State, Nigeria. The finding shows that most of the respondents were male and married and they were still at middle-age group On the basis of this study, the major problems faced by the respondents in the study area include, inadequate transport facilities, weather condition and high cost of input.

Based on the study findings, the study recommends the need for government to provide inputs such as chemicals (pesticides and herbicides) planting seeds etc at subsidized rates to farmers and also aim at solving major problem of vegetable production. Also, good road networks should be provided by the government (state or federal, where applicable) to aid easy movement of farm produce to the designated locations.

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A Five-Year Retrospective Study of Small Ruminant Cases Presented to the State Veterinary Hospital, Ibadan Nigeria

Wahab Y. A[1*], Akinlabi E. Y[2], Raheem K. A[3]

[1]Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.
[2]Department of Animal Health and Production Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.
[3]Department of Theriogenology, Michael Okpara University of Agriculture, Umudike, Nigeria.
[*]Corresponding author: wahabyunus.abiola@gmail.com

***Abstract*— A five-year retrospective study was conducted using clinical case record of sheep and goat presented for treatment at State Veterinary Hospital, Ibadan, Nigeria between July 2013 and June 2018. A total of 520 small ruminants were presented within this period.Simple descriptive frequency statistic was employed to determine disease prevalence. Out of the 520 small ruminants presented, 360(69.2%) were sheep and 160(30.8%) were goat. The most frequently diagnosed diseases in these animals were endoparasitism (31.3%), viral infections (18.3%), bacterial infections (15.6%), reproductive conditions (8.3%) and musculoskeletal disorders (7.7%). Prevalence of diseases among small ruminants revealed that endoparasitism (22.5%), viral infections (12.3%), bacterial infections (10.2%),musculoskeletal disorders (6.3%) and reproductive conditions (5.2%) were the most prevalent in sheep while endoparasitism (8.8%), viral infections (5.96%), bacterial infections (5.4%) and reproductive conditions(3.1%) were most prevalent in goats. The study has shown that endoparasitism, viral infections, bacterial infections and reproductive conditions are the leading problem ravaging small ruminants. Therefore, there is need for urgent intervention with special interest towards ensuring proper animal management to control the effect of these agents. It is recommended that livestock farmers should adopt best management practices that will minimize the vulnerability of small ruminants to disease. More so, there should be mass vaccination campaign against preventable diseases like PPR.*

***Keywords*— Disease, goat, prevalence, retrospective study, sheep.**

I. INTRODUCTION

Livestock sectoris one of the major contributors to the overall gross domestic product of most developing nation. It enhances the living of most livestock farmers as it help to generate steady incomes (Ajala *et al.,* 2008). Livestockespecially sheep and goat are very importantto the rural dwellers who rely solely on the turnover from this animals. They are widely distributed in the rural, urban and peri-urban region (Ajala *et al.,* 2008). To a very large extent, they serve as source of employment to an appreciable number of people, such as animal herders, feed millers, butchers, thereby generating wealth and as such contribute to the economy of the nation (Ogbaje *et al.,* 2012). Sheep and goat are very valuable for social function as they are used to fulfil cultural rites during wedding, naming and burial ceremony. Sheep is particularly used during religious festivities, as such they are often regarded as more valuable than goat (Aliyu *et al.,* 2005). Undoubtedly, the contribution of small ruminants to the overall daily protein consumption cannot be underestimated. They serve to augment beef which is the major source of meat for Nigerians (Ajala *et al.,* 2008).However, the small size of the animals and high market price of their meats makes the animals less demanded for regular meat consumption (Lawal-Adebowale, 2012). Nonetheless, little resources is required for purchasing stock animal, land, feed and other necessary requirements needed to start up the production coupled with relatively little risk and tendency to multiply owing to their reproductive efficiency (Omoike, 2006).This makes it more affordable for average poor livestock farmers which contribute larger percentage of the livestock keepers. However, these animals are poorly managed as they are allowed to roam freely on extensive and semi-intensive system. With this system of rearing, the animals are not properly monitored, most often they are faced with malnutrition, exposed to the adversity of extreme weather conditions with little or no access to veterinary care, making them so vulnerable to incidence of various diseases(Lawal-Adebowale,2012).

Diseases remain one of the major threats to the livestock industry. Livestock animals are constantly being threatened by various livestock diseases which in turn affect their optimal productivity (MacRae *et al.*,2005). This is as a result of its negative effect through morbidities, mortalities and abortions or through, quality and the cost of time and money in management of the diseases (Singh & Prasad, 2008).

Despite this worrisome impact on the economy, high prevalence of livestock diseases has often been reported in developing countries like Nigeria, they are mostly caused by microorganism such as bacteria, viruses, parasites, mycoplasma, ricketsia, protozoan and nutritional or managemental factors (Abiola *et al.,* 2016).For instance, parasitism has been reportedas one of the major agents causing serious problem in ruminant in developing countries especially where nutrition and sanitation are poor Odoi *et al.,* (2008), affecting health of millions of animals causing huge economic loss to livestock farmers (Ahmed *et al.,* 2010). Retrospective study of animal diseases is a rapid and cheap means to identify the strategy for effective disease control when analyzed statistically(Abiola *et al.,* 2016).This underscores the significant of this retrospective study of clinical diseases diagnosed at the State Veterinary Hospital, Ibadan, Nigeria. We envisioned that the data generated would give us the recent picture of pattern of disease occurrence which will be useful for epidemiological surveillance and to formulate policies towards proper intervention for disease control.

II. MATERIALS AND METHODS

Study Area

The study was conducted in Ibadan, the capital city of Oyo State, South-western region of Nigeria. The city lies at latitude 7 ˚23' N and Longitude 3˚ 56'E and it is located at the transition zone between the forest and grassland areas of the country. The state veterinary hospital is the only state government owned veterinary hospital centrally located at Mokola area of the cityand it serve as one of the major veterinary hospital affordable to small scale livestock farmers.

Methodology

This five years retrospective study was conducted based on the clinical record of sheep and goat presented to the State Veterinary Hospital, Ibadan between July 2013 and June 2018. The first part of the clinical case record contains information about the patient and the owner and the second part captures the history relating to the case presented, clinical signs observed, clinical parameter recorded, laboratory investigation conducted, disease diagnosed and treatment instituted. Diagnoses were often made based on history, clinical signs presented and laboratory analysis.

Statistical analysis

Data gathered were analysed based on species and disease conditions using simple descriptive statistic.

III. RESULTS

The result of this retrospective study revealed that overall 520 small ruminant cases were presented to the State Veterinary Hospital, Ibadan Nigeria between July 2013 and June 2018. Out of which, 260(69.2%) of the animal presented were sheep and 160(30.8%) were goats (Table 1).

Common clinical conditions diagnosed were classified according to Abiola *et al.,* (2016); bacterial infection (septicaemia, pasteurellosis, foot rot, tetanus), viral infection (Peste de Petit ruminante PPR, Orf, sheep pox), endoparasitism (helminthosis, haemoparasitism and verminous pneumonia), ectoparasitism (mange, tick, fleas and lice infestation), reproductive conditions (dystocia, placenta retention, uterine prolapse, vaginal prolapse, abortion, pyometra, metritis, vulvitis and mastitis), gastrointestinal conditions (bloat, ruminal impaction and rectal prolapse), musculoskeletal disorders (fracture, arthritis and trauma), respiratory conditions (pneumonia) and other conditions (poisoning, snake bite, malnutrition, conjunctivitis, dog bite, hernia, wound, keratitis, toxicosis and minerals deficiency).

Of the 360 cases of sheep diagnosed, endoparasitism was found to be most prevalent 117(32.5%) followed by viral infections 64(17.8%), bacterial infections 53(14.7%), musculoskeletal disorders 33(9.2%), reproductive conditions 27(7.5%), other conditions 24(6.7%), ectoparasitism 17(4.72%), respiratory condition 16(4.4%) and the least prevalent was gastrointestinal conditions 9(2.5%) (Table2).

Table 3 shows the prevalence of condition diagnosed in 160 goats presented. The result revealed that endoparasitism had the highest prevalence of 46(28.8%), followed by viral infections 31(19.4%), bacterial infections 28(17.5), reproductive conditions 16(10%), ectoparasitism 12(7.5%), other conditions 9(5.6%), musculoskeletal disorders 7(4.4%), gastrointestinal conditions 6(3.8%) while the least prevalent was respiratory condition 5(3.1%).

Table 4 gives the summary of cases diagnosed in sheep and goat presented for treatment at State veterinary hospital, Ibadan, Nigeria between July 2013 and June 2018. The total number of cases presented was 520 which include sheep 260(69.2%) and goats 160(30.2%). The result further revealed that endoparasitism had the highest overall prevalence rate of 163(31.3%), followed by viral infections 95(18.3%), bacterial infections 81(15.6%), reproductive conditions 43(8.3%), musculoskeletal disorders 40(7.7%), other conditions 33(6.3%), ectoparasitism 29(5.6%), respiratory infection 21(4.0%) and the least prevalent was gastrointestinal conditions 15(2.9%).

IV. DISCUSSION

From this study, it was revealed that cases of sheep were presented for treatment than goat. The probable explanation for this disparity may be due to the hardy nature of goat compare to the sheep. Goats are reported to be more resistant to common diseases than sheep (Peacock, 2006). It could also implies that more people are keeping sheep compare to the goat probably due to its high market value and the socio-religious value especially during Muslim festive period (Aliyu *et al.,* 2005).This finding is similar with the report of Peter *et al.,* (2015) whose study at the state veterinary hospital, Maiduguri, Nigeria recorded more casesof sheep than goats. It also corroborates Unigwe *et al.,* 2016 who had earlier reported that more cases of sheep were presented to Mokola veterinary hospital between July 2009 and June 2013. However, our finding is in contrast with the work of Abiola *et al.,*(2016) where they reported more cases of goat than sheep at the University of Ibadan veterinary teaching hospital. Meanwhile, our study recorded more cases when compare to the previous work conducted in this location. Unigwe *et al.,*(2016) had reported that a total of271 small ruminant cases were presented to Mokola veterinary hospital from January 2009 to June 2013. This significant increase in cases presented within similar period of study could be ascribed to the palpable increased sensitization by the government and the professional body of veterinarians through mass media and seminars for the livestock owners. This strategy has been proven to improve livestock production and disease management (Buhari *et al.,* 2015).

The current study also found that endoparasitism is the predominant condition affecting sheep and goat, consistent with what was documented in previous reports (Peter *et al.,* 2015; Abiola *et al.,* 2016; Unigwe *et al.,* 2016), these authors have reported endoparasitism as the most prevalent small ruminants disease in their retrospective studies.The preponderance of helminthosis cases among small ruminant could be traced to the nature of the rearing system in this study location where small ruminants are allowed to roam freely during which they are often exposed to this parasitic agent. This system of rearing coupled with malnutrition makes small ruminants more vulnerable to parasitic infection. Parasitism has been reported as one of the major agents causing serious problem in ruminant in developing countries especially where nutrition and sanitation are poor (Odoi *et al.,* 2008). The second most prevalent disease was viral infections predominantly peste des petit ruminante (PPR) infection. This finding is in agreement with the report of Diallo *et al.,* (2007) that PPR disease is enzootic in several countries of West Africa, contributing to high economic loss in small ruminant production. PPR is highly contagious in nature with case fatality rate of 100%, it is therefore a major concern among small scale livestock keepers who rely on small ruminants as sole source of income (Emikpe & Akpavie, 2011). It is a vaccine preventable disease and its vaccine is considered to be one of the most effective vaccines ever produced against animal diseases (Diallo *et al.,* 2007). Therefore, the prevalence of this disease in this location could be due to the fact that majority of the animal affected has no previous exposure to PPR immunization. The next most prevalent condition was found to be bacterial infections. This could also be connected to the free range or extensive system of rearing that predominates in this region. It also corroborates the report of Unigwe *et al.,* (2016), that high prevalence of bacterial infection in small ruminants may be due to the exposure of these animals to kitchen waste, decomposed dead animals and grazing on pastures and rangelands littered with various dead animate objects. Reproductive conditions followed bacterial infections in the order prevalence, with dystocia constituting majority of the reproductive conditions recorded in this study. The high occurrence of this condition is attributed to lack of proper monitoring of female animals especially those at reproductive ages, majority of this farmers has no breeding record, as such the animals are bred indiscriminately and often prematurely leading to dystocia. This is in tandem with the report of Abiola *et al.,*(2016), they reported high prevalence of dystocia as reproductive conditions presented to University of Ibadan veterinary teaching hospital.We also found that musculoskeletal disorder cases were moderately high and trauma (fractures) was the leading cause. This could be easily linked to the fact that small ruminants are often left unguided while they scavenge or graze around, thereby making them more vulnerable to automobile accidents. The least prevalent condition in this study was gastrointestinal conditions such as bloat and other conditions (poisoning, snake bite, malnutrition, conjunctivitis, dog bite, hernia, wound, keratitis, toxicosis and minerals deficiency). This observation is also consistent with the report of Abiola *et al.,* (2016), and this could mean that the owners have been managing these cases with usage of ethno-veterinary medicine (Sandabe *et al.,* 2006).

V. CONCLUSION

In this study, it is evident that endoparasitism, viral infections, bacterial infections and reproductive conditions are the leading problem ravaging small ruminants. This therefore calls for urgent intervention with special interest towards ensuring proper animal management to control the effect of these agents. It is recommended that veterinary services should be strengthened, accessible and affordable for low income livestock farmers. Mass vaccination against preventable disease like PPR should be routinely done. Livestock farmers should adopt best management practicesthat will minimize the vulnerability of small ruminants to disease. More so, livestock farmers should be sensitized on importance of keeping farm record especially breeding recordin order to mitigate the effect of economic loss associated with these conditions.

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Table 1: Prevalence of small ruminants presented to the StateVeterinary Hospital in Ibadan, Nigeria between July 2013 and June 2018.

Species	Number	Prevalence (%)
Sheep	360	69.2
Goat	160	30.8
Total	**520**	**100**

Table 2: Prevalence of condition/diseases of sheep presented tothe StateVeterinary Hospital in Ibadan, Nigeria between July 2013 and June 2018.

Diseases	Number of cases	Prevalence (%)
Bacterial	53	14.7
Viral	64	17.8
Endoparasitism	117	32.5
Ectoparasitism	17	4.12
Reproductive	27	7.5
Gastrointestinal	9	2.5
Respiratory	16	4.4
Musculoskeletal	33	9.2
Others	24	6.7
Total	**360**	**100**

Table 3: Prevalence of condition/diseases of goat presented tothe State Veterinary Hospital in Ibadan, Nigeria between July 2013 and June 2018.

Diseases	Number of cases	Prevalence (%)
Bacterial	28	17.5
Viral	31	19.4
Endoparasitism	46	28.8
Ectoparasitism	12	7.5
Reproductive	16	10
Gastrointestinal	6	3.8
Respiratory	5	3.1
Musculoskeletal	7	4.4
Others	9	5.6
Total	**160**	**100**

Table 4: Summary of prevalence of condition/diseases of sheep and goat presented tothe StateVeterinary Hospital in Ibadan, Nigeria between July 2013 and June 2018.

Diseases	Sheep(%)	Goat (%)	Total (%)
Bacterial	53(10.2)	28(5.4)	81(15.6)
Viral	64(12.3)	31(5.96)	95(18.3)
Endoparasitism	117(22.5)	46(8.8)	163(31.3)
Ectoparasitism	17(3.3)	12(2.3)	29(5.6)
Reproductive	27(5.2)	16(3.1)	43(8.3)
Gastrointestinal	9(1.7)	6(1.2)	15(2.9)
Respiratory	16(3.1)	5(0.96)	21(4.0)
Musculoskeletal	33(6.3)	7(1.3)	40(7.7)
Others	24(4.6)	9(1.7)	33(6.3)
Total	**360(69.2)**	**160(30.8)**	**520(100)**

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Growth response of pre-sprouted seedlings of sugarcane in the presence of the bacterium *Herbarspirillum frisingense*

Gabriel Vieira Reis, Susana Cristine Siebeneichler, Bruna Lazzaretti, Allan Sales de Sousa, Melkisedeck Almeida Resplandes, Raffael Batista Marques, Ádila Pereira de Sousa, Márcia Fernanda Rocha Santos, Laina Pires Rosa and Magno De Oliveira

Plant Ecophysiology lab, Universidade Federal do Tocantins, rua Badejós, Lote7, Chácaras 69/72, s/n - Zona Rural, Gurupi, 77402-970, Tocantins, BRAZIL Tel.: 6398130-1376

*Corresponding author: Gabriel Vieira Reis; E-mail: gabriel.reis@mail.uft.edu.br

***Abstract*— *The objective was to evaluate the effect of bacterium Herbaspirillum frisingense on growth of the seedlings pre-sprouted from sugarcane. After you select the varieties of sugarcane, the necessary treatments of nodal segment obtained from reeds, to germinate and be transplanted to the cells half in soil substrate of gully and other half in soil ofgullies more Jatropha pie, where was subsequently applied doses of bacteria (9,33x10^{7} ufc.ml^{-1}). Five months after this process, plants were harvested and evaluated the dry mass of shoot and root dry mass. Data were analyzed by Tukey, a 5%, where he met difference for root dry mass in relation to the presence of bacteria and to dry pasta from the shoot to the type of substrate. No statistical difference was observed between cultivars, even these being different cycles. In relation to interaction varieties vs. bacteria statistical difference was observed between the cultivars to root dry mass. The presence of the bacteria inside the plant tissues of seedlings of sugarcane was beneficial for the plant, promoting a greater root growth. The soil substrate of gully more Jatropha pie turned out better for the cultivars in general.*

Keywords*— Endophytic bacteria; seedling production; Saccharum officinarum L.

I. INTRODUCTION

The sugarcane (*Saccharum officinarum*), culture introduced in Brazil in the 16th century, has great economic importance in the country, according to cultivable areas and ideal soil and climate conditions the production, making the country a participant in World marketing with high competitiveness [1, 2].

According to the National Supply Company, Brazil is the largest producer of sugar cane with a productivity expected to yield the 2018/19 of 66.007 kg/ha with a harvested area of 30, 3 thousand hectares and a total estimated production 625, 96 million tons, much of this production will be destined to the production of ethanol is expected to reach a total of 154, 96 million liters [2].

It is known that much of the production is concentrated on the South and Midwest of the country, however, in the North, the Tocantins is regarded as the new agricultural frontier, offering areas, weather, and water conditions. The State owns one of the main natural elements that favor the productivity of sugarcane, the luminosity, since, under the conditions of the State climate factor that tends to increase the number of profiling [3].

The system of seedlings pre-sprouted (SPS), or results pre-germinated in a correct planting, with healthy seedlings and that there is no competition between plants online, resulting in better initial growth. The technique can be used by all producers, small medium and large, for this is a simple, low-cost methodology allowing the planting of reestablished seedlings, and avoiding competition between sounds plants [4].

To increase the productivity, are being carried out studies and research of bacteria capable of contributing to a better synthesis initial growth of seedlings pre-germinated. Endophytic bacteria have numerous functions for the plant, acting beneficial and showing satisfactory results and significant. Bacteria that inhabit the interior of the plant tissue can contribute effectively to the biological fixation of nitrogen, since the exchange is done directly, having this way a lesser competition for carbon sources, noting that not all micro-organisms are able to penetrate plant tissue[5].

Bacteria of the genus Herbaspirillum are described as diazotrophic, able to fix nitrogen from the atmosphere under microaróbicas conditions and use of this resource to meet all your demand for this nutrient when compared to the other species of diazotrophic bacteria these are the least of it. Representatives of this genus are considered compulsory and feature low endophytic bacteria survival in soil [5, 6].

Was used to perform this experiment to frisingense of the same genre, which behaves similar to H. seropedicae. For this are already found numerous studies because it is a bacterium that is associated with various agricultural plants and promotes your growth H. frisingense can be isolated within plant tissues disinfected surface, causing damage to the host [7].

Several studies have shown the potential of endophytic bacteria for biological control of diseases, pests, and in promoting the growth of the host plant, being beneficial to the plant. In view of the favorable conditions to sugar cane production, the use of diazotrophic bacteria combined with technologies such as pre-sprouted seedlings production are possible alternatives to increase the productive potential of the Tocantins.

II. MATERIALS AND METHODS

The experiment was conducted in the experimental area of the University Campus of Gurupi from the Federal University of Tocantins, located 280 m altitude, 11º43'S e 49º04'W, where were grown seedlings. We have selected three varieties of sugar cane, and varieties of the early cycle: CVSP077231, a median: CTC4, and a belated: IACSP955000.

At the end of the month of January 2015, the sugarcane separated and the preparation of nodal segments wheels was done according to the methodology presented by [4]. First, it was made the cut of the cane in 3 cm segments containing the node in a hand-crafted guillotine that allowed the cut in a standard size. These nodal segments have undergone heat treatment, subject to water, with constant temperature of 50 C for a period of 30 minutes. After the heat treatment, the nodal segments were placed in trays of sprouting, being separated by cultivating lined up and over to the top, where it contained as substrate: a bucket and a half of commercial substrate Bioflora®, half a bucket of sand and kept in a greenhouse in a period of 10 days, when he had already finished the sprouting.

Twenty days after sprouting the seedlings were transplanted to the cells where the half was planted in cells that had as soil substrate of gully Jatropha pie and the other half in cells just with the soil of gully. Resulting in a total of 275 cells. Data were collected as the greater length and diameter of the stem and soil samples to analyze the fertility of both substrates.

After the transplanting of seedlings of sugarcane to the cells has been prepared the bacteria solution $9{,}33x10^7$ ufc.ml were applied to seedlings, 5 ml of bacteria per cartridge, using an automatic pipette. 10 days after transplanting of seedlings to the cells the first pruning in all the seedlings and was done on a weekly basis due to your accelerated growth. The monitoring of seedlings development weekly.

It required the application of commercial fertilizer, MatoVerde® 15-15-20, in the proportion of 14 g of the product in 7 L of water to 25 ml in each cell, applied every two weeks and then monthly, with the aid of a 30 ml syringe.

Five months after transplanting was accomplished the collection of plants, separating them in the root system and aerial. The shoot was dried in an oven of forced circulation the 60 °C until constant weight and then the heavy semianalítica balance was. The roots were washed under running water with care to avoid losses and after washing, they were also dried in an oven of forced circulation the 60 °C until constant weight, for determining dry mass.

The data of the variables of dry pasta from the shoot and root dry mass were analyzed with the help of the Sisvar program, through the Tukey test, the 5% probability.

III. RESULTS AND DISCUSSION

Generally, the difference was found for root dry mass in relation to the presence of the bacterium; and for dry pasta from the shoot to the substrate type (Table 1).

To the implementation conditions of this study, the results of the combined analysis of variance (Table 1) not show significance ($p < 0.05$) for the triple interaction of the factors' substrate x cultivars x bacteria (BAC x SUB x CULT) for the characters evaluated, indicating that the bacteria interacted with the substrate in root dry mass and substrate interacted with the cultivars for the same variable.

Table1. Summary of the analysis of variance. ADM (Aerial Dry Mass) and RDM (Root Dry Mass) of cultivars of sugarcane in relation to presence of bacteria in the soil substrates of gully and gully solo more Jatropha pie.

Cause of variation	**GL**	**Mean Square**	
		ADM	**RDM**
Bacteria (BAC)	1	0,0002^{ns}	0,0655*
Substrate (SUB)	1	1,2117**	0,0065^{ns}
Cultivar (CULT)	2	0,0983^{ns}	0,0038^{ns}
BAC*SUB	1	0,0085^{ns}	0,0181^{ns}
BAC*CULT	2	0,0696^{ns}	0,0411*
SUB*CULT	2	0,1158^{ns}	0,0462*
BAC*SUB*CULT	2	0,0177^{ns}	0,0106^{ns}
Error	88	0,0526	0,0125
Average	----	0,9811	0,3835
CV (%)	----	23,38	29,18

* and ** significant at the level of 5 and 1% probability of error by Tukey test, respectively; NS not significant at the 5% level of error probability by Tukey test.

No statistical difference was observed between cultivars; even these being of different cycles, aprecocious, median and late(table1).

Table2. Average test to the dry mass of the shoot (ADM) seedling of sugar cane in the land for construction and ground substrates for construction more Jatropha pie.

Substrate	**Average**
Jatropha pie	1,0870 a
Soil of Gully	0,8751 b

Medium followed by the same letter doesn't differ by Tukey test at 5% probability.

For the substrate difference to the dry mass of the aerial part already in the root, there is no difference between the substrates (table 1), where the plants were grown with the substrate soil of gully had the higher fresh mass of the shoot (table 2). [8] evaluating different substrates is a difference for the parameters of above ground (leaves, diameter, and height) of sugarcane of early and medium-late cycle corroborating with the research data confirming that the growth of sugarcane suffers influences of a substrate.

For root dry mass, cultivars IACSP955000 showed no difference with the presence of the bacteria, perhaps because it is a cultivar of the late cycle requires a longer time to demonstrate the effect. The cultivars CVSP077231 e CTC4 presented larger in relation to average values without inoculation, showing that there.

*Table.3. Average values for the interaction and Grow Bacteria (BAC * CULT) to root dry mass (MDR), in seedlings of sugarcane.*

Cultivars			
Bacteria	**(IACSP955000)**	**(CVSP077231)**	**(CTC4)**
Without	0,3661 aA	0,3450 bA	0,3356 bA
With	0,3983 aA	0,4439 aA	0,4122 aA

Medium followed by the same letter, lowercase letters in columns and capitals do not differ by Tukey test at 5% probability.

The difference for each of the cultivars with and without bacteria can give by symbiosis established between them, thus showing the effect on two of them.

The canes grown on substrate Jatropha pie did not differ statistically between them- selves, and in soil of gully the best result was to cultivate CVSP077231, and the other did not demonstrate statistical difference among themselves (Table 4), perhaps because it is a cultivar of early cycle, growing faster than the other.

*Table4: Average values for the Substrate interaction and Growing (SUB * CULT) to root dry mass (RDM), in seedlings of sugarcane.*

	Cultivares		
Substrate	**IACSP955000**	**CVSP077231**	**CTC4**
Soil of Gully	0,3739 aB	0,4228 aA	0,3306 bB
Jatropha pie	0,3906 aA	0,3661 aA	0,4172 aA

Medium followed by the same letter, lowercase letters in columns and capitals; do not differ by Tukey test at 5% probability.

Jatropha pie consists of up to 69.7% of the mass of seeds and is a by-product of oil extraction composed of cellulose, hemicellulose, lignin, extractive s, water, nutrients, forbol and curcina esters with properties compared to other fertilizers organic, highlighting the amount of soluble in neutral detergent fiber (NDF), acid detergent soluble fiber (FDA), cellulose and crude protein [9], being because of those factors that it was better to the culture and specifically for cultivating CTC4, acting as a source of organic matter for the plant to more than just in soil of gully.

The bacterium promoted initial root system effect, perhaps due to greater endophytic colonization initially in the roots, as this just bacterium infects roots, stalks and leaves of grasses, not being found in leaves of sugar cane [10]. However, the mechanisms of these beneficial associations between plants and the bacterium Herbaspirillum frisingense are still little known. Working with different diazotrophic bacteria, [11], stated that the positive interaction of the bacteria with the root growth of Brachiaria brizantha plants that can contribute to the improvement of the global acquisition of nutrients and water to the plant and the production of biomass.

And that the diazotrophic bacteria colonizers, as h. seropedicae, promotes root growth through various factors such as greater enzyme nitrogenase activity (ARA), phosphate solubilization, hormone production 3-indole-3-acetic acid (AIA), among others as confirmed by [11]. It is known that this hormone has influence on plant growth, especially in the root system [12] which explains the greater root dry mass in plants inoculated with H. frisingense (table 1).

IV. CONCLUSION

There is a beneficial effect on the use of *Herbaspirillum frisingense* bacteria in sugar cane, promoting changes in plant growth, mainly in root development, independent of cultivar tested.

The substrate of Jatropha pie turned out better for the cultivars in relation to the soil of gully, due to have higher concentration of nutrients, providing better conditions for the development of plants.

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Effectiveness of broadcast Agricultural Programmes on Agricultural Development among Farmers in Akoko South West Local Government Area of Ondo state, Nigeria

Ogunsola J.O[1], Ogunsola A.J[2], Alarape A.B[3], Oloba O.G[4], Osalusi C.S[5]

[1,3,4,5]Federal College of Forestry, Jericho Hills, Ibadan, Oyo State, Nigeria
[2]Forestry Research Institute of Nigeria
Email: omotola_ogunsola@yahoo.com

***Abstract*— *The study assessed the effectiveness of broadcast agricultural programmes on agricultural development in Akoko South-West Local Government Area of Ondo State, Nigeria. A multistage sampling technique was used in selecting 140 respondents for the study. Specifically, socio-economic characteristics, source of information and perception of broadcast agricultural programmes were examined. Data were collected using a well-structured questionnaire and were analyzed using both descriptive and inferential statistical tools. The results of the study revealed that majority (82.6%) of the farmers are married and more than half of them are male (57.0%). Television (91.9%), Radio (89.9%) and farmers association (82.6%) were revealed to be the major sources of information. The results also shows that farmers have a highly positive (62.9%) of the effect of broadcast agricultural programmes on agricultural development. The results of the hypothesis shows a significant relationship between age (p=0.034), education (p=0.015) and income (p=0.026) and the perception of farmers on broadcast agricultural programmes. The study concluded that broadcast agricultural programmes is effective in ensuring agricultural development in the study area and recommended that policies should be made to make broadcast stations more effective and more adoptive to agricultural programmes.*
***Keywords*— Agricultural programmes, Broadcast, Nigeria, Radio, Rural farmers, Television.**

I. INTRODUCTION

Agriculture provides the main source of foreign exchange for Nigeria and is an important sector of the economy with high potentials for employment generation, food security and poverty reduction. Oluigbo, (2012) in Obiora & Emordi, (2013) noted that up to the early 1970s, agriculture accounted for over 80 percent of Nigeria's Gross Domestic Product (GDP) but the discovery of crude oil in the late 1960s and the huge financial gains benefitted from it made the government to shift its priority from agriculture to crude oil and relied on food importation as a means of feeding her citizens. Consequently, this development affected agricultural production and extension services. Sadly, the price of crude oil in the world market started falling in 2015 and Yusuf (2016), explained that over $21bn was lost in capital investment in 2015 alone due to the sharp decline in oil receipts, and exploration investments was drying up, thereby affecting foreign reserves and straining fiscal budget. Following the reduction, Nigerians became apprehensive of the effects and there were calls for diversification. For instance, Moghalu (2016) noted that despite the efforts of successive governments in Nigeria, there have been high rates of poverty, unemployment and infrastructure deficits, and counseled that government must build a sustainable economic future by looking beyond oil. Agriculture has been described as the mainstay of economic growth in Africa. It is the single largest contributor to the wellbeing of the rural poor in Nigeria, sustaining 90% and 70% of the rural and total labour force respectively (Akpabio, 2005). Despite the pivotal role of agriculture in the nation's economy, it contributes only 38% of the GDP (Gross Domestic Product).

Rural broadcasting is a recent concept in Nigerian broadcasting history and it is a phenomenon that is prevalent in the developing or emerging world. The concept takes cognisance of the larger percentage of the rural population when compared with the urban residents. Onabajo (2002) opined that development that does not include attitudinal change that will respect the dignity of labour and the propensity towards social change and a self-reliant economy is at best incomplete. Rural education is a subset of rural development and rural broadcasting can only but have one major focus which is that of transmitting developmental information to educate the rural citizenry with overall intention of developing their wellbeing, through introducing innovative ideas that will stimulate them to action. Rural development projects employing rural broadcasting should be made relevant to the needs of the societies. The nation's rural communities have been identified as not only the source of the country's food basket, but also the source of raw materials supply for the industrial sector (Amusat, 2012). Hence, different agricultural programmes aimed at improving agricultural production and rural development was embarked upon.The success of agricultural development programmes in developing countries largely depends on the nature and extent of use of mass media in mobilizing farmers for the need for development. Communication has been acknowledged for playing, a prominent role in the success of agricultural production and adoption of innovations. The planners in developing countries realized that the development of agriculture could be hastened with the effective use of mass media. Radio and Television have been acclaimed to be the most effective media for diffusing scientific knowledge to the masses. In the study area where literacy level is low, the choice of communication media is of vital importance.In this regard, the television and radio are significant, as they transfer modern agricultural technology to literate and illiterate farmers alike even in interior areas, within short time to enlighten farmers on the use of various technologies to boost agricultural development. The farmers can easily understand the operations, technology and instruction through the radio. Despite all these claims, little have been done to ascertain how effective these broadcast agricultural programmes have been in agricultural development especially in the study area and this study was aimed at revealing the perception of the farmers on how effective these programmes have been in addressing agricultural development.

II. METHODOLOGY

The study was carried out in Akoko South-West local government area in Ondo State, Nigeria. Akoko South-West Local Government Area lies roughly between latitude 7º 20'N and 7º 30'N and longitude 5º 30'E and 5º50'E. It is located on a dissected plateau and is made up of many undulating hills and valleys. It covers an area of about 30 square kilometers. The local government has a mean annual rainfall of 1,270mm and mean annual temperature of 21.1oc, which results in a very humid condition. Because of the favorable climatic condition, there is abundant vegetation cover. With a population of about 22, 9486 according to 2006 population census, the study area is made up of more than 10 villages. About two third of the population is engaged in farming and most of the farmers are illiterate with very low income. Because of the rural nature of the local government, Akoko South-West is one of the three (3) various hills and plateau that cover the whole area and this has generally affected the productive focal local government areas that UNICEF chose to sponsor projects in. A multistage sampling procedure was used in selecting respondents for this study. The first stage involved the purposive selection of Akoko South west local government area due to its rurality and dominance of farmers in the area. The second stage involved the simple random selection of 50% of the total number of wards in Akoko Southwest local government. The third stage involved the random selection of two (2) villages each from each of the wards giving a total of twelve (14) villages used for the study. The fourth stage involved the simple random selection of ten (10) farmers from each of the villages giving a total of one hundred and forty (140) respondents used for the study. Data for this study was collected using a well-structured questionnaire. Descriptive statistical tool and inferential statistical was used for this study. Descriptive statistical tools such as frequency, percentages e.t.c was used. Descriptive statistics was used to reveal the socio economic characteristic while, PPMC (Pearson Product Moment Correlation) was used to analyzing the hypothesis.

III. RESULTS AND DISCUSSION

The result on Table 1 shows the result of the socio economic characteristics of the respondents examined which includes age, sex, marital status, level of education, level of income per month, source of labour, household size, religion, years of farming and involvement in agricultural groups. It was revealed from the table that 29.8% of the respondents were within the age of (31-40) years, followed by those within (21-30) years with 23.5% of the respondents, and while those above 50 years are 23.1% of the respondents and 7.4% of the respondents were below 20 years of age. This is in line with Ofuoku*et al.* (2008) who also reported that most of the

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respondents involved in farming activities belonged to middle age category which is the active age wherein productivity increased. Also, about 57.3% of the respondents are male and 42.9% were female which implies that male are more involved in farming more than their female counterpart and this may be due to the fact that the male is the head of the family and he needs to provide for the needs of his family. This result corroborate with Ndaghu*et al.* (2009), who found that most farm families in the rural areas are headed by males and are responsible for most production decisions. In addition, the results reveals that 82.6% of the respondents were married, 7.4% of the respondents were single, 5.3% of the respondents were widowed while 2.7% of the respondents were divorced. This is also in line with Ndaghu*et al.* (2009) who reported that most farmers in the rural areas were married. Furthermore, the result shows that 46.6% of the respondents were Christians, 34.9% of the respondents were Muslims while 15.4% of the respondents were traditionalists. This result revealed that there is no religious belief when it comes to sourcing for information in agriculture. Also, 42.9% of the respondents were within the range of (6-10) household size, 38.9% of the respondents range between (0-5) household size, 8.0% of the respondents range between (11-15) household size, 6.7%of the respondents range between (16-20) household while 3.4% of the respondents range between (above 20) household. This table further shows that the educational achievement of the respondents as followed: Secondary education (51.0%), tertiary education (15.4%), Primary education (12.0%), Adult education (11.4%) and No formal education (10.1%) was achieved by the respondents in the study area. This reveal that those with Secondary education and tertiary education are dominant in the study area. This implies that educational level of the respondents will mostly determine the level of information adoption compare to the illiterates. This result also reveals that 55.7% of the respondents make use of family labour while 44.3% of the respondents were hired labour for their farm work. It is clearly shows in this result that the farmers were been gotten from family labor because it is a cheaper means and the kind of relationship that exists in the family because it maybe monogamy or polygamy kind of family. This implies that majority of the respondent usually married more than one wife so that they can give birth to more children which will help them in farming activities. Moreover it also shows in table 4.1 that 59.0% belong to agricultural group while 40.9% does not belong to any group. This implies that majority of the respondents are involved in agricultural group such as cooperative societies which make it easy for them to source for information about their agricultural activities in the farm and access credit facilities. Lastly, it shows in the table above that 22.8% of the respondent have farming experience below 5 years, 45.0% were (5-10) while 32.1% were above 10years which implies that increase in yield mostly determine by the level of experience about that activities. In addition in finally revealed in the table that 50.3% of the respondents estimated 10,000 as their monthly income while 34.9%, 8.7%, and 6.0% estimated 20,000, 30000 and others respectively. This result implies that farming occupation is highly profitable based on the finding in the study area.

TABLE 1: SOCIO ECONOMIC CHARACTERISTICS OF THE RESPONDENTSIN THE STUDY AREA

Variables	**Frequency**	**Percentage**
AGE		
Below 20	11	7.4
21-30years	35	23.5
31-40years	40	29.8
41-50years	23	15.4
>50	30	23.1
SEX		
Male	76	57.0
Female	64	42.9
MARITAL STATUS		
Single	11	7.4
Married	123	82.6
Divorce	1	2.7
Widow	3	2.0
Widower	2	5.3

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RELIGION		
Christianity	65	46.6
Islamic	52	34.9
Traditional	23	18.4
HOUSEHOLD SIZE		
0-5	56	38.9
6-10	60	42.9
11-15	9	8.0
16-20	10	6.7
> 20	5	3.4
LEVEL OF EDUCATION		
No formal education	15	10.1
Secondary education	70	51.0
Adult education	17	11.4
Primary education	15	12.1
Tertiary education	23	16.4
SOURCE OF LABOUR		
Family labour	77	55.7
Hired labour	63	44.3
ARE YOU A MEMBER OF ANY AGRICULTURAL GROUP?		
Yes	82	59.0
No	58	40.9
YEARS OF FARMING EXPERIENCE		
Below 5 years	34	22.8
5-10 years	67	45.0
> 10 years	39	32.1
INCOME PER MONTH		
#10,000	75	50.3
#20,000	43	34.9
#30,000	13	8.7
others	9	6.0
Total	**140**	**100**

Results from table 2 shows the findings based on the sources of agricultural information among rural farmers in the study area, where the result reveal that 91.9% of the respondent are using Television and 89.9% of respondent are using Radio to source for information this is in line with (Ajayi, 2003) that the use of radio and television has been found to be a major source of information to farmers in South West of Nigeria. It was also shown that majority of the respondent did make use of Farmers association (82.6%) as source of information while (80.9%) of the respondent make use of the Neighborhood, Result also shows that 64.1% of the respondent got their information from internet and 62.4% are making use of newspaper as their source of information in the study area (Adekoya, 2000). Furthermore, the result reveal that most of the farmers (60.4%) make use of the extension agent and Agricultural research institute in sourcing for information, which show that the extension workers and research institute may be closed to the farmers in the study area, also 59.7% of respondent make use of market, likewise 47.3% of the respondent make use of posters in getting information in the study area.

TABLE 2: Sources of information among rural farmers in the study area

Variable	Yes F (%)	No F (%)
Agricultural research institute	90(60.4)	50(39.5)
Neighborhood	119(80.9)	21(19.1)
Radio	134(89.9)	6(10.0)
Television	137(91.9)	3 (8.0)
Farmers association	123(82.6)	17(17.4)
Newspaper	93(62.4)	47(37.5)
Internet	94(64.1)	46(35.9)
Market	83(59.7)	57(40.3)
Poster	69(47.3)	71(52.7)
Extension agent	84(60.4)	56(39.6)

The results on table 3 shows the agricultural information available among the rural farmers in the study area where the result reveal that 89.9 % of the respondent confirmed that information on pest and disease control measure were available to them through broadcast agricultural programmes and 89.3% of respondents said Information on the teaching on agronomic cultural practice on crop varieties, Information on how and when to plant were available to them through broadcast agricultural development programmes. It also revealed 86.6% of the respondent got Information on improved ways of processing and storing agricultural products available through broadcast agricultural development programmes while (82.6%) of the respondent said information on how and when to harvest Agricultural products were available to the through the same programme. Result also shows that 31.5% of the respondent did not get information on improved animal breeds through broadcast agricultural development programmes. 68.5% of the respondents got information on improved animal breeds available to them through broadcast agricultural development programmes.

TABLE 3: Information available for the farmers in the study area.

VARIABLE	FREQUENCY	PERCENTAGE %
Information on pest and disease control measure		
Available	134	89.9
Not available	6	10.0
Information on improved crop varieties		
Available	125	83.9
Not available	15	16.1
Information on the teaching on agronomic cultural practice on crop Varieties		
Available	133	89.3
Not available	7	10.7
Information on Fertilizer applications		
Available	123	82.6
Not available	17	17.4
Information on improved ways of processing and storing agricultural products		
Available	129	86.6
Not available	11	13.4
Information on how and when to plant		
Available	133	89.3
Not available	7	10.7

Information on method of livestock rearing		
Available	105	70.5
Not available	35	23.5
Information on improved animal breeds		
Available	102	68.5
Not available	38	31.5
Information on improved agricultural processing and storage machines		
Available	118	79.2
Not available	22	20.8
Is there information on how and when to harvest Agricultural products		
Available	123	82.6
Not available	17	17.4
TOTAL	**140**	**100**

The results from table 4 shows the perception of the respondents towards knowledge acquired through broadcast agricultural development programmes in the study area. The respondents agreed that Constant listening to broadcast agricultural development programmes on radio and television contributes to their farm's productivity with mean value 4.56, also the mean value 4.12 shows that the Listening to broadcast agricultural programmes has helped in improving and knowing how and when to plant my seeds for maximum yield, likewise the mean value 4.08 agreed that Farmer's in the study area tends to aquire more knowledge in watching farm broadcast programme on television rather than radio, this is in line with (Dauda, 2009) that if the information pass through good medium to the farmers will yield a good result in their production. The result also shows that Broadcast agricultural development programmes teaches the farmer 's on how to select viable seeds for planting with mean value 3.96, and the mean 3.93 for Information on weed control has reduces the cost of labour. Listening to broadcast agricultural development programmes is time wasting with mean value of 3.77. Also the results reveals that broadcast agricultural development programmes equipped the farmer's on how to cultivate crops in the study area with mean value of 3.65.

TABLE 4: PERCEPTION OF THE RESPONDENTS TOWARDS KNOWLEDGE ACQUIRED THROUGH BROADCAST AGRICULTURAL PROGRAMMES.

VARIABLE	**SA F (%)**	**A F (%)**	**U F (%)**	**D F (%)**	**SD F (%)**	**Mean**
Constant listening to broadcast agricultural Development programmes on radio and television contributes to my farm's productivity	45(33.2)	72(51.3)	5(10.1)	8(5.4)	-	4.56
Broadcast agricultural programme provides relevant and current farm information.	48(32.2)	79(59.0)	10(6.7)	3(2.0)	-	3.81
Agricultural information content on the farm Broadcast programme are easy to utilize at the farm level.	37(24.8)	92(67.7)	4(2.7)	7(4.7)	-	3.76
No new farming techniques are learned from the broadcast agricultural development programmes.	27(18.1)	35(26.5)	14(9.4)	64(46.0)	-	3.77
Television farm broadcast programmes are useful only for the elite farmers.	27(18.1)	41(27.5)	7(7.7)	65(46.6)	-	3.69

Radio farm broadcast programmes cannot lead to easy adoption of new farming practices and techniques by non-literate farmers.	22(18.8)	27(20.1)	22(14.8)	69(46.3)	-	3.94	
A farmers' religion does not influence listening to broadcast agricultural development programmes.	32(25.5)	72(50.3)	10(6.7)	26(17.4)	-	3.77	
Farmers' social and economic attainment in the community determines which farm broadcastprogramme they listen to or watch.	24(19.1)	71(50.7)	30(20.1)	15(10.1)	-	3.70	
Advertisement and jingles played during farm broadcast programme interfere with the smooth running and understanding of the information content.	37(24.8)	72(54.3)	17(11.4)	14(9.4)	-	3.81	
A farmer is easily convinced to adopt a recom-mended innovation from listening to or viewing farm broadcast programmes.	26(17.4)	38(25.5)	14(9.4)	62(47.6)	-	3.71	
To obtain information from broadcast agricul-turalprogrammes is expensive and time consuming.	26(17.4)	43(28.9	3(3.0)	68(50.6)	-	3.73	
Information on improved crop varieties has been able to increase my farm productivity.	27(18.1)	92(66.7)	16(11.7)	5(3.4)	-	3.46	
Information on fertilizer has been able to help improve my crop yield.	42(28.2)	93(68.4)	4(2.7)	1(0.7)	-	3.49	
Listening to broadcast agricultural programmes has helped in improving and knowing how and when to plant my seeds for maximum yield.	23(19.4)	114(78.5)	3(2.0)	-	-	4.12	
Broadcast agricultural development programmes equipped the farmer's on how to cultivate?		5(3.4)	119(80.9)	16(15.7)	-	-	3.65
Broadcast agricultural development programmes teaches the farmer 's on how to select viable seeds for planting.		34(22.8)	94(66.1)	5(6.4)	7(4.7)	-	3.96
Listening to broadcast agricultural development programmes is time wasting.		11(7.4)	43(28.9)	19(13.8)	67(50.0)	-	3.77
Information on weed control has reduces the cost of labour	22(19.8)	104(70.8)	9(6.0)	5(3.4)	-	3.93	
Farmer's tend to aquire more knowledge in watching farm broadcast programme on television rather than radio	15(10.1)	114(80.5)	5(3.4)	6(6.0)		4.08	

Broadcast agricultural development programmes didn't bring about any development to the farmers productivity	26(17.4)	38(25.5)	76(57.0	-	-	3.47

IV. CONCLUSION

Based on the findings from the study, it was discovered that broadcast agricultural programmes is effective in ensuring agricultural development in the study area. This is evident in the dominant perception of the rural farmers who reported positive disposition towards the effectiveness of broadcast agricultural programmes on agricultural development coupled with their high level of exposure to agricultural broadcast programmes in the study area. Despite all these attributes, farmers in the study area still had challenges in accessing these broadcast agricultural programmes on a regular basis owing to various factors such as: Inadequate or irregular power supply, short airtime and frequency of airing, language barrier, poor signal reception. In addition, level of education and income all had an influence on the sources of agricultural information the rural farmers prefer to use. Also, age, level of education and income all contributes synonymously to the perception of farmers on knowledge acquired through broadcast agricultural programmes. There is also a high level of significance of the depth of exposure of farmers to their disposition towards agricultural broadcast programmes as a developmental tool in the study area. This further reiterate the importance of broadcast agricultural programmes in enhancing sustainable agricultural development as it facilitates farmers' access to information and new technologies.

V. RECOMMENDATIONS

Based on the findings from the study, the following recommendations were made:

a. Research messages should be translated into the simplest language possible and translated to the prevalent language.
b. Radio stations and television channels should provide forum for questions and answers on aired program.
c. Radio stations/broadcasters should become independent of government in terms of management and programs and be more adoptive to their requirements.
d. Farmers should procure transistor radio sets, operated by battery to enable them listen to radio when there is power failure.

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Analysis of the Relationship of Factors Affecting Customer Satisfaction at PT. PLN (Persero) Kotabaru Area

Sigit Hari Wibowo[1], Emmy Sri Mahreda[2], Muhammad Husaini[3], Wahyuni Ilham[4]

[1]Program Studi Magister Pengelolaan Sumber Daya Alam dan Lingkungan Program Pascsarjana Universitas Lambung Mangkurat, Indonesia
e-mail : sigithari123@gmail.com
[2]Fakultas Perikanan Universitas Lambung Mangkurat, Indonesia
e-mail : emmy.sri92@yahoo.com
[3]Fakultas Pertanian Universitas Lambung Mangkurat, Indonesia
e-mail : husaini_abang@yahoo.com
[4]Fakultas Kehutanan Universitas Lambung Mangkurat, Indonesia
e-mail : wfcilham@yahoo.de

***Abstract*— *This study aims to determine and analyze the Quality of Service through variable assurance, empathy, age, gender, education, occupation, length of subscription, and power customer on customer satisfaction at PT. PLN (Persero) Kotabaru Area. This type of research uses explanatory research types. The object of research in this study is PT. PLN (Persero) Kotabaru Area. The sampling technique used was purposive sampling using the Slovin formula, where the number of samples was 400 people. Data analysis in this study used Correlation Analysis. Correlation Test research results prove that the empathy variable has the highest correlation that is equal to 0.586, is at the level of "Medium", this means that the empathy variable of PT. PLN (Persero) Kotabaru Area to customers makes customers feel satisfied with PT. PLN (Persero) Kotabaru Area.*
Keywords*— *Correlation, service quality, variable.

I. PRELIMINARY

Electricity is the source of human life, because without the presence of electricity, it would be difficult for people to survive. To be able to continue to live, then the need for electricity must be met. Therefore, the supply of electricity will be borne, including the Indonesian people, especially the people of South Kalimantan, specifically Kotabaru, Tanah Bumbu and its surroundings.

PT PLN (Persero) is a state-owned company appointed to meet the electricity needs of the Indonesian people. Service productivity is the ability of service producers to use inputs to provide services to meet customer expectations.

The increase in the number of customers is increasing day by day, is a positive increase to support the potential of PT. PLN (Persero) Kotabaru Area, but in practice the community still complained about the services provided by PT. PLN (Persero) Kotabaru Area. Complaints that cause disruption to the electricity network, changes in service to dispensation services that occurred in the past involving consumers, such as errors in recording meters, delays in service of new installations and powered by power, being late in making electricity normalize, and also power outages using suddenly because it needs to be done by the community.

In addition to the need for electricity continues to increase, there are also some complaints against PT. PLN (Persero) Kotabaru Area is replaced by services received by customers such as, power outage schedules that have no certainty of completion time, house-to-house cable connections, and interference from electric power substations. As a company engaged in the sale of electrical energy, PT. PLN (Persero) Kotabaru Area is required to maximize the competence of employees to always be ready and ready to use.

The level of service quality (Service Quality) cannot be agreed based on the point of view. Therefore, in formulating the strategies and programs of institutions oriented to the interests of customers. Services provided by PT. PLN (Persero) Kotabaru Area can not be separated from the quality of service consisting of Assurance and Emphaty.

According to Fatmawati (2010), one of the functions of government that is now increasingly highlighted is the community that is managed by government institutions that provide public services. Improving the quality of public services by the government is now increasingly prominent, even a priority for the community. The problem that is often criticized by the public or service recipients is the perception of "quality" inherent in all aspects of service.

Customer satisfaction is a requirement for a company that survives and is very important because maintaining customer satisfaction will create a good image for the company itself, there is also a good relationship between the company and the customer.

According to a brief consideration with consideration to the customer that has been done in Kotabaru District Regarding some questions regarding delays in service, consideration of time and service costs, frequent power outages, illegal electricity to a certain number of people who use the information needed to help themselves by collecting additional fees in every house when carrying electricity payment receipts required by customers and the PLN itself. This phenomenon will cause negative challenges from customersBased on the preliminary facts discussed above, it is very necessary to discuss further, therefore this study is given the title "Analysis of the Relationship of Factors Affecting Customer Satisfaction at PT. PLN (Persero) Kotabaru Area ".

Formulation of the problem

Based on the background of the problem that has been described, then the problem is formulated as follows:

1. Are the factors of service quality (Guarantee, Empathy, Age, Gender, Education, Employment, Length of Subscription and Power) correlated with Customer Satisfaction at PT. PLN (Persero) Kotabaru Area?
2. What level of customer satisfaction does the customer receive from PT.PLN (Persero) Kotabaru Area?

Research purposes

From the background of the above questions, the purpose of this study are:

1. To analyze the close relationship between Customer Satisfaction and the factors that influence it (Guarantee, Empathy, Age, Gender, Education, Employment, Length of Subscription and Power) at PT. PLN (Persero) Kotabaru Area.
2. To find out the level of Customer Satisfaction) at PT. PLN (Persero) Kotabaru Area.

THEORETICAL BASIS

Service Quality

Service quality can be measured by comparing perceptions between expected service and expected and perceived service by consumers (Gronroos, 1982; Parasuraman et al, 1990). In measuring service quality, according to Kotler (1994) it must start from recognizing the needs / interests of consumers and ending with consumer perceptions. This means that the picture of quality must refer to the views of consumers and not to the service provider, because consumers consume and enjoy services. Consumers deserve to determine whether the service is of good quality or not.

Efforts to maintain the quality of service, thereby aiming to provide satisfaction to the community, so that community satisfaction with PLN services in the Kotabaru Area can be used as an indicator of the performance of staff of PLN Kotabaru Area staff. The quality of public services is thus interpreted as community satisfaction with the services received by comparing the expectations of the community with the reality based on speed of service time, fairness in service, as well as time efficiency and service costs.

Some of the descriptions above regarding service quality, then in an effort to achieve research objectives, namely to find out the service quality of the Kotabaru Area PLN that is used to measure the quality of the service is by referring to the opinion expressed by Parasuraman, et.al. in Kotler (2003: 455).

Customer satisfaction

Customer satisfaction is the customer's response to the discrepancy between the level of interest before and the actual performance felt after use. One factor that satisfies consumers or customers is the perception of service quality that focuses on five service dimensions, namely responsiveness, reliability, empathy, guarantee, and direct evidence. Customer satisfaction, in addition to being influenced by perceived service quality is alsodetermined by product quality and price (Rangkuti, 2002: 30).

Gaspersz (1997: 34) states that basically customer satisfaction can be defined simply as a condition where the needs, desires and expectations of customers can be fulfilled through the products consumed.

Hypotheses

Based on the conceptual framework above the hypotheses in this study are as follows:

1. Suspected factors of service quality (Assurance, Empathy, Age, Gender, Education, Employment, Length of Subscription and Power) are closely correlated to Customer Satisfaction at PT. PLN (Persero) Kotabaru Area.
2. It is suspected that the customer has a strong level of satisfaction with PT.PLN (Persero) Kotabaru Area.

II. RESEARCH METHODS

This research uses explanatory research type. According to Sugiyono (2009: 18) explanatory research is research that explains the causal relationship between variables that influence hypotheses. This is in accordance with the purpose of the study, which is to explain the causal relationship that occurs between the independent variables with the dependent variable by testing the hypothesis.

The object of research in this study is PLN customers located in the work area of PT. PLN (Persero) Kotabaru Area.

Sample

The sample is part of the number and characteristics possessed by the population. (Sugiyono, 2009: 122). In this study, the sampling technique used was purposive sampling. Purposive sampling, namely sampling carefully selected by taking research objects that are selective and have specific characteristics.

The sample distribution will be divided into 3 (three) regions, namely Kotabaru, Batulicin and Satui, for the Kotabaru 150 customers, Batulicin 150 customers and Satui 100 customers.

III. RESULTS AND DISCUSSION

From the questionnaire that has been collected can be seen general description of respondents seen from various characteristics below:

Table 1. Gender of respondents

No.	Jenis Kelamin	Frekuensi	Persentase (%)
1	Laki-Laki	280	70%
2	Perempuan	120	30%
Jumlah		400	100%

Table 2. Age of Respondents

No.	Usia	Frekuensi	Persentase (%)
1	< 25	49	12,3%
2	26-30	42	10,5%
3	31-40	80	20,0%
4	41-50	134	33,5%
5	>50	95	23,8%
Jumlah		400	100%

Table 3. Education of respondents

No.	Usia	Frekuensi	Persentase (%)
1	SD	79	19,8%
2	SMP	67	16,8%
3	SMA-SMK	160	40%
4	Diploma	41	10,3%
5	S1-S2	53	13,3%
Jumlah		400	100%

Table 4. Respondent occupations

No.	Pekerjaan	Frekuensi	Persentase (%)
1	Wirausaha	6	1,5%
2	PNS	138	34,5%
3	Swasta	87	21,8%
4	TNI	42	10,5%
5	Polri	127	31,8%
Jumlah		400	100%

Table 5. Length of subscription of respondents

No.	Lama	Frekuensi	Persentase (%)
1	< 5 tahun	255	63,7%
2	> 5 tahun	145	36,3%
Jumlah		400	100%

Table 6. Respondent subscription power

No.	Lama	Frekuensi	Persentase (%)
1	450 watt	76	19%
2	900 watt	240	60%
3	1300 watt	56	14%
4	2200 watt	13	3,3%
5	≥3500 watt	15	3,8%
Jumlah		400	100%

Table 7. Correlation test results

Variabel	*Corellation*	Tingkat Hubungan
Assurance (X_1)	0,452	Sedang
Empathy (X_2)	0,586	Sedang
Umur (X3)	0,053	Sangat Lemah
Jenis Kelamin (X4)	0,127	Sangat Lemah
Pendidikan (X5)	0,074	Sangat Lemah
Pekerjaan (X6)	0,011	Sangat Lemah
Lama Berlangganan (X7)	0,102	Sangat Lemah
Daya (X8)	0,036	Sangat Lemah
Nilai R = 0,617		

Source: Primary Data Processed Results,

From the table above it can be seen that the correlation value of each variable, only assurance and empathy have a "Medium" relationship level, while other variables have a "very weak" relationship level. Of all the Emphaty variables, the highest correlation value is 0.586.

Hypothesis Test Results

Hypothesis 1

Allegedly the factors of service quality (Assurance, Empathy, Age, Gender, Education, Employment, Length of Subscription and Power) correlate closely with

Customer Satisfaction at PT. PLN (Persero) Kotabaru Area.

Correlation analysis results, it is known that the largest correlation value is Emphaty of 0.586, showing that of the eight variables the greatest relationship between Emphaty variables with Customer Satisfaction variables where other variables that are considered influential are controlled or made fixed (as control variables) are moderately correlated because they are in intervals of 0.400 - 0.599. This means that empathy from the PLN to the customers makes customers feel satisfied with PT. PLN (Persero) Kotabaru Area.

Hypothesis 2

Based on Table 7 can be seen from the R value of 0.624. This is the level of satisfaction felt by customers of PT. PLN (Persero) Kotabaru Area. This value is at the level of "STRONG" correlation, between 0.600 - 0.799. This is a good achievement for PT. PLN (Persero) Kotabaru Area, but its services must be improved so that the community or customers really feel the same and even satisfaction.

IV. CONCLUSION

From the results of the study described in the previous chapter, then in this concluding discussion the authors can draw the following conclusions:

A. Assurance, Empathy, Age, Gender, Education, Employment, Length of Subscription and Power have a correlation with Customer Satisfaction at PT. PLN (Persero) Kotabaru Area.
B. Service quality factors (Assurance, Empathy, Age, Gender, Education, Employment, Length of Subscription and Power) are closely correlated to Customer Satisfaction at PT. PLN (Persero) Kotabaru Area. It is known that the variable Emphaty has the highest correlation that is equal to 0.586 is at the level of "Medium", this means that empathy from the PLN to the customers makes customers feel satisfied with PT. PLN (Persero) Kotabaru Area.
C. Based on the value of R, 0.624. This is the level of satisfaction felt by customers of PT. PLN (Persero) Kotabaru Area. This value is at the level of "STRONG" correlation, between 0.600 - 0.799. This is a good achievement for PT. PLN (Persero) Kotabaru Area, but its services must be improved so that the community or customers really feel the same and even satisfaction.

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Adoption Behaviour of Beneficiary and Non-Beneficiary (FLD) Farmers of Green Gram Cultivation Khargone District of Madhya Pradesh

Mr. Bhupendra Chouhan[1], Dr. Sandhya Choudhary[2], Dr. Abhay Wankhede[3], Dr. K. S. Kumar[4]

[1]M.Sc. Agricultural Extension & Communication Final Year Student 2019
[2]Professor, Agricultural Extension & Communication, College of Agriculture, Indore
[3]Associate Professor, Agricultural Extension & Communication, College of Agriculture, Indore
[4]Professor, Agricultural Statistics, College of Agriculture, Indore

Abstract— *India produces variety of pulses including chickpea 39.00 per cent, pigeon pea 21.00 per cent, green gram 11.00 per cent, black gram 10.00 per cent, lentil 7.00 per cent, field pea 5.00 per cent and other of around 14.00 million tonnes annually from an area of around 23.63 million hectares, with an average yield of around 650 kg per hectare, which is one of the largest producing country in the world. The major pulses producing states are Madhya Pradesh 23.00 per cent, Uttar Pradesh 18.00 per cent, Maharashtra 14.00 per cent, Rajasthan 11.00 per cent, Andhra Pradesh 9.00 per cent and Karnataka 6.00 per cent where pulses are predominantly grown as rainfed crops. The Front Line Demonstration (FLD) is to demonstrate newly released crop production and protection technologies and its management practices in the farmers' field under different agro-climatic regions and farming situation. the impact of Krishi Vigyan Kendra in terms of adoption of improved agriculture production practices, a sample of 60 non-beneficiary farmers was selected from control villages. Thus, a total of 120 farmers will be selected as respondent for the study purpose. Out of total beneficiaries, 45.00 per cent had medium adoption level, followed by 33.33 per cent had high and only 21.67 per cent had low adoption level of green gram production technology*.

Keywords*— *Adoption Behavior, Beneficiary, Non-Beneficiary, Front line Demonstration (FLD), Farmers, Green Gram.

I. INTRODUCTION

Green gram is an important pulse crop in our country after chickpea and pigeon pea, cultivated in three different seasons' viz., kharif, rabi and summer. India is the largest producer of green gram that accounts for 54% in the world production and covers 65% of the world acreage and it is grown on about 3.70 million hectares with annual production of 1.57 million tonnes. Green gram is grown in Khargone district (M.P.) that occupies 9905 hectares area and the total production was 3900 tones with average productivity of 3.70 quintal per hectare. For development of production and productivity of green gram in Khargone district, FLDs are being executed by personnel of KVK program from 2010.

Front line demonstrations (FLDs) have been proved the best means for creating awareness of new development in technology generation and to assess the various socio-economic variables for affecting the adoption level of farmers as the regular feedback is a necessary component of these demonstrations. KVK in Khargone district has been organizing FLDs on green gram, therefore keeping in view the researchers keen interest to know adoption behaviour of beneficiaries' and non beneficiaries in cultivation of green gram, the study was conducted with the following objective

Objective

1. To compare the adoption behavior of green gram production technology among beneficiaries and non-beneficiaries (FLD) Farmers

II. REVIEW OF LITERATURE

Jatav (2010) reported that majority of FLD respondents (53.33%) had medium level of scientific

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temperament, while 44.44 per cent had high and only 2.22 per cent had low level of scientific temperament.

Kangali (2012) revealed that in case of adopter of frontline demonstration of chickpea growers, majority of the farmers (50.00%) possessed partial adoption of total chickpea production technology considered in the study followed by (40.00%) farmers had full adoption and (10.00%) farmers had low adoption of chickpea production technology respectively.

Kumari (2015) reported that majority of the FLD beneficiaries (58.00%) were having high adoption of wheat production technology. Whereas majority of non-FLD beneficiaries (50.00%). were having medium adoption of wheat production technology.

Singh (2017) reported that adoption of an improved package of practices in wheat cultivation recorded higher B:C ratio (1.92) as compared to FP (1.63). Yield enhancement and higher net returns observed under FLDs of improved technologies in wheat. Thus, the productivity of wheat could be increased with the adoption of recommended improved package of practices.

III. METHODOLOGY

The present study was conducted in Khargone district M.P. For this study purposive sampling technique was adopted, where FLD was conducted by Krishi Vigyan Kendra Khargone (M.P.) during 2018-19.Khargone District constitutes of nine blocks namely Barwaha, Bhagwanpura, Bhikangaon, goganwa, Kasrawad, Khargone, Maheshwar, Segaon and Ziranya. Out of these blocks, two block Khargone and kasrawad had been taken by the KVK for green gram FLD. Therefore this block was selected purposively for the study.Khargone and Kasrawad block comprises of 92 villages Panchayat. Out of these six villages were taken by the KVK for green gram FLDs in two block. All the six villages were selected purposively for the study. Finally at last stage a comprehensive list of all the beneficiary farmers from each selected village was prepared with help of records of Krishi Vigyan Kendra. From each sample village, 60 beneficiary farmers will be selected through randomly sampling method as respondents for the study purpose. Apart from this, in order to assess the impact of Krishi Vigyan Kendra in terms of adoption of improved agriculture production practices, a sample of 60 non-beneficiary farmers was selected from control villages. Thus, total of 120 farmers will be selected as respondent for the study purpose.

IV. RESULT & DISCUSSION

Adoption behaviour of green gram production technology among beneficiaries and non-beneficiaries:

Table 1: Distribution of beneficiaries and non beneficiaries according to their adoption behavior of green gram production technology, n=120

S. No.	Categories	No. of beneficiaries	No. of non beneficiaries
1.	Low	13 (21.67%)	31 (51.67%)
2.	Medium	27 (45.00%)	18 (30.00%)
3.	High	20 (33.33%)	11 (18.33%)
Total		**60**	**60**

Table shows that out of total beneficiaries, 45.00 per cent had medium adoption level, followed by 33.33 per cent had high and only 21.67 per cent had low adoption level of green gram production technology.

The table also revealed that out of 60 non-beneficiaries farmers, higher percentage of the non beneficiaries i.e., 51.67 per cent belonged to medium adoption group of green gram growers

Table.2: Practice wise adoption level of green gram growers about green gram production technology

S.N.	Practices	Beneficiaries level of adoption						Total score	Mean score
		High		Medium		Low			
		F	%	F	%	F	%		
1	Selection of land	24	40.00	20	33.33	16	26.66	128	1.13
2	Improved varieties	35	58.33	20	33.33	05	8.33	145	2.41
3	Seed rate	30	50.00	18	30.0	12	20.00	138	2.30
4	Seed treatment	22	36.66	19	32.66	19	32.66	123	2.05
5	Bio fertilizer management	15	25.00	22	36.66	23	38.33	90	1.50
6	Manure & fertilizer management	27	45.00	20	33.33	13	21.66	154	2.56
7	Spacing	18	30.00	25	41..66	17	28.33	121	2.01

8	Weed management	25	41.66	17	28.33	18	30.00	127	2.11
9	Insect & pest management	20	33.33	18	30.00	22	36.66	118	1.96
10	Disease management	20	33.33	26	43.33	14	23.33	126	2.10

S.N.	Practices	Non beneficiaries level of adoption						Total score	Mean score
		High		Medium		Low			
		F	%	F	%	F	%		
1	Selection of land	10	16.66	22	36.66	28	46.66	**102**	1.7
2	Improved varieties	08	13.33	19	32.66	33	55.00	95	1.58
3	Seed rate	09	15.00	24	40.00	27	45.00	102	1.7
4	Seed treatment	14	23.33	21	35.00	25	41.66	109	1.81
5	Bio fertilizer management	11	18.33	18	30.00	31	51.66	100	1.66
6	Manure & fertilizer management	16	26.66	23	38.33	21	35.00	115	1.91
7	Spacing	12	20.00	16	26.66	32	53.33	116	1.93
8	Weed management	10	16.66	14	23.33	36	60.00	94	1.56
9	Insect & pest management	08	13.33	11	18.33	41	68.33	87	1.45
10	Disease management	12	20.00	17	33.33	31	51.66	101	1.68

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Evaluating Micropropagation of Kashan Damask Rose, Yasooj Aromatic Rose and Their Hybrid

Sahar Mirzaei[1]*, Abbas Ghamari Zare[2], Sonia Jafary[3]

[1]Assistant Professor, Ornamental Plants Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran.
*corresponding author email: sahar_mirzaei81@yahoo.com
[2]Associate Professor, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
[3]Master of Science, Department of Agriculture, Payam Noor University, Tehran, Iran.

Abstract**—*According to the value of damask rose and aromatic rose, crossing of Kashan damask rose × Yasooj aromatic rose has high importance in terms of its essential oil performance, rose water quality and its usage as a medicinal plant. With considering propagation problems of this plant, mass reproduction with tissue culture can help in a short time. The samples of hybrid plants and their parents were collected from Research Institute of Forests and Rangelands. After pre-sterilization and sterilization, explants were established on the MS medium. When length of explants became around 2 cm, were transferred to MS medium with 3 different treatments for shooting and it was indicated that best shooting treatment for all 3 genotypes was MS medium with IBA (0.1 mg/l), 2ip (0.5 mg/l) and BA (0.5 mg/l). G22 genotype produced better shoots, so it was established on 8 different rooting treatments and finally MS media with IBA (2 mg/l) showed best result (100%). Then explants of (G22×G4) and G4 genotype also were established on the best rooting media and produced 28% and 0% roots, respectively. All the rooted plantlets were transferred to plastic pots containing cocopeat+ perlite+ vermicompost with the ratio of 2:1:0.5 and placed in the laboratory for compatibility. Totally it was concluded that between 3 genotypes (crossed (G22×G4), damask rose as the paternal line (G4) and (G22) aromatic rose as the maternal line), G22 had the highest results of micropropagation, G4 had lowest results and crossed plant (G22×G4) was in the interstitial situation.*
Keywords**—Aromatic rose, Crossing, Damask rose, In vitro.**

I. INTRODUCTION

Damask rose is one of the most important roses and famous plants in the horticultural history of the world (Heidari *et al.*, 2005). Damask rose with the scientific name of *Rosa damascena* Mill. and English name of Persian rose, is from Rosacea family. This species has leaves consisted of seven and often nine serrate leaflets and it is a hybrid of *R. gallica* and *R. cunina* (Tabaei Aghdaei *et al*., 2009). Flowers are pink, somewhat big, mostly compacted in an inflorescence with few flowers, short pedicle and large petals (Mirheidar, 1996). Damask rose is one of the most important medicinal and aromatic species, which is cultivated in some parts of the world. Iran has been known as the source of this valuable plant (Chevallier, 1996). In the past, Persia was the main center of rose and other medicinal plants. Most of workshops for producing rose water were placed in Fars province (Meimand and Firuzabad cities). Today, Isfahan province, especially Kashan city, is one of the most important centers of cultivating damask rose, producing rosewater and different odors. In addition to Kashan, in other provinces such as Fars, Kerman and Azarbayejan sharghi also it has been cultivated (Tabaei Aghdaei & Rezaei, 2004). Damask rose has been placed in the category of roses (Bealis, 1990). Most important essence source of rose it is in their petals, which had different uses from last centuries. Petals of damask rose and it is products, reduce the amount of blood cholesterol. The plant is resistant to salt and drought, even it can be grown in the poor soils. In addition, it has been considered as an oily rose species (Gunes, 2005). Present day's damask rose is being used in the preparation of herbal medicines (Nikbakht, 2003). Volatile oil compounds or an essence, which is used for making odors, also it is used as tranquilizer, anti-depressant and anti-inflammatory (Haji-akhondi and Baligh, 2002). Iran is the most important producer and exporter of rosewater and damask rose essence (Guenther, 1952). Products of damask rose are dry flower, rosewater and essence (Tabaei Aghdaei *et al*., 2009). Debergh and Maene (1981) and Soomro *et al*., (2003) reported that, some of rose species hardly propagate by classic methods, because rooting is weak in some cuttings. In the other hand, in vitro culture of explants is a suitable and fast propagation method for rose varieties. Nikbakht *et al*., (2004) reported that usual method of propagating damask rose is cutting. However, in this method, the risk of viral and bacterial disease diffusion is high and it is not a safe method for supporting high recommendation of healthy shrubs and trees. Vander Salm *et al.,* (1994) reported that propagating seedlings by tissue culture is a suitable way for achieving plant material, which are free of disease and having new characteristics. According to high requirements for re-cultivation of damask rose lands, breeding them with native plants and propagating them by tissue culture is the best way to achieve hybrids with excellent characteristics. Therefore, this research work was carried out to investigate the micropropagation of Kashan damask rose × Yasooj aromatic rose (G4×G22), Kashan damask rose (G4) and Yasooj aromatic rose (G22).

II. MATERIAL AND METHODS

2.1 Pre sterilization and sterilization

Shoot tips of Kashan damask rose, Yasooj aromatic rose and their hybrids, was used for microporopagation process (Ghamari Zare, *et al.*, 2001). Pre sterilization was done with water and washing liquid, by brushing the samples and then sterilization was done with two different treatments, according to Table. 1.

Table 1. Sterilization treatments

Code	No.	$HgCl_2$ (0.1%)		NaOCl (1.5%)	
		D. (min)	H. (%)	D. (min)	H. (%)
G4	20	5	80	10	55
G22×G4	22	5	90	10	82
G22	20	5	75	10	75

*G4: Damask rose, G22: Aromatic rose, G22×G4: Hybrid, H: Healthiness, D: Duration

Three genotypes of G4, G22 and G22×G4 were sterilized with two different treatments, $HgCl_2$ (0.1%) for 5 min and NaOCl (1.5%) for 10 min. The number of sterilized samples of G4 and G22 was 20 and hybrid G22×G4 was 22. After one month, treatments were evaluated and the best sterilization treatment was recognized.

2.2 Establishment in the shooting media

In this stage, healthy sterilized explants, which had a bud with length of 1- 1.5 cm were selected. Explants of all the samples (G4, G22 and G22×G4) were exposed to 3 different treatments (Table. 2) to recognize the best shooting treatment. Each treatment contained 5 replications and each replication consisted of 5 explants. Complete Randomized Design was used to analyze the shoot proliferation treatments.

Table 2. Shoot proliferation treatments

Media	BA (mg/L)	2ip (mg/L)	IBA (mg/L)
GM3	0.5	0.5	0.1
GM5	3	-	-
GM6	1.5	1.5	0.1

*Base media: MS media (Murishig & Skooj, 1962) + 100 mg/L Ascorbic acid

2.3 Establishment in the rooting media

In this stage, the best genotype in the case of shooting was recognized and transferred to modified MS media (half macro elements, twice phosphate and 100 mg/L Ascorbic acid) with 8 different treatments, 5 replications and 5 explants in each replication. After analyzing data by Complete Randomized Design, the best rooting treatment was recognized and used for all genotypes.

Table 3. Rooting treatments

Treatments	Hormones	Hormones (mg/L)
GMr1	NAA	0.2
GMr2	IBA	0.2
GMr3	NAA	1
GMr4	IBA	1
GMr5	NAA	2
GMr6	IBA	2
GMr7	IBA، GA	1 & 2
GMr8	-	-

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2.4 Establishment of the seedlings in the soil

In order to access compatibility of the seedlings, all rooted seedlings were transferred to plastic pots, which contained soil (cocopeat + perleat + vermicompost with the ratio of 2:1:0.5 respectively). Pots were covered with a transparent plastic and some pores were created on the plastic, gradually. After 20 days, all the plants were compatible.

2.5 Analysis of data

Experiments data entered in the Excel software and analyzed by SAS software, in the form of Complete Randomized Design.

III. RESULTS

2.6 Sterilization

Between all the three genotypes, highest percentage of healthy samples (G22×G4 (90%), G4 (80%) and G22 (75%) was obtained from sterilizing in $HgCl_2$ (0.1 %) solution. Percentage of healthy seeds which sterilized with NaOCl (1.5%) was G22×G4 (82%), G22 (75%) and G4 (55%). In both of the treatments, G22 showed 75% of healthiness. Totally, G22×G4 showed highest percentage of healthiness in compare with other genotypes, in both of the treatments.

2.7 Shoot proliferation

Between all the three genotypes, highest shoot length was obtained from G22 in GM3 media. Comparison of GM5 and GM6 media showed that, G22 and G4 produced better and longer shoots in GM6 media and G22×G4 in GM5 media (Fig.1).

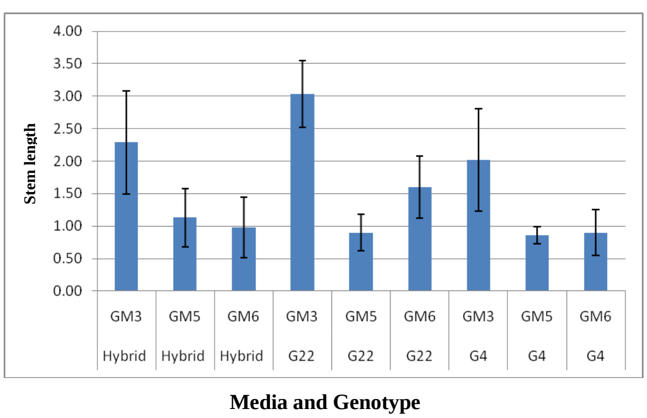

Fig. 1. Effect of different media on shoot length (cm) in G4: Damask rose, G22: Aromatic rose, G22×G4: Hybrid rose.

Analysis of variance showed that G4 and G22×G4 had higher shoot proliferation in GM5 and GM6 media, respectively. However, G22 produced more shoots in GM6 and GM5, respectively. All the genotypes produced lowest shoot proliferation in GM3 media (Fig. 2).

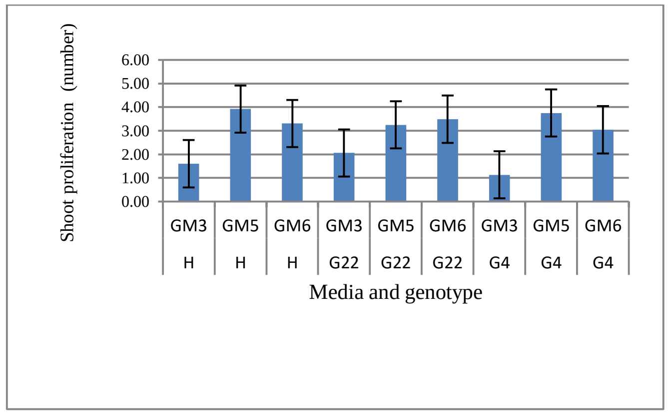

Fig. 2. Effect of different media on shoot proliferation (number.) in G4: Damask rose, G22: Aromatic rose, G22×G4: Hybrid rose.

2.8 Establishment of plant samples in the media

Totally, all the genotypes were established in three different media (Fig.3, 4, 5 & 6) and there was no significant difference between them. G22 showed 100% establishment in all media. G22×G4 showed 100% establishment in GM3 and GM5 and 90% in GM6. G4 showed 100% establishment in GM5, 80% in GM6 and 68% in GM3.

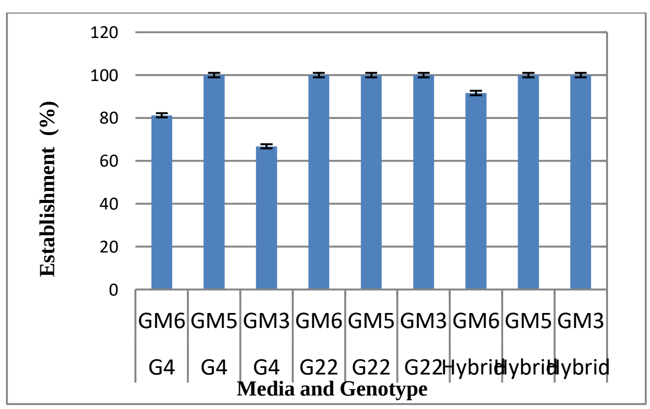

Fig. 3. Effect of three different media as main factor and three genotypes of roses as secondary factor on soil establishment

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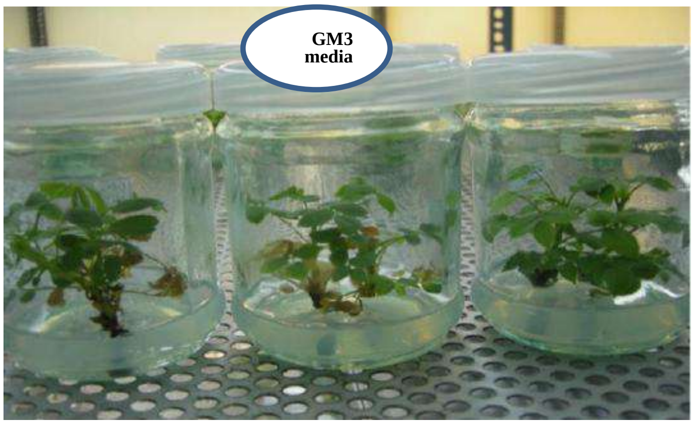

Fig. 4. Establishment of G22, G4 and G22×G4 on GM3 media for shoot proliferation

Fig. 5. Establishment of G22, G4 and G22×G4 on GM5 media for shoot proliferation

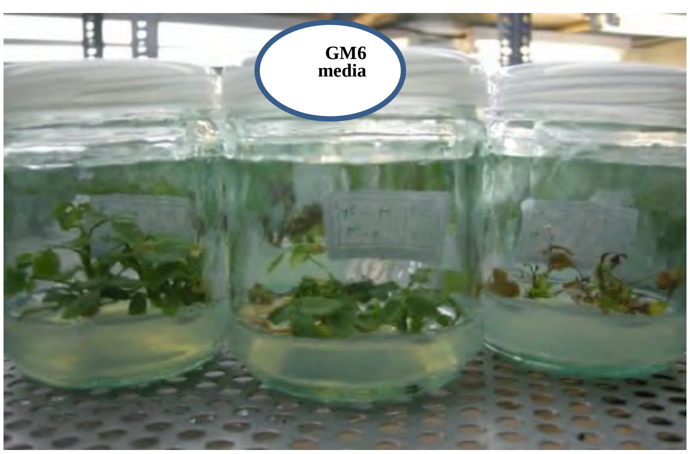

Fig. 6. Establishment of G22, G4 and G22×G4 on GM6 media for shoot proliferation

2.9 Rooting of plant samples in the media

According to the results of shoot proliferation, it was shown that aromatic rose (G22) had the best shooting. So, it was cultured in 8 different media to recognize the best rooting media. G22 produced 100% roots in GMr4, GMr5 and GMr6 rooting media, in GMr1 83%, in GMr2, GMr3 and GMr8 50%. There was no rooting in GMr7 media (Fig.7). Speed of rooting was faster and its density was higher in GMr6 in compare with GMr4 and GMr5 (Fig.8). On the base of results, GMr6 showed best rooting. So, rooting of G22, G4 and G22×G4 were compared in GMr6 rooting media. G22 and G22×G4 were rooted 100% and 28%, respectively. However, G4 did not produce any root (Fig.9).

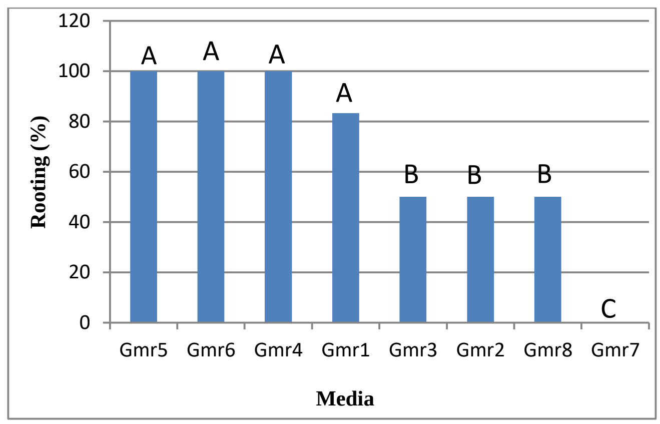

Fig. 7. Effect of 8 rooting media on G22 genotype

G22 G4 G22×G4

Fig. 8. Establishment of G22, G4 and G22×G4 on GMr6 rooting media

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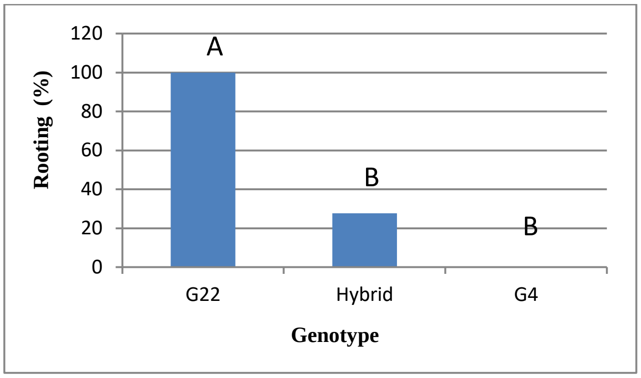

Fig. 9. Rooting of G22, G4 and G22×G4 on GMr6 rooting media

2.10 Establishment of plant samples in the soil

In this stage, rooted seedlings were transferred to plastic pots, which contained soil (cocopeat + perleat + vermicompost with the ratio of 2:1:0.5 respectively). Pots were covered with transparent plastic and some pores created on the plastic, gradually. After 20 days, all the plants were compatible. G22 showed highest incompatibility in the soil (Fig. 10).

Fig. 10. Establishment of G22 in the soil

IV. DISCUSSION

In some research works, success of micropropagation in rose cultivars has been reported (Pati *et al*., 2001 & 2010, Rout *et al*., 1999). Success of these methods for rose cultivars, it is dependent on their species and genetically field. Some cultivars can not grow through in vitro condition and their propagation happens slowly (Kornova & Michailova, 1994). Also, micropropagation of damask rose was done by Khosh – khui and Sink (1982), and because of its problems in shoot proliferation still it is continuing (Kumar *et al*., 1999). Most of the studies are on phyto-chemical aspects, such as methods of extracting the essence and analyzing the essential oils. Therefore, in order to extend the cultivation of damask rose flowers, solving problems of growers and introducing new varieties, which are superior in all aspects of qualitative and quantitative characteristics of flowers, essence and flowering period, this research work was laid out in the Research Institute of Forests and Rangelands. For this purpose, 80 genotypes of damask rose, were collected from different parts of the country and established in the institute. Jabbarzade and Khosh-Khui (2005) reported that explants of *Rosa damasena* Mill. was sterilized with chlorox (10%) for 15 min, NaOCl (1.5%) for 10 min and $HgCl_2$ (0.1%) for 5 min. results showed that in all the studied genotypes, $HgCl_2$ (0.1%) showed better results. Allahverdi *et al.*, (2010) also found that sterilization of Damask rose explants with $HgCl_2$ (0.1%) for 5 min was best treatment, which are in agreement with our findings. Our results, in case of using growth regulators was in agreement with findings of Rout *et al*., (1999), who showed that cytokinins are effective on blossoming buds and BA has significant effect on shoot proliferation in the in vitro condition. Nak-Udom *et al*., (2009) also studied micropropagation of *R. hybrida* L., they cultured the explants on MS media containing 3 mg/l BA and 0.003 mg/l NAA, which produced plantlets with best shoot proliferation and complete rooting. These results are same as our results for G4 and G22×G4, but G22 got its best results in GM6 media with 1.5 mg/l BA, 1.5 mg/l 2ip and 0.1 mg/l IBA. Nikbakht *et al*., (2005) studied the in vitro culture of *Rosa damasena* Mill., species of Azaran and Ghamsar, with 32 treatment levels contained BA (0, 1, 2 & 3 mg/l), GA_3 (0, 0.1, 0.25 & 0.5 mg/l) and NAA (0 & 1 mg/l). Best shoot proliferation was achieved in Azaran species with BA (1 & 2 mg/l), GA_3 (0.1 mg/l) and NAA (0 & 1 mg/l) and in Ghamsar with BA (1 & 2 mg/l), GA_3 (0.1 mg/l) and NAA (0 mg/l). Their results were in agreement with our results in case of G4 genotype, which showed the best results of shoot proliferation with BA treatment. BA has been used for many ornamental plants in laboratory condition. Wang *et al.*, (2002), Carelli and Echeverrigary (2002) and Alsemaan (2013), studies micropropagation of *Rosa damasena* Mill., they studied effect of different concentrations of BA (0, 0.5, 1 & 2 mg/l) and GA_3 (0, 1 & 2 mg/l) on shoot proliferation and concluded that BA (2 mg/l) and GA_3 (2 mg/l) had highest effect for best shoot proliferation. Alseman (2013) used half strength of MS media, 3 gr/l activated charcoal and IBA (0, 1, 2 & 3 mg/l) for rooting and found that IBA (2 mg/l) had highest effect on rooting of plantlets. These results are in agreement with our findings, which showed that in all three genotypes, BA (3 mg/l) in shoot proliferation and IBA (2 mg/l) in rooting had the best effect. Tabesh *et al*., (2013), used activated charcoal (3 mg/l) for removing phenol compounds of *Rosa damasena*, but we used ascorbic acid (100 mg) instead of activated charcoal. Also, Jabbarzadeh and Khosh-Khui (2005), showed that IBA (0.1 mg/l) and BA (2.5 & 3 mg/l) were best treatment for shoot proliferation of *Rosa damasena* Mill. In our research also, BA (3 mg/l) was best for shoot proliferation, but IBA (2 mg/l) had highest effect for rooting.

V. CONCLUSION

Totally our results showed that, between all three genotype, G22 (mother parent) had the best micropropagation results, G4 (father parent) was the worse one and G22×G4 hybrid was between them. However, in some characters such as stem length, shoot proliferation and rooting percentage, G4 was better than G22. Best rooting was recorded for G22 and G4 did not produce any root.

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Fig molasses: Processing Essentials and Organoleptic Properties

Ossama Dimassi[1*], Fatimah Rizk[1], Ehssan Sharif-Askari[2], Mohamed Rached[2], Raymond Akiki[3]

[1]Department of Nutrition and Food Science, Lebanese International University, Beirut, Lebanon
[2]Department of Biomedical Sciences, Lebanese International University, Beirut, Lebanon
[3]Department of Business Administration, Lebanese International University, Beirut, Lebanon
*Corresponding authors: odimassi@gmail.com

Abstract— *Local varieties of fig fruits (genus Ficus) are classified by their morphological characteristics mainly color of outer layer versus color inside. This has resulted in three categories, namely green outer layer with both white and red color inside and with outside and inside red color. Fig molasses produced following a traditional Lebanese recipe, using overripe-fresh-figs, was scrutinized to assess technical details such as fig molasses per fig conversion values (FMFCV) (kg/100kg figs) and organoleptic properties. Part of the prepared figs had pH adjusted to around 4.2 using citric acid. Concerning FMFCV there was no significant differences between molasses produced using the different fig cultivars. Water was added to the strained figs and a second extract was produced. FFMCV recognized from the first straining was significantly higher than that value recorded from the second straining. There was no significant difference between the two products concerning pH, water activity and ash content. Fig molasses produced from pH-adjusted figs had a significantly higher FFMCV, lower pH and higher ash content compared to those produced from non-pH-adjusted ones; however, no significant difference water activity was detected. Concerning organoleptic properties there was no significant difference between all the fig molasses produced. The use of the overripe figs shows how traditional methods have innate food waste reduction concept. Lower pH, higher FFMCV and no significant difference in the sensory attributes favor the use of the citric acid in the recipe since in would increase the production efficiency and would prolong the product shelf life.*

Keywords— ***Fig; Fig molasses; Fig to fig molasses conversion value; Strained figs; pH.***

I. INTRODUCTION

Fig fruit, scientifically named “Ficus carica”, is cultivated in most Mediterranean countries mainly in mild and dry areas (Polat & Caliskan, 2008).

Many fruit trees such as fig, pinus, walnut, pomegranate, carob and pistachio are well adapted to the lebanese enviroment are not sufficiently exploited and are generaly neglected and found in marginal lands or peripheries of the orchards (Stover, Aradhya, Ferguson, & Crisosto, 2007). Although they represent a great potential to the local and regional market (Migdadi et al., 2007).

Fig has long been consumed in the dried form. Therefore, most of the research has been directed towards dry fig culture ((Polat & Özkaya, 2005).

In Lebanon as well as the Mediterian region and southern Arabia figs has always been an important part of the diet since they are an important spurce of carbohydrates, contain essential amino acids and are rich in vitamins B1, B2 and C and minerals (Flaishman, Rodov, & Stover, 2008) (Vallejo, Marín, & Tomás-Barberán, 2012). Furthermore, the use of figs in cosmetics is well established (Aburjai & Natsheh, 2003)

Fresh figs are highly perishable and prone for microbial spoilage even at cold temperatures. Figs falls in the category of climacteric fruits and to some extent sensitive to ethylene which stimulates softening and increase decay severity, if kept at temperatures equal to or higher than 5ºC (Gözlekçi, Erkan, Karasahin Yildirim, & Şahin, 2008).

The high perishability of the fruits makes the storage for long unachievable thus processing into dried fruits, jam or molasses is done (Flaishman et al., 2008) (Jawandha, Singh, Kaur, & Arora, 2016). Molasses traditionally were also produced from many other ingredients such as carob and all were a way to have access to the nutritional benefits outside the production season and as natural sweeteners (Dimassi, Fawaz, & Rached, 2019; Dimassi, Rached, Fawaz, & Akiki, 2019). Moreover, fig molasses is a natural food that was produced traditionally (Gözlekçi

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et al., 2008). Molasses were used to conserve the very ripe figs which would be less fit for drying.

The aim is to study the characterize fig molasses to enable marketing and to standardize the production in order to increase the product efficiency. To assess the physicochemical properties, water activities, pH ash content for the 70 degrees brix molasses for both molasses produced with and without the addition of the citric acid (Kuchi, Gupta, & Tamang, 2014) (Cevrimli, Kariptas, & Ciftci, 2009) of the three cultivars are identified in this study. In addition, the most important indicator for the efficiency of production, namely the Kg figs to Kg fig molasses produced is calculated and accordingly it can be used in future feasibility studies concerning this product. Furthermore, to assess the overall acceptability of the fig molasses produced a sensory evaluation was conducted.

II. MATERIAL AND METHOD

2.1 Fig Molasses Sample Preparation

The materials used in this study are figs, which were morphologically classified into three categories namely green outer layer with white inside (GW), green outer layer and red color inside (GR) and with red color outside and red color inside (RR).

Ripe fresh fig fruits were collected washed. Water (1:1 ratio) was added to the cleaned fig fruits and boiled until fig fruits became soft, able to be mashed. After that, the boiled ingredients were strained using a colander and cheesecloth such that the juice is separated from cooked figs. This fig molasses produced was placed in a pot and subjected to heat treatment until reaching sticky consistency close to that of honey (adjusted to 70ºBrix). On the other hand, the resulting strained cooked figs were recovered with water (one fourth the strained weight), cooked again, strained using a cheesecloth and then boiled for a second time to reach the desired brix.

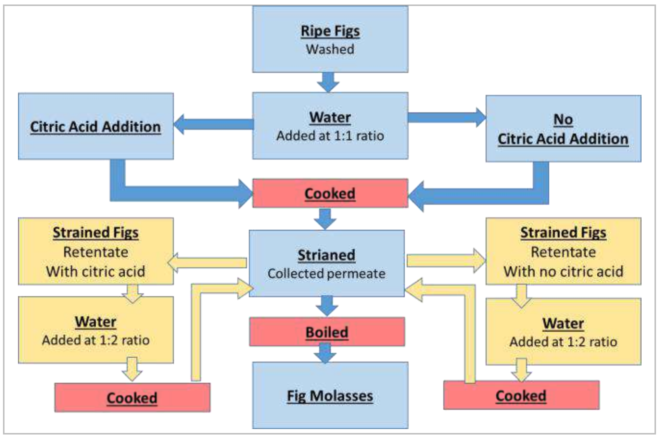

Fig.1: Fig molasses production flow

2.2 Materials

Brix Value Analysis: Brix Value was measured using Portable hand held RFM700 refractometer (Bellingham and Stanley LTD. United Kingdom).

Weight determination: Weight was measured using Portable electronic balance Model 727 was used to measure the weight with an accuracy of ±1 gr (Jata Hogar).

pH analysis: Microcomputer based pH/conductivity/TDS/salinity and temperature pocket meter Model pH/EC80 was used to measure the pH (Jenco VisionP).

Water activity: It was determined using AQUALAB Pawkit Water Activity Meter. Samples were flattened to cover the bottom of the cup and then water activity was measured at room temperature (Nielsen, 2010).

Ash Determination: Ash was determined using the AOAC 942.05 method.

2.3 Methods

2.3.1 Effect of different cultivars on the fig molasses physicochemical properties and fig molasses production efficiency

Fig molasses from each cultivar, GW, GR and RR, was produced separately and tested for ash content, pH and water activity. Furthermore, the production efficiency of each cultivar was recorded by taking the initial weight of the fresh ripe figs and the weight of the fig molasses produced thus having the Kg fig molasses produced from100 Kg fresh ripe figs.

2.3.2 Comparing Fig molasses produced from fresh ripe figs and those produced from the byproducts (strained figs) of the primary production

Fig molasses produced from fresh ripe figs were produced and tested for ash, pH and water activity and most importantly fig molasses per 100kg figs. These values were compared to the fig molasses produced from strained figs which is the byproduct of the primary production. No sensory analysis was conducted on fig molasses produced from the byproduct of the primary production due to time and quantity constrains.

2.3.3 Effect of citric acid addition on the physicochemical properties and the production efficiency

Fig molasses produced from fresh ripe figs were compare to those molasses produced without the addition of citric acid in terms of ash, pH and water activity and most importantly fig molasses per 100kg figs.

2.3.4 Sensory attributes

The sensory attributes include: sweetness with 0 having no sweetness and 5 having highest sweetness, sourness with 0 having no sourness and 5 having the highest sourness, color intensity with 0 being the brightest and 5 being the darkest one, aroma with 0 being the unpleasant and 5 being the most pleasant one, overall acceptability with 0 having lowest acceptability and 5 having the highest acceptability. In addition to that, the sensory score of each fig molasses was calculated by taking the mean of the different sensory attributes.

2.4 Statistical analysis

All tests and analysis were run in triplicates and averaged. General linear repeated measure model performed via SPSS (statistical Package for the Social Sciences, version 17.0) was used to study the fig molasses produced from different cultivars in terms of ash content, pH, water activity, kg fig molasses per 100kg figs and sensory score.

Furthermore, paired t-paired test was used to assess the difference between the fig molasses resulting from the fig cultivars with and without citric acid addition and Chi square was used to study if there is significant different in the score frequency distribution of each score per fig cultivar with and without citric acid. Thirty panelists did the sensory evaluation. Spearman correlation was used to assess the correlation between the different sensory attributes.

III. RESULTS

3.1. Effect of different fig cultivars

In this study no difference was detected in the ash content and pH of the fig molasses produced from the three local fig cultivars chosen for this study. There was, however, a significant difference in water activity between the molasses done from GR and that of the molasses done from the RR figs, while there was no significant difference in the water activity between the molasses done from the GW and that of molasses done from the GR and RR.

Table 1 Ash content, pH and water activity of the different fig cultivars

	GW	GR	RR
	Mean±SE	Mean±SE	Mean±SE
Ash Content %	$1.26^{a}\pm0.15$	$1.06^{a}\pm0.15$	$1.13^{a}\pm0.15$
pH	$4.74^{a}\pm0.07$	$4.76^{a}\pm0.07$	$4.82^{a}\pm0.07$
Water activity	$0.83^{ab}\pm0.02$	$0.78^{a}\pm0.02$	$0.887^{b}\pm0.02$

- Means with different letters among rows are significantly different
- GW is green outside layer and white content inside; GR is green outside layer and red content inside; RR is red outside layer and red content inside

As for the production-efficiency, it was accesses by measuring the Kg fig-molasses produced per 100 Kg figs. There was no significant difference noticed between the amounts of molasses produced per Kg of the fig cultivar (Fig.2).

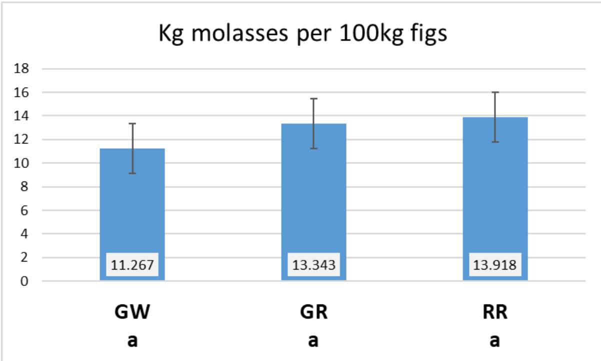

Fig.2: Effect of different fig cultivars on fig molasses production efficiency

(GW is green outside layer and white content inside; GR is green outside layer and red content inside; RR is red outside layer and red content inside)

3.2. Fig molasses produced from fresh ripe figs and those produced from the byproducts (strained figs) of the primary production

Following the procedure described in figure 1, we have fig molasses produced by the addition of 1 to 1 ratio of distilled water to fresh ripe figs (primary product) and those produced by the addition of 1 to 4 ratio of distilled water to strained figs (secondary product).

The ash content, pH and water activity of the of the primary and secondary product molasses did not differ significantly

Table 2 Ash content, pH and water activity of primary product and secondary product

	Primary Product*	Secondary Product*
	Mean±SE	Mean±SE
Ash Content %	$1.214^{a}\pm0.108$	$1.024^{a}\pm0.16$
pH	$4{,}723^{a}\pm0.047$	$4.876^{a}\pm0.07$
Water activity	$0.826^{a}\pm0.016$	$0.838^{a}\pm0.024$

- Means with different letters among rows are significantly different

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- *: Primary product is fig molasses produced by the addition of 1 to 1 ratio of distilled water to fresh ripe figs; Secondary product is fig molasses produced by the addition of 1 to 4 ratio of distilled water to strained figs

In terms of the production efficiency indicator, the molasses produced from the fresh ripe figs (primary product) showed a significantly higher yield compared to molasses yield when strained figs were used (secondary product) (Fig. 3).

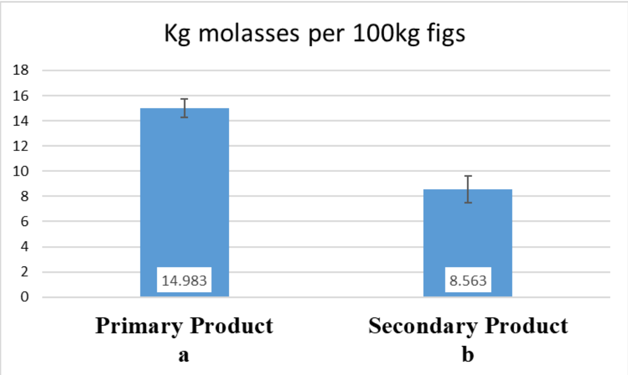

Fig.3: Comparison between primary and secondary product molasses in terms of production efficiency (Primary product is fig molasses produced by the addition of 1 to 1 ratio of distilled water to fresh ripe figs; Secondary product is fig molasses produced by the addition of 1 to 4 ratio of distilled water to strained figs)

3.3. Effect of citric Acid addition

Fig molasses, irrespective of the cultivar, done with the addition of citric acid to the figs before being cooked (Fig. 1) showed a significantly lower ash content and pH when compared to those done without the addition of citric acid. (Table 3). However, the water activity of both molasses showed no significant difference.

Table 3 Ash content, pH and water activity of fig molasses done without and with addition of citric acid

	Without Citric Acid	With Citric Acid
	Mean±SE	Mean±SE
Ash Content %	$1.309^a±0.108$	$0.833^b±0.16$
pH	$5.078^a±0.047$	$4.167^b±0.07$
Water activity	$0.815^a±0.016$	$0.86^a±0.024$

- Means with different letters among rows are significantly different

Concerning the chosen indicator of production efficiency citric acid addition resulted in a significantly higher fig molasses yield (Fig. 4).

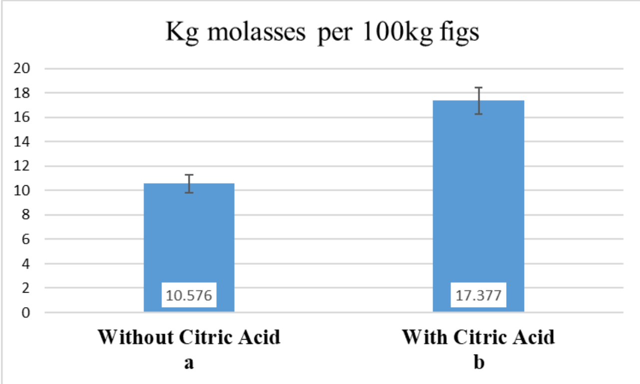

Fig.3: Effect of citric acid addition on fig molasses production efficiency

3.4. Sensory attribute results of the different fig molasses produced

The procedure followed in the production of fig molasses resulted in six fig molasses to be tested for their sensory attributes namely those molasses produced from the three local cultivars with and without citric acid addition (Fig. 1). Noting that molasses produced using the strained figs were not included in this study due to time and quantity constrains.

The sweetness, sourness and the aroma sensory attribute of fig molasses from the three chosen cultivars in Lebanon with and without the addition of citric acid showed no significant difference (Table 4). The mean values are between 3 and 4.

Using chi square no significance was detected between the frequencies of score between the different sensory attributes recorded for the three chosen cultivars with and without citric acid. The highest frequency of panelists, for the above-mentioned sensory attributes, chose score 4 followed by score 3 then by the score 5 ending by the choice of the scores 2 and 1 being the lowest (Table 5).

Table 4 Sweetness, Sourness and aroma score means of fig molasses from different fig cultivars with and without citric acid

Fig molasses source	Sweetness	Sourness	Aroma
	Mean±SD	Mean±SD	Mean±SD
GW	$3.70^a±0.99$	$3.73^a±0.98$	$3.60^a±0.93$
GR	$3.73^a±0.94$	$3.67^a±0.96$	$3.63^a±0.89$
RR	$3.83^a±1.02$	$3.77^a±1.01$	$3.50^a±0.86$
GW + Citric	$3.57^a±1.07$	$3.20^a±1.16$	$3.40^a±1.07$
GR + Citric	$3.47^a±0.97$	$3.20^a±1.10$	$3.00^a±1.01$
RR + Citric	$3.73^a±0.87$	$3.67^a±1.12$	$3.00^a±0.80$

- Means with different letters among rows are significantly different

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- GW is fig cultivar with green outside layer and white content inside; GR is with green outside layer and red content inside; RR is with red outside layer and red content inside

As for the color and overall acceptability scores of fig molasses produced from the different fig cultivars with and without citric acid, no significant difference could be detected (Table 6).

Table 5 Percentage of score choice by panelists per a sensory attribute

Score	Sweetness	Sourness	Aroma	Color	Ov.Ac*
1	1.67	2.22	1.11	1.11	1.11
2	9.44	17.22	11.67	8.33	15.00
3	30.56	26.11	36.11	17.78	22.78
4	36.67	33.89	35.56	50.00	42.78
5	21.67	20.56	15.56	22.78	18.33
Sum	100	100	100	100	100

- *: Is overall acceptability

Concerning the panelist choice frequency no significant difference was detected between the different fig molasses and the highest percentage of panelist chose score 4 for color followed by score 5 then score 3 and consequently 2 and 1 being the lowest. As for the overall acceptability, like all other sensory attributes the choice of score 4 was the highest followed by score choice 3 then choice of score 5, 2 and 1 consequently (Table 5).

Table 6 Color and overall acceptability score means of fig molasses from different fig cultivars with and without citric acid (CA)

Fig molasses source	Color	Over all Acceptability
	Mean±SD	Mean±SD
GW	$3.70^{a}\pm0.99$	$3.73^{a}\pm0.98$
GR	$3.73^{a}\pm0.94$	$3.67^{a}\pm0.96$
RR	$3.83^{a}\pm1.02$	$3.77^{a}\pm1.01$
GW + CA	$3.57^{a}\pm1.07$	$3.20^{a}\pm1.16$
GR + CA	$3.47^{a}\pm0.97$	$3.20^{a}\pm1.10$
RR + CA	$3.73^{a}\pm0.87$	$3.67^{a}\pm1.12$

- Means with different letters among rows are significantly different
- GW is fig cultivar with green outside layer and white content inside; GR is with green outside layer and red content inside; RR is with red outside layer and red content inside

As for the sensory score, which is the mean of all the sensory attribute scores, there was no significant difference detected between the fig molasses produced from the different cultivars with and without citric acid (Fig. 4). Which complies with all the sensory analysis done before.

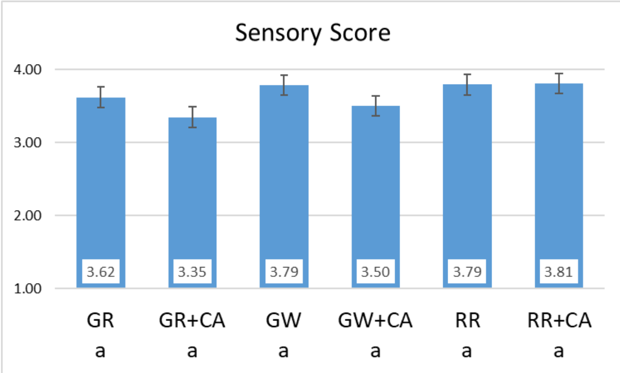

Figure 4 Sensory scores of fig molasses from different cultivars with and without citric acid (CA)
(GR is green outside layer and red content inside; GW is green outside layer and white content inside; RR is red outside layer and red content inside)

Applying spearman correlation it was found that all sensory attributes were positively and significantly correlated with each other. The overall acceptability was positively and highly correlated with the sweetness and sourness of the fig molasses. It was positively, significantly but medium correlated with the color and aroma sensory attributes.

Table 7 Spearman correlation of the different sensory attributes

	Sweetness	Sourness	Color	Aroma	Ov. Ac
Sweetness	1	0.673**	0.286**	0.332**	0.758**
Sourness		1	0.390**	0.388**	0.720**
Color			1	0.456**	0.494**
Aroma				1	0.560**
Ov. Ac+					1

- ***: highly significant p<0.01*
- *+: Is overall acceptability*

IV. DISCUSSION

As for the physicochemical properties of fig molasses produced from the different fig cultivars there was no significant difference in terms of Ash content, pH and water activity. This is also true when comparing the kg molasses per kg figs. This suggests that we can collect ripe figs from different cultivars with no need of sorting especially that no significant difference was detected when comparing the score of sensory attributes of fig molasses produced from different cultivars.

Furthermore, the fig molasses from fresh ripe figs and those done from strained figs did not differ did not differ significantly in terms of Ash content, pH and water activity. Therefore it is advisable to do the secondary production since it will increase the production by one third. Just sensory analysis should be investigated later, although the physicochemical properties suggest no difference.

As for the results concerning the citric acid addition, it lead to lower ash content and, expectantly, lower pH although the water activity was not significantly different suggesting that it solubilized organic tissues (Cevrimli et al., 2009). Furthermore, it resulted in an increase in the yield of fig molasses by 1.64 times making the production more efficient. Furthermore, citric acid addition lowered the pH to less than 4.6 rendering it a high acid food and thus according to FDA standards safer.

Since fig molasses in traditionally done from extra ripe figs it targets the utilization of those figs which are difficult to utilize otherwise, thus reducing food wastage and showing the wisdom of the ways used in our heritage.

V. CONCLUSION

The results of this study suggested the addition of citric acid and the utilization of the byproduct of the primary fig molasses production namely the strained figs to increase the efficiency of production and the increase of fig molasses yield by more than one third. The no difference of the sensory scores among cultivars and with and without the citric acid addition strengthened the usage of citric acid, since it also makes the fig molasses produced in the high acid food category therefore considered safer and easier to conserve.

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Radiation use Efficiency in Rice Crops under Different Numbers of Ttillers and Nitrogen Fertilizer Applications

Sugiono[1,2]*, Husni Thamrin Sebayang[2] and Agus Suryanto[2]

[1] Institute for Agricultural Technology (AIAT) East Java Indonesia
[2] Faculty of Agriculture, Universitas Brawijaya, Malang, East Java, Indonesia
*coreesponding: astro_bptp@yahoo.co.id

***Abstract*— The most important in agricultural activities is the utilization of solar radiation energy, nutrients and water. Plants with enough water and nutrients, Under optimal conditions, nutrition and water are fulfilled, the crop efficient use radiation is determined by the interception of light and the pattern of spread within the plant canopy.Setting number of tillers will affect distribution of radiation in rice. In addition to the radiation use efficiency by setting different number of tillers, nitrogen fertilizer, Urea, is very influential on the growth of plant organs. The aim of the research was to study the effect of different number of tillers and nitrogen fertilizer application on solar conversion energy in rice crops. The research design used a split plot design in which dose of nitrogen fertilizer as the main plot and different number of tillers as subplots. The results showed that urea at doses of 100 and 150 kg ha-1 was able to provide RUE of 1.37 and 1.39%, which is higher than 0 and 50 kg ha-1 .Result conversion efficiency treatment number tillers 10 and 15 per plant is 1.34, increase by 1.37% compare treatment number tillers 5 per plant.*

***Keyword*— lowland rice, nitrogen, number of tillers, radiation use effciency.**

I. INTRODUCTION

Rice is the most important crops. In Indonesia, rice is the main food commodity to supporting human carbohidrat necessity. Almost the region in Indonesia cultivated rice, especiallythe region that have good irrigation. Plant growth and quality depends on the interaction between environmental factors and plant genetic factors. Genetic factors are related to plant spesific characteristics. In addition to genetic planting factors which are one of the reliable components and a significant contribution in increasing national rice production, climate factors also influence the increase in rice production. According to Suprapto et al. (2013) that interaction between rice and environmental factors affecting the rice growth. In rice cultivation there are still many obstacles in improving rice growth and yield both in terms of nutrients in other management. To improve rice growth by using the right dose of fertilizer. In line with the development and advancement of fertilizer technology and changes in nutrient status in the soil, the existing fertilizer recommendations need to be further examined and refined.

The excessive number of tillers decrease rice yields. The excessive number of tillers indicates that the crop is overmuch fertile during the vegetative phase. Many Indonesian farmers provide additional fertilizer during the vegetative phase. Indonesian farmers assume that increasing the number of tillers also increase yields. Optimum phase of tillers produced is generally at the age of 50-60 DAP. Setting the number of tillers in a rice family determines the quality of the rice plant family. The quality of rice clumps affect the number of population per unit area and yield. Setting the number of tillers aims to determine the optimum number of tillers.Basically giving the possibility of plants to grow well without competition of taking water, nutrients, and sunlight. Good pattern of cultivation layout is important to optimizing efficiency use radiation of sunlight for photosynthesis.

The aim of this research was to study and determine the radiation use effciency (RUE) in lowland rice by regulating the number of tillers and applying nitrogen fertilizer in the generative phase of rice crops.

II. MATERIAL AND METHOD

The experiment was laid in split plot design with three replicate. Factorial experiment consist of 2 treatments, as a main plot is N (Urea) fertilizer dose from farmers recommendations (N1), 50 N kg ha-1 (N2), 100 N kg ha-1 (N3) and 150 N kg ha-1 (N4). Second treatment as sub plot is number of tillers consist of 5

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tillers (J5), 10 tillers (J10) and 15 tillers (J15). number of tiller controlling at 45DAP.

Varieties in this research use Inpari-42. Observation before fertilizer application at 50 DAP was used as an initial reference for rice growth. Observations in this experiment consist of 2 parameter, growth and yield parameters. Growth parameter are the number of tillers, leaf area and leaf area index. Yield parameters are yield (harvest) and energy conversion efficiency. In addition, secondary data was collected at the Karangploso BMKG station to get rainfall intensity and solar radiation data.

Solar energy conversion indicate percentage (%) of the light energy which is converted into photosynthesis contained in dry matter plants. RUE calculating by different dry weight of plants, at a certain time period by multiplying the coefficient of combustion (4000 cal g-1).Results of these calculations then divided by the total intensity of solar radiation in a certain time period which is multiplied by PAR. The RUE calculation formula according to Suprapto et al. (2013).

$$\text{RUE} = \frac{\Delta\ W.K}{I.t.PAR} \times 100\%$$

The Radiation Use Efficiency (RUE) was described by several components such as difference of plants dry weight (ΔW) (g m-2) in a period (t), Coefficient of burning heat (K) (4,000 cal g-1), Intensity of daily radiation (I) (cal m2 day-1), a period of a specific time (day) (T), and photosynthetic Active Radiation (PAR) (0.45).The result data were statistically analyzed using analysis of variance (Anova) to campare the differences between the different treatments.The means were separated by using least significant difference (LSD) test at 5% and 1 % level of significance.

III. RESULTS

The results showed fertiliizer dose and number of tilles have no interaction growth, harvest and energy conversion efficiency parameters. Each treatment showed that the dose of N fertilizer affected the number of leaves at the age of 65 HST, while the treatment of the number of tillers affected the number of leaves at 65 and 80 HST (Table 1).The leaf area index at 65 HST is affected by the number of tillers. J15 leaf area index at the age of 65 HST 1.67 higher than J5 and J10, leaf area index respectively 0.94 and 1.27 (Table 2).

The yielfd results of harvested in Tables 3 and 4 giving a dose of Nitrogen fertilizer were not significantly different in the components of the Total Dry Weight, Number of Panicles per Clump, Harvest Results, Results, Productive Tiller, Weight 1000 Seeds. But the Spikelet filling percentages (%) were significantly difference. Number of tillers were significantly affect at parameter Total Dry Weight (g plant-1), yield results per plant (g of clump-1) and yield (t ha-1).

Table 5 shows the use of RUE value of N1 is 1.22% not significantly different from N2 which is 1.29%. N1 is lower than N3 and N4. The RUE value of N3 is 1.37%, not significantly different from N4, which is 1.39%. RUE N4 higher than N1 and N2. In the treatment of J5 the RUE was 1.24% lower than that of J10 and J15, respectively 1.34 and 1.37%. Whereas RUE J10 and J15 are not significantly different.

Table 1. Effect of Nitrogen Fertilizer Dose and Number of Tillers on Number of Rice Tillers

Dose N Fertilizer (kg ha^{-1})	**Number of Tilers on Observation Age (DAT)**			
	50*	**65**	**80**	**95**
0	26.56	12.22 a	27.25	23.25
50	26.44	12.88 a	27.22	23.92
100	26.00	14.44 b	27.95	23.92
150	26.33	14.83 b	27.12	24.25
LSD 5%	ns	1.07	ns	ns
Number of Tillers	**Number of Tilers on Observation Age (DAT)**			
	50*	**65**	**80**	**95**
5	26.50	9.58 a	26.04 a	21.44
10	26.50	13.33 b	28.69 c	24.69
15	26.00	17.88 c	27.42 b	25.38
LSD 5%	ns	0.87	1.24	ns

Note: The numbers in each column and row followed by the same letter are not significantly different at the LSD test level of 5%, DAT: days after transplanting, *: observations before N fertilizer and number of tillers treatment. ns: non significant.

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Table 2. Effect of nitrogen fertilizer dose and and number of tillers on leaf area index of rice

Dose N Fertilizer (kg ha^{-1})	Leaf Area Index on Observation Age (DAT)			
	50*	65	80	95
0	2.95	1.30	3.30	2.62
50	2.97	1.34	3.60	2.80
100	2.95	1.18	3.70	2.78
150	2.97	1.35	3.74	2.83
LSD 5%	ns	ns	ns	ns
Number of Tillers	**Leaf Area Index on Observation Age (DAT)**			
	50*	**60**	**80**	**95**
5	2.98	0.94 a	3.55	2.70
10	2.96	1.27 a	3.51	2.75
15	2.94	1.67 c	3.70	2.82
LSD 5%	ns	0.21	ns	ns

Note: The numbers in each column and row followed by the same letter are not significantly different at the LSD test level of 5%, DAT: days after transplanting, *: observations before N fertilizer and number of tillers treatment. ns: non significant.

Table 3. Effect of nitrogen fertilizer dose and number of tillers on total dry weight, number of panicles per clump and yield per clump

Dose N Fertilizer (kg ha^{-1})	Harvest Component		
	Total Dry Weight (g plant^{-1})	Number of panicles per clump	Yiel per Clump (g clump^{-1})
0	83.96	15.78	52.96
50	88.13	16.20	54.15
100	91.40	16.98	56.89
150	91.06	18.01	58.30
BNT 5%	ns	ns	ns
Number of Tillers	**Harvest Component**		
	Total Dry Weight (g plant^{-1})	**Number of panicles per clump**	**Yiel per Clump (g clump^{-1})**
5	85.34 a	15.88	52.36 a
10	87.85 ab	16.47	56.01 ab
15	92.72 b	17.88	58.36 b
LSD 5%	5.21	ns	4.02

Note: The numbers in each column and row followed by the same letter are not significantly different at the LSD test level of 5%, ns: non significant.

Table 4. Effect of nitrogen fertilizer dose and number of tiller on yield, productive tillers, weight of 1000 seeds and spikelet filling percentages

Dose N Fertilizer (kg ha^{-1})	Harvest Component			
	Yield (t ha^{-1})	Productive Tillers (%)	Weight of 1000 seeds (g)	Spikelet filling percentages (%)
0	4.37 a	56.80	27.39	88.41 c
50	4.64 a	56.01	27.38	87.47 bc
100	4.79 ab	59.24	27.05	85.57 a
150	5.15 b	63.29	26.87	85.75 ab
LSD 5%	0.47	ns	ns	1.84
Number of Tillers	**Harvest Component**			

	Yield (t ha^{-1})	Productive Tillers (%)	Productive Tillers (%)	Biji Bernas (%)
5	4.39 a	57.35	26.81	86.76
10	4.83 b	57.29	27.18	85.76
15	4.99 b	61.88	27.52	87.89
LSD 5%	0.26	ns	ns	ns

Note: The numbers in each column and row followed by the same letter are not significantly different at the LSD test level of 5%, ns: non significant.

Table 5. Effect of nitrogen fertilizer dose and thinning saplings on radiation use efficiency (RUE)

Dose N Fertilizer (kg ha^{-1})	RUR (%)
0	1.22 a
50	1.29 ab
100	1.37 bc
150	1.39 c
LSD 5%	0.13
Number of Tillers	**RUE (%)**
5	1.24 a
10	1.34 b
15	1.37 bc
LSD 5%	0.09

Note: The numbers in each column and row followed by the same letter are not significantly different at the LSD test level of 5%, ns: non significant.

IV. DISCUSSION

Plant growth parameters showed no interaction between the dose of urea fertilizer and the number of tillers. However, the growth parameters that observed after reducing the tillers per plant (J5,10 and J15) and fertilizing at 50 HST showed that generally affected by the number of tillers and urea fertilizer doses. To produce optimum plant growth and high yields requires the provision of adequate nitrogen supply and population regulation in rice crop (Tabri, 2010). Plant fertilizers at the right dose and in accordance with the needs of plants so that there is a balance of nutrients in the soil that causes plants to grow and develop properly and provide optimal results. In addition, the high number of tillers per plant affects on nutrient competition. Then the low number of tillers affects on less efficient fertilization.

The result after reducing the number of tillers is increase in the number of tillers. After reducing, cutting tillers causes the tillers regrowth. According to Purwanto (2009), the ability to produce tillers affected by genetic, each rice cultivar has different capabilities to produce tillers. However, environmental factors and cultivation affect the number of tillers too. The rate of tiller production stops when the N content in the leaf blade becomes 2%, P 0.03% and K 0.5%. The rate of tillering increases linearly with increasing N content to 5%, P to 0.2% k to 1.5% above this value does not affect the formation of tillers. The results showed that no interaction between the dose of urea fertilizer and the number of tillers on Inpari-42. The highest productivity of Inpari-42 is 5.15 tons at 150 kg ha^{-1} nitrogen treatment. Schulze and Caldwell (1995) revealed that urea with the appropriate dosage would increase the nitrogen content in the rhizosphere, increase the distribution of nitrogen by compensating and increase the efficient use of nitrogen. Higher urea fertilizer would be toxic to crop then decrease growth and yield. Yield loss is factor that affect the low yield per hectare. Yield loss at the time of harvest solve by several factors, such as harvest age, air content, and tools, and good procedure harvest. In addition, too old age with low water content causes the grain fall off easily during harvest (Lubis et al., 2013).

Higher use of urea fertilizer at the vegetative end phase of the rice plant yields increased results compared to BIJI BERNAS. Increasing nitrogen reduces plant nutrient uptake (Mashur, 2004). When nutrient macros such as P and K is inhibited would reduce the quality and quantity of yield. Dobermann and Faithurst (2000) recorded that deficiency P decreasing yield quality of grain and poor kalium (K) uptake increase the number of empty grain per plant. Higher urea increase the empty grain to reach 19.17% means the percentage of pithy seeds only reaches 80.83% (Ambarita et al., 2018).

The efficient use of solar radiation is the ability of plants to convert energy into biomass. Biomass is a plant's response to the absorption of radiation by the plant canopy. The results showed that RUE in rice crops was affected by the dose of urea fertilizer and the number of tillers. The value of RUE in the study was 1.22 to 1.39%. RUE value of local varieties of rice that is 0.64 to 1.71% and RUE greatly affect the yield (Zaini et al.,). The research of Ahmad et al. (2009), showed that the RUE values in the two varieties below 2.00%, according to of Lubis et al. (2013) average RUE of 10 rice varieties are of 1.13%, while the research of Varon and Diaz (2015) RUE in rice plants was 1.39 to 1.58%. The value of RUE and the interception of PAR into biomass is also influenced by the population number (Liu et al., 2012), management of care such as fertilization and irrigation (Wang et al., 2015) and the type of variety used (Schneider et al., 2016).

The amount of energy used by canopy affected by plant populations. High population number is negatively affect on growth, grain and yields, due to competitive effects, both on vegetative and reproductive development phase (Ahmad, 2005). Each varieties have different capability to use solar radiaton. Radiation that reaches the plant not be fully utilized, because the responsiveness of plants to radiation. Therefore the efficiency of solar radiation in plants needs to be improved. The greater the amount of energy available will increase the amount of photosynthesis (Zervoudakis et al., 2012).

V. CONCLUSSION

N Fertilizer (Urea) and number of tillers in the rice cultivation affects the growth and yield. Reducing tillers to 5 tillers per clump, it showed a yield of 4.39 t ha^{-1} which was the lowest yield. Giving a very large urea fertilizer reduces the quality of bernas seeds, which is 85.57%. There is urea fertilizer application and regulation of the number of tillers in the efficiency of Inpari-42 rice solar radiation energy conversion. The RUE value obtained is around 1.22 to 1.39%.

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Assessing the impact of Farming Systems and Land use change on Dryland plant Biodiversity: A case study of Mwala and Yatta sub Counties in Machakos County, Kenya

Mutua U.M[1], Kisangau D[2], Musimba N[3]

[1] South Eastern Kenya University
Email: urmutua@gmail.com
[2] Department of Life Sciences, School of Pure and Applied Sciences,South Eastern Kenya University
Email:pdkisangau@seku.ac.ke
[3] Department of Rangeland Agriculture,South Eastern Kenya University

Abstract*— The study focused on assessing the impact of farming systems and land use change on dryland biodiversity and documented the views, knowledge and practice of the farmers on the role of biodiversity in the semi-arid midlands of Eastern Kenya. A descriptive survey design was employed to collect data on famers' views, knowledge and practices from 120 respondents from four locations in Mwala and Yatta Sub Counties in Machakos County. Nested Quadrat method was employed to determine levels of loss of plant live forms in the cultivated and uncultivated areas in the four locations. The collected data was then analyzed using simple descriptive statistical such as percentages, frequency and means. Other methods used in the analysis included Logistic regression, Pearson Chi-square and t-tests. The study established that Households in the study areas understand the benefits of non-crop tree species (100%) and therefore grow the tree species (72%) and also conserve the indigenous species (88%). Results from multivariate logistic regression analysis further showed that the age and level of education of the respondents were the strongest statistically significant factors affecting the farmers' knowledge on above ground biodiversity and its relevance to crop production ($p < 0.005$). It was also established that mixed farming system was the main farming system practiced by 98% of the households in Mwala and Yatta sub counties, with crops and livestock on the same farm. It was established that average population of plant live forms (grass, shrubs and trees) in the study sites was found to be significantly different between cultivated and uncultivated zones in the four locations ($p <0.005$). It is concluded that human activities such as farming increases loss of plant live forms and interferes with above ground biodiversity and reduces the effectiveness of crop-livestock integration in the production systems due to reduced grazing areas.

***Keywords*— Biodiversity, Land use, Farming Systems.**

I. INTRODUCTION

According to the Convention on Biological Diversity (CBD) the most comprehensive global framework for conservation of biodiversity, the word "Biodiversity" was coined from the words 'biology' and 'diversity' and is defined as the totality of genes, species and ecosystems. It is thus distinguished into genetic diversity, species diversity and ecosystem diversity. At species level, it is construed to include plants, animals and micro-organisms that are and form the life support system of the earth. It can be measured by the types of different species, or the genetic variations within and between them and how they interact with each other (Institute of Economic Affairs, 2011)

Globally, concerns about the changes in land use and ground cover emerged due to realization that land surface processes influence climate and that change in these processes impact on ecosystem goods and services (Lambin et al., 2003). The impacts that have been of primary concern are the effects of land use change on biological diversity, soil degradation and the ability of biological systems to support human needs.

Crop yields have declined, forcing people to cultivate more and more land to meet their needs (Kaihura and Stocking, 2003).

In the early 2000s, approximately 30% of Kenya was affected by very severe land degradation (UNEP, 2000)and an estimated 12 million people, or a third of the Kenya's population, depended directly on land that is being degraded (Bai, et al., 2008). The droughts of 1970-2000 accelerated soil degradation and reduced per-capita food production (GoK, 2002). According to Muchena (2008), land degradation estimate is increasing in severity and extent in many areas and that over 20% of all cultivated areas, 30 per cent of forests and 10% of grasslands are subject to degradation. The expansion of cropping into forested and water catchment zones accounts for much of this degradation. The damage to soil, loss of habitat, change of land use, water shortages and siltation leads to reduced ecosystem services. Since the 1972 United Nations Conference on Human Environment held at Stockholm, Sweden, the Government of Kenya has continued to reinforce formulation of policies and strategies that would address land degradation. As Murage, et al., (2000)noted, farmers' perceptions and experiences are paramount when planning to implement an enterprise counteracting the on-going land degradation. Moreover, recent diagnostic participatory approaches are increasingly showing that farmers clearly perceive and articulate differences in the levels of soil fertility on their farms.

According to Intergovernmental Panel on Climate Change (2001), in Africa agriculture has been the main contributor to current economy ranging from 10% to 70% of Gross Domestic Product (GDP) and is highly affected by land degradation leading to exploitation of natural resources like forests, settlement and cultivating of fragile land, like hills and sloppy areas. Due to the information gap among people in Africa on land conservation, this has led to mismanagement of natural resources causing land use change, although this has been highly challenged by global warming throughout the world. Biodiversity can contribute directly to food security, nutrition and the well-being of rural communities by providing a wide range of plant and animal food products from both domesticated and wild sources. It can also play a role in maintaining important ecosystem services while contributing to enhanced resilience and stability of rural social-ecological systems. Biodiversity can be viewed as a safety-net to vulnerable households experiencing shocks caused by droughts or market volatility (FAO, 2011). Biodiversity can be greatly affected, by decisions made by various stakeholders about land-use and agricultural, livestock and natural resource management practices.

Many smallholder famers focus on increasing land productivity and crop yields without paying much attention on what happens to biodiversity. The farmers have little or no information on the relevance of non-crop species and the surrounding biodiversity on sustainable and increased crop production. In the efforts of encouraging sustainable dryland biodiversity, use of local knowledge and practices is a key element for success. Land ownership and changes in land uses may affect the distribution of non-crop tree species and general biodiversity. The choices people make in terms of what they plant influences the diversity and abundance of crop and non-crop trees. Population growth has led to land fragmentation in the study area leading to opening up of uncultivated land for crop production. Farming clears plants, interferes with biodiversity and reduces the effectiveness of crop-livestock interactions due to reduced grazing areas. Cultivation also enhances decomposition of organic matter indirectly affecting above ground biodiversity. Farming systems introduced to small holder farmers like mono-cropping and use of inorganic fertilizers can significantly reduce agrobiodiversity by affecting above ground biodiversity. So far, there are very limited studies done on dryland areas, and particularly in the proposed study area focusing on the relationship between biodiversity and land use change and farming systems, and their relevance to improved crop yields for small scale farmers. Therefore, the purpose of this study was to establish the relationship between different farming systems, land use change and the dryland biodiversity in the study areaand recommend the appropriate way forward. Specifically, the study sought to: assess the effect of farming systems and land use change on above ground dryland biodiversity for cultivated and uncultivated areas of Mwala and Yatta Districts; and document the views, knowledge and practice of farmers regarding the status and role of dryland biodiversity in crop production.

II. MATERIALS AND METHODS

2.1 Description of the study area

This study was carried out in Agro-ecological zone (AEZ) Low Midland (LM) 4 and 5 in Mwala and Yatta Sub Counties respectively in Machakos County. The study was executed in two locations in each of the two Sub Counties. These are Kavumbu and Kyawango Locations in Mwala Sub County, and Katangi and Ndalani Locations in Yatta Sub

County. Population density in the study area varies with the agro-ecological zones, and ranges from 40 to 100 person/km^2 (Jaetzold et al, 2006).

2.2 Data collection and analysis

Semi-structured questionnaires were used to gather information on the level of understanding of the farmers on dryland plant biodiversity and its relevance to crop production. Respondents were identified using simple random sampling method. Information on farmers' understanding on biodiversity and its link to crop production was recorded during the interviews. The different farming systems practiced by the farmers and their effects on above ground plant biodiversity were also recorded. A total of 120 respondents were interviewed in the two Sub Counties with 30 in each location. To determine the level of reduction in plant diversity, the dead stumps and the living trees were compared at the sampling points. All the farms which were sampled during the transect walk were geo-referenced. The method was pre-tested before the actual field work started to minimize errors during the actual work.

Data was analyzed using the Statistical Package for Social Sciences (SPSS) where Simple descriptive statistical such as percentage, frequency, mean, mode and median were generated and presented in form of tables and graphs. Descriptive statistics were used in report presentation to bring out the dominant knowledge, attitude (feelings or perceptions) and practice of the farmers regarding the status and role of dryland plant biodiversity on crop production. Logistic regression was also used to determine the effects of social-economic factors on the farmers' knowledge on above ground biodiversity and Pearson Chi-square analysis for the association between socio-economic factors and farming activities. Data on plant live forms between the cultivated and uncultivated lands was subjected to t-tests and analysis of variance (ANOVA) using mixed model in Statistical Package for Social Sciences (SPSS 20). Differences between variable means in the cultivated and uncultivated lands was examined using least significant difference (LSD) at the 5% level of significance.

III. RESULTS AND DISCUSSIONS

3.1 Households' information and demographic characteristics

Findings from the study showed that 82% of the households in the study areas were male headed while 18% were female headed households. The average age of the respondents was 51 years and thus respondents were mature enough to share quality knowledge and experiences on different aspects of plant biodiversity. Most of the respondents had primary (54%) and secondary (29%) as their highest levels of education. 86% of the respondents practiced farming as the main household occupation while the others were employed (7%) and traders (7%) as shown in Table 1.

Table 1: Households' general and demographic characteristics

Variable	Statistics	Percentage (%)	Mean
Age (Yrs)			51
Household head	Male	82	
	Female	18	
Marital status of household head	Married	84	
	Unmarried	6	
	Separated/ divorced	3	
	Windowed	8	
	Never married	0	
Education level of household head	None	13	
	Primary	54	
	Secondary	29	
	Above secondary	4	
Occupation of household head	Employed	7	
	Farmer	86	
	Trader	7	

Results from multivariate logistic regression analysis further showed that the age and level of education of the respondents were the strongest statistically significant factors affecting the farmers' knowledge on above ground biodiversity and its relevance to crop production ($p< 0.005$). Respondents' knowledge on above ground biodiversity and its relevance to crop production was found to increase with increasing age and levels of education ($p< 0.005$). Other demographic factors such as gender, marital status and occupation were found to insignificantly effect on the knowledge on above ground biodiversity and its relevance to crop production ($p> 0.005$) (Table 2).

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Table 2: Multivariate logistic regression analysis for the association between socio-demographic factors and knowledge on above ground biodiversity and its relevance to crop production

Variable	B	S.E.	Wald	df	*p* value
Age	.059	.025	5.581	1	.018
Gender	1.179	1.066	1.222	1	.269
Marital status	19.302	13397.664	.000	1	.999
Education	.940	.449	4.377	1	.036
Occupation	.763	.628	1.477	1	.224
Constant	-47.446	46730.118	.000	1	.999

Variable(s): Age, Gender, Marital status, Education, Occupation

3.2 Farmers' knowledge on importance of non-crop tree species

Most HH (88%) understand the benefits of non-crop tree species and therefore grow them and also conserve the indigenous species. In the study area, Mwala Sub County practiced higher levels of diversity in terms of growing of non-crop tree species and conservation of indigenous species an indication that farmers in that area are more resilient and providing for in-situ conservation. Farmers understand the importance of biodiversity as most of them reported benefits such as environmental conservation, soil conservation and soil fertility improvements, medicinal and nutritional purposes, beauty and ornamental purposes, provision of firewood, fencing posts, timber and other building materials (Table 3). According to Rerkasem et al., (2009), in many traditional agricultural landscapes, the wild and cultivated areas are integrated under a management system to complement each other. Various forms of forests and individual trees are cared for, managed and used for food, fuel, medicine, timber and various other necessities.

Table 3: Purposes of non-crop tree species in the farm

Purposes of non-crop species	Frequency	Percentage (%)
Firewood	41	24.8
Timber/building materials	41	24.8
Shade	25	15.2
Environmental conservation	14	8.5
Fencing	12	7.3
Wind brake	10	6.1
Soil conservation	6	3.6
Charcoal	5	3.0
beauty/amenity/Ornamental	3	1.8
Income	2	1.2
Draw rainfall	2	1.2
Nutritional value	2	1.2
Medicine	1	0.6
Nitrogen fixation/soil fertility	1	0.6

3.2.1 Land ownership and utilization

Land ownership in the study areas was found to be evenly distributed among HH with majority of HHs owning 2-5 acres of land. However, majority of the HHs cultivated 2-3 acres of their land. This is an indication that some of the land might have been put under non-crop tree species for the purpose of diversification. It was also established that the amount of land cultivated significantly depends on the size of land owned by the household ($p < .005$).Crops grown by farmers include maize, green grams, beans, sorghum, millet, tomatoes, cassava and pigeon peas. The crops were intercropped with fruit trees such as mangoes and oranges tree as reported by 93.2% the respondents. Land utilization is significantly associated with the respondents' occupation and land owned ($p < 0.005$) as shown in Table 4. Other studies conducted elsewhere in tropical regions have shown, for instance, that farmers with larger farms are willing to manage trees for timber production (Sebastian etal. 2014). More timber trees can be retained or planted in pastures, especially in linear plantings such as living fences, farm boundaries and along internal roads and paddock divisions (Plath etal. 2010); Esquivel etal. 2014).Farmers produce timber even in small-scale fallows (Marquardt et al. 2013; Robiglio et al. 2013)

Table 4: Pearson chi-square analysis for the association between crops growing and other socio-economic factors

Variable	Chi-square	df	p value
Occupation of respondent	25.697	15	.041*
Education level of respondent	15.332	15	.428
Amount of land owned by household	35.582	15	.002*
Land ownership status	5.715	5	.335

*. The Chi-square statistic is significant at the .05 level.

3.2.2 Farming systems

Mixed farming systems is the main farming system practiced by Households in the study areas, with crops and livestock on the same farm. Households grow and keep a variety of

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crops and animals. The main cropping systems identified during the study included agroforestry, intercropping, mono-cropping and livestock keeping. Intercropping fruit trees and other farm crops encouraged planting of non-crop trees and therefore increasing plant biodiversity. 94% of farmers in the study areas had maintained the same trend of intercropping trees and crops for the last five years (Table 5). According to Selvaraju et al., (2006), many communities harvest wild vegetables, fruits, tubers and other edibles from the forest during the year, especially during the season of greatest food scarcity and use them as food. The establishment of more trees in different land uses can also increase the fuel-wood supply and avoid the extraction of wood from forests (Ndayambaje etal. 2013). Most on-farm production of fruits is lost due to poor market development (Almendarez et al. 2013); home consumption of fruits and other edible products from woody species is critical for food security, as has been shown in many agro-ecological zones e.g. in dryland Africa (Kehlenbeck and McMullin 2015;. Despite the demonstrated contributions of non-crop tree species to domestic consumption, modest income generation, reduction of vulnerability to contingencies, conservation of tree biodiversity and carbon sequestration, more efforts are needed to promote the establishment of trees at the farm level (Lovell etal. 2010). The potential role of incentives such as payments for ecosystem services (Rudel etal. 2016), and the creation of conditions to increase the net incomes and cash in smallholder farm economies need to be assessed and promoted (Etongo etal. 2015). Providing farmers with sound technical advice on non-crop tree species and farmer managed regeneration may also increase the role of trees on farmers' livelihoods (Regmi and Garforth 2010; Oeba etal. 2012; Iiyama etal. 2017). Econometric studies show that the decision to grow trees is not necessarily the same as deciding the number of trees grown. Land certification, as an indicator of tenure security, increases the likelihood that households will grow trees, but is not a significant determinant of the number of trees grown. Other variables, such as risk aversion, land size, adult labor availability, and education of household head, also infuence the number of trees grown (Mekonnen and Damte 2011).

Table 5: Main farming systems in each location

Farming system	Kyawango (%)	Katangi (%)	Ndalani (%)	Masii (%)
Intercropping	0	52	57	47
Shifting cultivation	0	0	0	0
Mono-cropping	71	22	11	20
Strip cropping	0	0	4	0
Livestock keeping	11	67	39	50
Agro-forestry	93	33	43	37
Mixed cropping	7	44	68	3

The main livestock kept include cattle and goats (ranging from 1-10) compared to sheep, pigs and donkeys. About 88% of the HHs interviewed kept over 5 chicken. The farmers practice intensive livestock production on cattle, goats, sheep, donkey and poultry. The number of livestock kept was significantly associated with the farming system used ($p < .005$) as shown in Table 6.

Table 6: Number of livestock kept

Livestock	None	1 - 4	5 - 10	10 - 14	15 and above
Cattle	23%	60%	15%	0%	2%
Goats	8%	33%	31%	11%	17%
Sheep	74%	16%	6%	3%	1%
Poultry	7%	5%	28%	30%	30%
Pigs	100%	0%	0%	0%	0%
Donkeys	67%	32%	2%	0%	0%

Different farming systems contribute differently in the efforts of maintaining above ground biodiversity in the study area. According to Maitima, et al, (2004), in forest and communal grazing areas, adjacent to mixed farming areas, plant and animal biodiversity may decrease because of over-grazing. According to FAO (2011) [30], Biodiversity can contribute directly to food security, nutrition and the well-being of rural communities by providing a wide range of plant and animal food products from both domesticated and wild sources. It can also play a role in maintaining important ecosystem services while contributing to enhanced resilience and stability of rural social-ecological systems.

3.3 Effect of cultivation on above ground biodiversity

The rapid loss of species due to human activities and its important implications for ecosystem functioning, services and human well-being have prompted biodiversity research to grow into one of the most active fields in ecological research during the last 20 years (Loreau et al. 2001,2002; Hooper et al.2005). The dominant grass species identified in the study areas were *Dactylocteniumspp* and *Acanthospernum spp*. The most dominant shrub species in the study sites included *Indigoferaspicata*, *Gnidialatifolia* and *Orthosiphon spp*. The dominant tree species were *Acacia spp*, *Combretumspp* and *Terminalia spp*. Generally, the study established that the population of plant live forms in the study sites was higher in the un-cultivated areas than in the cultivated areas (Table 7)

Table 7: Population of plant live forms in the study sites

Study site	Plant live forms	Cultivated	Un-cultivated
Masii	Grass per M^2	4.33	14.67
	Shrubs per Ha	833.33	2083.33
	trees per Ha	200.00	466.67
Kyawango	Grass/ herbs per M^2	3.67	10.33
	Shrubs per Ha	1875.00	2291.67
	trees per Ha	300.00	600.00
Katangi	grass herbs per M^2	6.33	10.00
	Shrubs per Ha	625.00	3750.00
	trees per Ha	100.00	300.00
Ndalani	grass herbs per M^2	7.67	13.00
	Shrubs per Ha	625.00	2916.67
	trees per Ha	150.00	533.33

The average population of plant live forms (grass, shrubs and trees) in the study sites was found to be significantly different between cultivated and uncultivated zones ($p < 0.005$) in the four locations (Table 8).

Table 8: T-test results comparing plant population in cultivated and uncultivated zones

Plant live forms	t	Df.	Mean Difference	Std. Error Difference	*p*-value
Grass per M2	-5.849	22	-6.5	1.111	0.000
Shrubs per Ha	-3.466	20	-1697.917	489.866	0.002
Trees per Ha	-3.742	19	-286.111	76.469	0.001

Aboveground and below ground compartments of terrestrial ecosystems have traditionally been studied in isolation from one another (Wardle et al. 2004[36]), hampering a holistic understanding of ecosystem functioning. The interactions between human activities and agricultural production influence plant community dynamics and composition (Klironomos 2002; Wurst et al. 2008[34]; Bardgettand Wardle 2010).

IV. CONCLUSIONS

HHs in the study areas demonstrated to have knowledge on the importance non-crop tree species and majority practiced high levels of diversity. The HHs grow dryland crop species and conserved indigenous trees/non-crop species in their farms and therefore are more resilient and providing for in-situ conservation. Maintenance of high levels of inter- and intra-species diversity is a strategy to decrease vulnerability and enhance resilience to climate change and associated stresses. Adaptation activities include the maintenance and reintroduction of traditional varieties, the adoption of new species and varieties to meet newly developed production niches, and the development of ways of ensuring that materials remain available and adapted. In the efforts of encouraging sustainable dryland biodiversity, use of local knowledge and practices is a key element for success. It is concluded that farmers understand the importance of biodiversity conservation and are keen to ensure that natural vegetation is conserved for it benefits such as environmental conservation, soil conservation and soil fertility improvements, medicinal and nutritional purposes, beauty and ornamental purposes, provision of firewood, fencing posts, timber and other building materials.

Land ownership and changes in land uses in the study areas affected the distribution of non-crop tree species and general biodiversity. Findings showed that households owned 2-5 acres of land and utilized about 2-3 acres of their land in cultivation. There was a significant variance in the average population of plant live forms (grass, shrubs and trees) between cultivated and uncultivated zones in the four locations. The rates of reduction in plant diversity in the study area was also found to differ significantly between the cultivated and uncultivated areas. Results from the analysis of the data collected showed that cultivation has reduced above ground plant biodiversity of grass and herbs, shrubs, and tree saplings significantly. Degradation of habitats due to changes in land use is the immediate most serious threat to dryland biodiversity. This is further exacerbated by climatic factors at both local and global scales. The choices people make in terms of the production system influences the diversity and abundance of crop and non-crop trees. Therefore, it is concluded that cultivation significantly reduces above ground plant diversity. Loss of plant diversity in the study areas was highly characterized by reduced biodiversity where this has been majorly contributed by human and animal activities.

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God Bless You All

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The Influence of Pesticides on the Biology and Physiology of the Land Snail *Bulimulus tenuissimus* (Orbigny, 1935)

Iza Patrício[1], Florence Gonçalves Martins[2], Tatiane Cristina dos Santos Bonfim[2], Mauricio Carvalho de Vasconcellos[1], Jairo Pinheiro[3], Marta Julia Faro[4], Clélia Christina Mello-Silva[1]

[1]Grupo de pesquisa em Saúde e Educação Ambiental Crítica com Ênfase nas Relações Parasitárias do Laboratório de Avaliação e Promoção da Saúde Ambiental, IOC, FIOCRUZ, Av. Brasil 4365, Manguinhos, 21040-900, RJ, Brazil
Email: izapattricio@gmail.com, mau@ioc.fiocruz.br, clelia@ioc.fiocruz.br
[2]Curso de Pós-Graduação em Ciências Veterinárias, Instituto de Veterinária, UFRRJ, BR 465, km 47, 23897-000, Seropédica, RJ, Brazil
Email: florencegmartins@gmail.com, tatianecdsb@hotmail.com
[3]Departamento de Ciências Fisiológicas, Instituto de Ciências Biológicas e da Saúde, UFRRJ, BR 465, km 47, 23897-000, Seropédica, RJ, Brazil
Email: jairopinheirodasilva@gmail.com
[4]Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, IOC, FIOCRUZ, Av. Brazil 4365, Manguinhos, 21040-900 RJ, Brazil
Email: martajulia.ioc.fiocruz@gmail.com
Corresponding author: clelia@ioc.fiocruz.br

***Abstract*—** *Some terrestrial gastropods are agricultural pests and are exposed to toxic products capable of causing physiological and biological alterations. The action of some herbicides and molluscicides has been studied on freshwater snails, but there are few studies of terrestrial species. This study analyzed the biological and physiological responses of Bulimulus tenuissimus exposed to the natural molluscicidal latex of Euphorbia milii var. hislopii and the synthetic herbicide Roundup® Original. A total of 210 snails were divided and exposed for 24 hours to the products (latex or herbicide in concentrations of 1,000 ppm and 100,000 ppm) and type 2 water. Of this total, 150 exposed animals were analyzed biochemically after 24 hours, and the others were observed during 30 days for analysis of the mortality rate. After 24 hours, some snails presented escape mechanisms such as epiphragm formation, cephalopedal mass retraction and burial. The latex did not cause metabolic alterations but the herbicide altered the carbohydrate metabolism of B. tenuissimus, reducing energy substrates in the sites analyzed (hemolymph, digestive gland and cephalopedal mass). Regarding the protein metabolism, there was no alteration after exposure to both analyzed substances. After 30 days of exposure, higher mortality was recorded for the groups exposed to the herbicide. These findings suggest that B. tenuissimus is resistant to the molluscicidal latex of Euphorbia milii var. hislopii more than the herbicide Roundup® Original.*

Keywords*— *Bulimulus tenuissimus, carbohydrate metabolism, Euphorbia milii var. hislopii, Roundup® Original.

I. INTRODUCTION

Agrotoxics are substances used to increase agricultural productivity through pest and disease control [1]. However, their indiscriminate use can cause serious damage to the environment and to human health, as well as putting selective pressure for development of resistance by pests [2].

Brazil has been the largest consumer of agrotoxics in the world since 2008 according to data from the Brazilian Institute of Geography and Statistics (IBGE) [3], with numerous active principles and commercial formulations, which can be classified according the action on the target organism and the chemical group to which they belong [2]. Of the agrotoxics used in Brazil, 56% are herbicides [4]. Among the commonly used Roundup® stands out, which has as its active ingredient Glyphosate (N-(phosphonomethyl) glycine) combined with a surfactant that facilitates transport by the plant. It is a non-selective herbicide, effective in the total destruction of weeds [5], and in the control of plants in reforestation, gardens and aquatic plants in fish farming tanks and in lakes [6]. From the Roundup® herbicide several formulations were commercialized by the Monsanto Company, one of them is Roundup® Original, which presents polyoxyethylene amine surfactant (POEA), which helps the herbicide to penetrate the leaves of plants [7]. Some herbicides have deleterious effects on limnic mollusks, interfering at the cellular and physiological, molecular level to reduce the survival rate and reproductive activity [8, 9, 10].

Molluscicide is the classification of agrotoxic for control of mollusks present in gardens, fields and greenhouses, including those that are intermediate hosts of parasites in aquatic environments, such as snails that transmit schistosomiasis. Poisoning by such substances can cause osmotic imbalance, and as a consequence two mechanisms that cause mortality may occur: retraction of the cephalopedal mass with the release of hemolymph and/or abnormal projection out of the shell [11], called "distress syndrome".

Molluscicides can be synthetic or natural. Chemical control of terrestrial species occurs mainly through synthetic molluscicides. Although effective, these compounds also act on non-target organisms, causing significant changes in ecosystems [12]. As a consequence, there is increasing demand for readily biodegradable substances as an alternative to synthetic molluscicides [13].

The latex of *Euphorbia milii* var. *hislopii* (N. E. B.) (Euphorbiaceae), popularly known as crown of thorns, Christ plant, or Christ thorn, is considered one of the most promising Brazilian molluscicides. In laboratory and field conditions it complies with the recommendations of the World Health Organization (WHO) for use as a natural molluscicide, in addition to being biodegradable and less hazardous to non-target organisms [14], with effective results on limnic mollusks and amphibians [15].

The land snail *Bulimulus tenuissimus* (Orbigny, 1935) is present in many Brazilian regions [16], where it is considered an agricultural pest. It is also the only species in the family Bulimulidae cited as an intermediate host of some parasites of poultry [17]. Recently, studies showed that this mollusk is host of the nematode *Angiostrongylus cantonensis* (Chen, 1935) [18,19], a rodent lung parasite capable of infecting humans and causing meningitis, characterized by eosinophilic inflammation [20].

Studies of synthetic or natural substances for the control of terrestrial species are scarce, as well as the physiological changes induced by these substances. For this reason, this study analyzed the biological and physiological responses of *B. tenuissimus* exposed to the natural molluscicide latex of *E. milii* var. *hislopii* and the synthetic herbicide Roundup® Original.

II. MATERIAL AND METHODS

2.1. Determining concentrations of *E. milii* var. *hislopii* latex and Roundup® Original

The crude latex extract was collected in the Ilha do Governador district (22°48´09´´S/ 43°12´35´´W) of the city of Rio de Janeiro, Brazil. Determination of the concentrations of the two substances was based on the procedures described by Vasconcellos and Amorim [21], and based on pre-tests, concentrations of 1,000 ppm (parts per million) (0.1%) and 100,000 ppm (10%) for both substances were chosen (mg/L for latex and ml/L for herbicide).

2.2. Formation of groups

The parents of the snails used were obtained in a garden located in the municipality of Seropédica, Rio de Janeiro, Brazil (about: 22º 44' 38" S; 43º 42' 27" W). A total of 210 hatched mollusks were kept in the Laboratory for Evaluation and Promotion of Environmental Health of Fiocruz in Rio de Janeiro, where they were monitored until reaching sexual maturity (110 days).

For the 24-hour and 30-day follow-up, the snails were divided into five groups, with 30 and 12 specimens each, respectively: two groups exposed to latex (L) at concentrations of 1,000 ppm and 100,000 ppm (L1,000 and L100,000, respectively); two groups exposed to Roundup® Original (R) (R1,000 and R100,000, respectively); and one control group sprayed with type 2 water.

2.3. Exposure

For the 24-h follow-up, 150 mollusks were exposed individually in disposable flasks with a capacity of 150 ml containing 50 g of sterile soil moistened with water. Addition of the compounds was done by spraying the concentrations prepared for each product using 5 ml glass hand sprayers. Each mollusk received 2 ml of the product corresponding to its group, under a fume hood. Twenty-four hours after exposure, the specimens were observed and dissected for the collection of biological samples.

For the 30-day follow-up, the exposure occurred plastic terrariums with 12 cm in diameter and 8 cm in depth, containing 3 cm of moistened sterilized soil as substrate. Each terrarium received 24 ml of the substance corresponding to its group through glass sprayers: 2 ml of the corresponding substance was sprayed onto each mollusk, under a fume hood. They were observed every

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other day for 30 days (four weeks) after exposure to determine the mortality rate.

In both cases, the control groups were sprayed with 2 ml of type 2 water.

2.4. Collection of hemolymph and dissection after 24 hours

After 24 hours of exposure, 150 snails (30 specimens from each group) were dissected. The hemolymph was collected by extravasation from the apex rupture of the shell, collected in microtubes, kept in an ice bath at 10 °C and then stored at -20 °C in a freezer until the biochemical analyses [18].

The specimens were dissected in Petri dishes and the cephalopedal mass and digestive gland tissues were kept separately in an ice bath at 10 °C during collection to avoid enzymatic degradation of carbohydrates. Subsequently, the samples were weighed and stored at -20 ºC in a freezer until use.

2.5. Biochemical analysis

The hemolymph was analyzed with a BioSystems A15 automatic biochemical analyzer, with reading through the use of reagent kits for biochemical analysis prepared for glucose, total protein and lactate dehydrogenase (LDH E.C. 1.1.1.27).

Glycogen extraction from the digestive gland and cephalopedal mass was performed as described by Pinheiro and Gomes [22] and quantified through the 3,5 dinitrosalicylate (DNS) technique [23], with results expressed as mg of glucose/g tissue, fresh weight.

2.6. Maintenance of the terrariums

The feed consisted of 3 g ± 1 g, of chayote (*Sechium edule*) and carrot (*Daucus carota*), plus lettuce leaves (*Lactuca sativa*) *ad libitum*. *In natura*. The feed was supplemented with bird feed enriched for growth with calcium carbonate in proportion of 3:1 [24], served in plastic containers (3 cm in diameter and 0.5 cm in depth [25]. All feed was changed every two days. The soil sprinkled with the substances was kept in the terrariums until the end of the experiment (30 days).

2.7. Mortality records

The terrariums were observed every two days, for 30 days (four weeks), for quantification and removal of dead snails. The mortality rate was expressed as a percentage (%).

2.8. Statistical analyses

The results were expressed as mean ± standard error of the mean, and were submitted one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test (α= 5%) to compare the means (GraphPad InStat and GraphPad Prism, v. 6., Prism Inc.).

III. RESULTS

After 24 hours of exposure, exhaust mechanisms, characterized by increased mucus release and presence of epiphragm, were observed in the snails of all groups exposed to both toxic products. Also, in the L1,000 group, burial behavior occurred, and in the R1,000 group, cephalopedal mass retraction was observed.

Significant reductions of 29.99%, 24.21% and 12.21% in the glycogen content of the digestive gland were observed in the groups L100,000 (17.09 ± 0.29), R1,000 (18.5 ± 0.66) and R100,000 (21.43 ± 0.77), respectively, in relation to the control group (24.41 ± 0.37). However, a non-significant increase was observed of 7.57% in the group L1,000 (26.26 ± 0.66) in relation to the control group (Fig. 1A).

The pattern was repeated for glycogen content in the cephalopedal mass, with significant reductions of 37.9%, 60.63%, 28.66% in groups L100,000 (18.96 ± 0.02), R1,000 (12.02 ± 0.14) and R100,000 (21.78 ± 0.03), respectively, in relation to the control group (30.53 ± 0.08). And group L1,000 (35.83 ± 0.14) presented a significant increase of 17.36% compared to the control group (Fig. 1B).

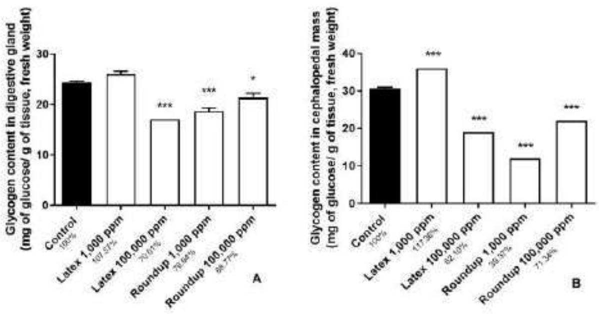

Fig. 1. Changes in the glycogen content (mg of glucose/g tissue, fresh weight) in Bulimulus tenuissimus tissues exposed to two concentrations of natural molluscicide latex of E. milii var. hislopii and the synthetic herbicide Roundup® Original. ***A*** *Digestive gland.* ***B*** *Cephalopedal mass (p value<0.0001, strongly significant).*

(P<0.001***, strongly significant/ P<0.01**, significant / P<0.05*, weakly significant difference/ P>0.05 no significant difference in relation to the control group).

The glucose concentration in the hemolymph showed significant reductions of 72.08% and 74.99%, respectively, in the L1,000 (6.33 ± 0.88) and R100,000 (5.67 ± 1.2) groups in relation to the control (22.67 ± 1.2). The decrease of 29.42% observed in group R1,000 (16 ± 0.0) was not significantly different than the control. In addition, a non-significant increase of 27.92% in the L100,000 group (29 ± 3.05) was observed compared to the control group (Fig. 2A).

The analysis of lactate dehydrogenase (LDH) activity in hemolymph showed increases in activity of 48.43% and 65.85% in L100,000 (142 ± 43.15) and R1,000 (158.67 ± 2.73), respectively, after 24 hours of exposure. Moreover, reductions of 46.69% and 38.68% of this activity were observed in L1,000 (51 ± 6.03) and R100,000 (58 ± 12.44), respectively. However, no significant differences were observed in relation to the control group (95.67 ± 12.55) (Fig. 2B).

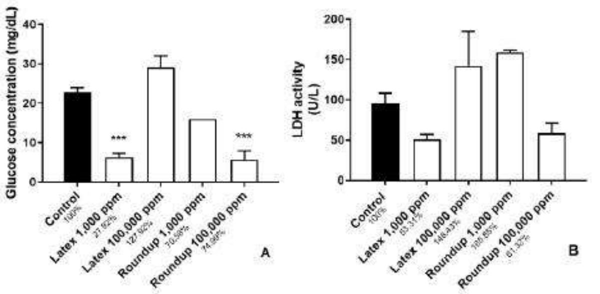

Fig. 2. ***A*** *Glucose concentration expressed in mg/L (P<0.0001, strongly significant) and* ***B*** *Lactate dehydrogenase (LDH) activity expressed in U/L (P= 0.0153, significant) in Bulimulus tenuissimus hemolymph after 24 hours of exposure to Euphorbia milii var. hislopii latex and the synthetic herbicide Roundup® Original.*
(P<0.001***, strongly significant/ P<0.01**, significant / P<0.05*, weakly significant difference/ P>0.05 no significant difference in relation to the control group).

Exposure to the toxic substances caused non-significant reductions in circulating concentration of protein in the hemolymph of 33.35%, 1.27% and 28.19% in the L1,000 (17.33 ± 2.85) L100,000 (25.67 ± 3.48) and R100,000 (18.67 ± 4.70) groups, respectively, compared to the control group (26 ± 3.46). In R1,000 there was a 33.34% increase in this parameter (34.67 ± 0.3) (Fig. 3).

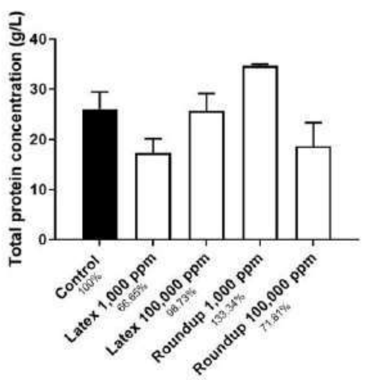

Fig. 3. Circulating protein concentration, expressed in g/L of Bulimulus tenuissimus hemolymph after 24 hours of exposure to Euphorbia milii var. hislopii latex and the synthetic herbicide Roundup® Original.

(P<0.001***, strongly significant/ P<0.01**, significant / P<0.05*, weakly significant difference/ P>0.05 no significant difference in relation to the control group).

Dead mollusks were found in terrariums after 24 hours in the R100,000 group, after 48 hours in the R1,000 group, 96 hours in the L100,000 group and 168 hours in the L1,000 group.

For mortality of *B. tenuissimus* after 30 days (720 hours), the exposure to the synthetic herbicide in the groups R1,000 and R100,000 caused mortality rate of 91.67%. In the L1,000 and L100,000 groups, these rates were 75% and 83.33%, respectively, for the exposed snails. The control group had a 75% mortality rate (Table 1).

Table 1. Mortality rate of Bulimulus tenuissimus accompanied for 30 days after exposure to Euphorbia milii var. hislopii (L) latex and the synthetic herbicide Roundup® Original (R).

Hours of exposure (h)	Exposed groups				
	Control	L1,000	L100,000	R1,000	R100,000
24	-	-	-	-	1
48	-	-	-	1	-
72	-	-	-	-	-
96	-	-	1	1	2
168	2	3	4	3	2
336	-	2	2	3	3
504	3	1	2	1	2
720	4	3	1	2	1
Total no. of dead snails	9 (75%)	9 (75%)	10 (83.33%)	11 (91.67%)	11 (91.67%)
N- Survival percentage (%)	3- 25%	3- 25%	2- 16.63%	1- 8.33%	1- 8.33%

IV. DISCUSSION

Land mollusks can present physiological and behavioral strategies to ensure their survival under unfavorable conditions, such as estivation, retraction of the cephalopedal mass, and burial [26, 27], all of which can favor survival after exposure to molluscicides [28].

In this study, the first reaction to the toxic substances, observed after 24 hours of exposure, was increased release of mucus in all terrariums containing exposed mollusks. According to the literature, increased mucus production and secretion is one of the first reactions of gastropods to stress, among them chemical irritation caused by molluscicidal products [29]. Other strategies, such as cephalopedal mass retraction and epiphragm formation, were also observed in the present study after stress, corroborating the finding of D'ávila and collaborators, who examined *Bradybaena similaris* (Férussac, 1821), *Leptinaria unilamellata* (d'Orbigny, 1837) and *Subulina octona* (Brugüière, 1789) at a constant temperature of 35 °C for 48 hours and observed cephalopedal mass retraction after 12 h of exposure and the presence of epiphragm in four replicates of *B. similaris* with 24 hours of exposure [30].

Exposure to toxic substances can alter the physiological homeostasis of an organism [31,32,33]. Silva et al. [34]., exposing *B. similaris* to the LC_{50} of the aqueous extract of *Solanum peniculatum* L. (Solanaceae) for 72 h, observed after 24 h an increase in the carbohydrate content of the digestive gland and the concentration of glucose in the hemolymph, and a significant reduction of carbohydrates in the cephalopedal mass. According to the authors, this profile demonstrates the existence of metabolic compensation for the regulation of homeostasis in the organism [34].

In the present study, the L1,000 group showed an increase in glycogen content in the digestive gland and cephalopedal mass, and a significant reduction of glucose concentration in the hemolymph. In the L100,000 group, there was a significant reduction of the glycogen content in the tissues and an increase in the glucose concentration in the hemolymph. In this respect, according to Pinheiro et al. [35], mollusks mainly use glycogen stores in the digestive gland and muscle tissue to maintain normoglycemia in the hemolymph. This explains our observation that the snails in group L100,000 had tendency to stabilize the glucose in the organism through gluconeogenesis to maintain homeostasis. In the L1,000 group, the energy metabolism was maintained through the consumption of free glucose in the hemolymph, causing accumulation of glycogen in the tissues, to guarantee the regulation of glycemia.

Unlike latex, the exposure to the herbicide Roundup® Original caused reductions in glucose concentration in the hemolymph and glycogen content in the digestive gland and cephalopedal mass of *B. tenuissimus*. Barky et al. [36], when exposing *Biomphalaria alexandrina* to the LC_{10} of the herbicides Atrazine (0.33 ppm) and Roundup® (0.84 ppm) for four weeks, observed an increase in glucose concentration in the hemolymph and reduction of glycogen content in the tissues of these animals, a different pattern than the present study.

LDH is an enzyme involved in the activation of anaerobic metabolism, and its measurement can be used to understand energy production by mollusks, which can occur either aerobically or anaerobically. Silva et al. found a significant increase in LDH activity in *B. similaris* after 24 h exposure to the LC_{50} of the aqueous extract of *S. paniculatum*. According to the authors, acceleration of anaerobic metabolism occurred, a possible consequence of intoxication [34]. In the current study, the results obtained show that the different concentrations of the molluscicide latex and herbicide did not significantly alter LDH activity, so that aerobic metabolism was maintained.

Faced with a deficiency of energy substrates first consumed in the body, mollusks can search for proteins as an alternative means of obtaining energy [37]. In this study, the total protein concentration in the exposed groups did not differ significantly from the control group, showing that it was not necessary to use this source for homeostatic maintenance.

Regarding the mortality of *B. tenuissimus* during 30 days of exposure, the first deaths occurred in 24 and 48 hours for the groups exposed to the herbicide. Mortality after exposure to the latex was slower, with the first death occurring after exposure for 96 h. Mortality was recorded until the end of 720 h at both concentrations. Afonso-Neto et al. [38], submitting a terrestrial gastropod to the latex of three Euphorbiaceae species, verified that *E. milii* var. *hislopii* was able to kill 100% of the specimens within 24 hours after exposure to the different dilutions, and highlighted the decrease of the latex effect revealed by 25% mortality after exposure to the concentration of 1: 1,000 ml/ml of the product.

The WHO determines that the concentrations used for natural molluscicides in aquatic mollusks are 20 ppm for plant extracts and 100 ppm for the raw plant) [39], and the literature does not find dosage recommendations for land mollusks species [40]. In this study, the concentrations used for the molluscicide exceeded the limit determined by the WHO. In our work, the concentrations of herbicides were equal or higher than those used by other authors, using the profile mollusk vs. herbicide [8, 9, 10, 36]. The specimens of *B. tenuissimus* was able quickly recovery of homeostasis.

The use of agricultural chemicals in plantations is common [41], in Brazil. The applications of these substances are justified by the fact that about 10% of the harvest is lost due to the presence of different agricultural pests in the cultivation system [42]. Because of this, the resistance or resilience to pesticides found in this work in *B. tenuissimus* can be explained in two ways: the first related to the habit of mollusks to feed on vegetations with pesticides and the other through the generation of phenotypes of resistance induced by selective pressure imposed by the frequent use of pesticides. The resilience and persistence of invertebrates is explained in the literature [43, 44] and according to Brigante et al. [45] are characteristics of biondicator species such as land mollusks.

V. CONCLUSION

In our study, *B. tenuissimus* showed resistance to high concentrations of pesticides. The snails were able to survive exposure to Roundup® Original at both concentrations used in this work, but the energy metabolism was altered. New studies on the behavioral, physiological and epigenetic effects of pesticides on these land snails are already underway. These studies are necessary because these mollusks have wide geographical distribution, are considered agricultural pests and are intermediate hosts of *Angiostrongylus cantonensis*, etiological agent of eosinophilic meningoencephalitis.

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Effect of Invasive Pest *Phenacoccus manihoti* Matile-Ferrero (Hemiptera; Pseudococcidae) in Cassava

Nila Wardani[1], Aunu Rauf[2], I Wayan Winasa[2], Sugeng Santoso[2]

[1]Lampung Assesment Institute for Agriculture Technology, Jl. ZA. Pagar Alam No. IA, Bandar Lampung,
email: wardaninila@yahoo.co.id
[2]Department of Plant Protection, Bogor Agricultural University, Dramaga Campus, Bogor, West Java, Indonesia 16680

Abstract**—***Heavy damage by the cassava mealybug, Phenacoccua manihoti Matile-Ferrero (Hempiptera: Pseudococcidae) caused symptoms of bunchy top, shortened and distorted nodes, leaf drops, and inhibition of plant growth. The consequences of these pests can cause yield losses of 80%. This study was to determine the development of P. manihoti and cassava yield loss. Field observations indicated that symptoms of bunchy top appeared as early as 8 weeks after planting (wap) and rose quickly started 16 wap, at the same time with the advent of the dry season (May-June). Level of infestation developed faster on variety Jimbul; at 18 wap all plants had bunchy tops. While on varieties Roti and Manggu, 100% infestation occurred at 30 and 36 wap, respectively. There was a correlation between early infetation with plant height ang yield. Cassava plants infested during early stage were shorter and the yield lower, compared to those infested at further stages. Lower yields of variety Jimbul (0.94 kg/tree) than variety Manggu (3.16 kg/plant), was thought to be related to heavy infestation which occurred during early stage.*
Keywords**—*****Cassava, mealybug, level of infestation, Phenacoccus manihoti..***

I. INTRODUCTION

Plant lice from the family Pseudococcidae are often referred to as mealybug. Damage to plants by these pests due to stilet suction on the leaves can cause leaf wrinkles, some types can act as vectors of plant viruses or cause chlorosis due to the presence of saliva (saliva) which is toxic [1, 2].

Plant lice often invade previously uninfested areas by sending plant material from one area to another. Population explosions often occur if they are not accompanied by natural enemies or there are no natural enemies in new areas. The population will remain stable in new areas because of the control of natural enemies both predators and parasitoids [1]. The important role of the natural enemy of white lice has been widely reported in the Americas and Africa [3, 4, 5].

One of the mealybug pests that attack cassava plants in Indonesia is *P. manihoti*. *P. manihoti* came from South America and entered Africa in the 1980s. This pest first entered the Southeast Asian region was Thailand in 2009, which caused heavy attacks, possibly having spread to Laos and Cambodia. In Indonesia it was first discovered in the West Java area at the end of 2010 [6, 7].

Adult *P. manihoti* is oval in shape, pink in color covered with waxy white flour, body size is more or less long (1.10-2.6 mm) and wide (0.50-1.40 mm) [8]. Often found at the top of the plant which results in the tops becoming wrinkled and dwarfing bunchy tops. Heavier attacks occur in the dry season than in the wet season [9]. In Colombia *P. manihoti* and *P. hereni* can cause a 68-88% reduction in production and in Africa can cause yield losses of up to 80% [10].

It is known that in Asia, Indonesia is one of the biggest cassava producers after Thailand [11]. In the country, cassava is widely used as raw material for food and non-food industries. Currently, in addition to food and feed, cassava can be developed as an alternative energy source (biofuel). By using bioethanol as a premium mixture (Premium mix E 10) for transportation. Bioethanol is sourced from starchy and sugary plants such as corn, cassava, sweet potatoes, sago and sugar cane. Production costs for each liter of ethanol from cassava are cheaper compared to other raw materials so that the development of cassava-based industries is quite prospective [12].

Although in Indonesia the level of damage and severe attacks have never been reported, but this pest has the potential to cause cassava yield loss if not controlled. For this reason, this study aims to determine the development of *P. manihoti* attacks, cassava losses due to these pests.

II. METHODOLOGY

The study was conducted on farmers' land in the villages of Ngampar, Cikeas and Sukaraja, Sukaraja District. The research took place from February 2012 to February 2013.
The level of attack is known to be taken as a sample in the form of 10 plots of cassava plantations taken by purposive sampling in each village (Ngampar, Cikeas, and Sukaraja). From each plot of cassava plantations 20 samples were observed, with diagonal sampling technique. Then observed plants that were attacked by *P. manihoti*, and which were not attacked
Observation of the level of attacks carried out in the garden of cassava farmers in the village of Ngampar which has been attacked by the pest *P. manihoti*. The area of farmers' gardens that was used as observation plots ranged from 1000-2000 m2. Observations were made on three types of local cassava varieties namely Bread, Manggu and Jimbul varieties. The population of each cassava variety that was chosen as the selected sample was Bread variety with a total population of 1504 plants, 472 Manggu plants and Jimbul 629 plants. Cultivation techniques are applied in accordance with what is done by farmers, namely monoculture cultivation with a spacing of 1 m x 1 m, fertilizing is done using manure and urea fertilizer. The plantations observed were not applied with pesticides.
Observations to determine the development of *P. manihoti* attacks on cassava plants (rainy season and dry season) were carried out on all plant populations with observational intervals every two weeks. The initial effect of white lice infestation on the production and observation of natural enemies was carried out on 40 plant samples for each observed variety, with observational intervals once every two weeks. The observations were tabulated and the tendencies compared between the three varieties observed.

III. RESULTS AND DISCUSSION

P. manihoti survey

The attack from *P. manihoti* at three locations in Sukaraja District was very high. The highest attacks occurred in Sukaraja Village 99.5% and Ngampar Village (99.4%), the lowest in Cikeas Village (73.5%). Although different, but the level of attacks in this district is relatively the same as the average attack of almost one hundred percent.
The difference in the level of *P. manihoti* attacks can be caused by several factors. Like most insect pests, mealybug population dynamics are influenced by biotic and abiotic factors that will shape the characteristics of pest populations, such as density, rate of birth, rate of death, distribution pattern, biotic potential, and behavior [13]. One of the abiotic factors that affect mealybug populations is environmental conditions such as climate, especially rainfall, temperature and humidity. The population is strongly influenced by climatic conditions so that population abundance is higher in arid regions compared to areas with high rainfall (wet) [9]. Hotter conditions with lower humidity, is a more suitable place for the development of *P. manihoti*. The high attack from mealybug is seen with the symptoms of banchy top and stem distortion.

Increased symptoms of *P. manihoti* attacks

Observation of the development of *P. manihoti* attacks begins when the plants are 6 weeks after planting (wap) until the plants are harvested. In the field, all varieties appear to show symptoms of attack, but differ in severity and severity of symptoms of attack (Figure 1). This condition shows that there are differences in the resistance of the three cassava varieties observed, as well as the results of the study of the resistance of 4 cassava varieties in Thailand, between the Kasetsart 50 varieties, Huaybong 60, Rayong 9 and Rayong 72, all varieties attacked by *P. manihoti*, differences occur at the level of pest attacks [14].
The development of *P. manihoti* is strongly influenced by environmental factors, especially rainfall. Figure 2 presents the rainfall that took place during the study. Attacks increase rapidly starting in June, or after the plants are 15 weeks after planting (wap), and reach a peak in September / October. The relatively hot temperature and low rainfall during this period caused the population of *P. manihoti* to develop faster, especially in Pseudococcidae [15].

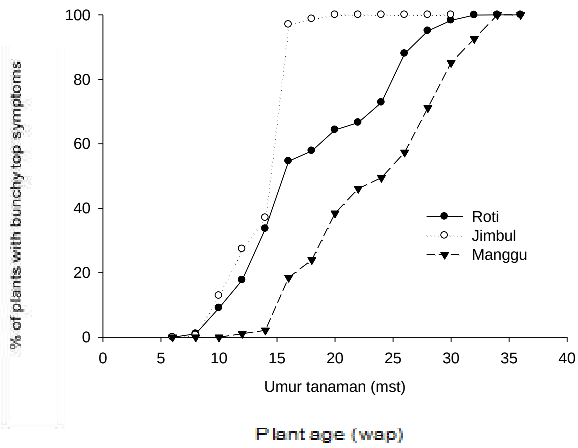

Fig.1: Development of P. manihoti's attack on three cassava varieties

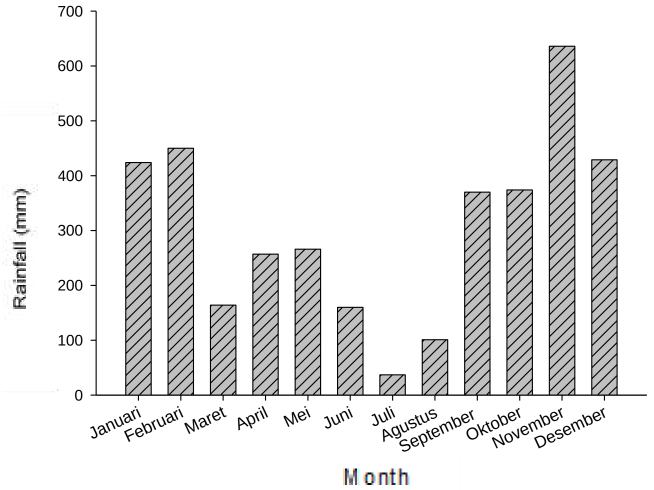

Fig.2: Rainfall around the study site [16]

P. manihoti attacks begin with the symptoms of an initial attack, where the shoots of the cassava plants begin to wrinkle, the initial symptoms usually occur around 7-10 days, then with an increase in the flea population, the symptoms of the attack quickly become a bunchy top, ie, the shoots wrinkle and collect. This symptom lasts relatively long (20-30 days). When lice reach a high population (approximately 200-1000 individuals in one shoot), then the shoots of plants will lose leaves (deciduous leaves). The shoots will remain leafless until it rains. If it rains, it will re-sprout at the top of the plant, due to lice attack will leave the trunk with a short internode or like a wrinkle, sometimes even the stems become bent and curved, this phenomenon is known as distortion on the stem. This distortion can be seen along the stems of cassava plants and can indicate how many times there was an attack on a cassava tree during its growth period.

The lowest attack rate occurred around November to February, at this time it was seen that the intensity of rainfall that occurred was quite high at 429-636 mm / month [16]. The presence of rain that falls almost every day in these months causes lice in plants to be carried mechanically by rainwater and die, so the population is decreasing. With the rain, the shoots of the cassava plants that have fallen leaves sprout again.

The mechanism of the death of plant lice in the presence of rain water can be used as an idea to control lice using water such as falling rain using sprinklers. The principle used by this system is to put pressure on the water in the pipe and emit it into the air so that it resembles the rain that then falls on the surface of the soil or plants.

For cassava irrigation has not been done much, but with the increasingly high economic value of cassava, the provision of irrigation either by drip irrigation or sprinkler may be developed, and irrigation by using sprinkler is an alternative that can be used. Because besides being able to provide irrigation to plants but it can also function mechanically to reduce the isolation of white lice. In this case research on discharge, the water pressure needed to be able to reduce the flea population with sprinkler irrigation remains to be done. Watering aims to provide additional water to rainwater in sufficient time and when plants are needed. In general, irrigation is useful for facilitating soil management, regulating soil temperature and microclimate, cleaning or washing the soil of dissolved salts or high acids, cleaning dirt or garbage in waterways, and inundating the soil to eradicate pests and pests disease [17].

The three varieties observed were local varieties, but only the Manggu variety had clear origins. The other two varieties are unclear, but their characteristics can be approached with varieties that have been released by the Ministry of Agriculture. Roti varieties tend to be the same as Adira-4, and Jimbul is closer to Malang-2 varieties. Manggu variety is produced by the cassava plantation in Lido, Cijeruk, Bogor, which is capable of producing 80-120 tons / ha, with an age of 11 months and 32% starch.

Three varieties were observed, the difference in the level of attack occurred mainly due to the initial difference in the occurrence of the attack, and the speed at which the attack developed, until all plants were attacked 100% (Figure 1). The average initial occurrence of mealybug attacks in Roti and Jimbul varieties is at the age of 8 wap, while Manggu varieties at 12 wap, the attack rate increases with increasing age of the plant. From Figures 1 and 2 it is also seen that the peak of *P. manihoti's* attack on the Roti variety occurred around September, the Jimbul variety in June to July, and the Manggu variety in October. It can be seen that Jimbul varieties are relatively vulnerable to dry conditions due to low rainfall which causes the plants to be heavily attacked. In the Roti and Manggu varieties at the peak of the dry season, they are better able to survive so that new plants are attacked one hundred percent when the rainy season.

The rate of increase in attacks differs from the three varieties. From Figure 1, it can be seen that for Roti and Manggu varieties the rate of attack development is relatively constant, but in Jimbul varieties there is a surge in the development of attacks when the plant ages 14-16 wap. In Roti and Manggu varieties, the entire plant population was attacked when the plant was older than 34 wap, whereas in Jimbul varieties at 18-20 mst the entire plant population was attacked by *P. manihoti*. From the physical symptoms in the field it was also seen that the

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Jimbul variety showed more severe symptoms of attack compared to the other two varieties. Severe attacks (bunchy top) on the Jimbul variety occur at the age of 18 wap, on the Roti and Manggu varieties slower ie at 28 and 38 wap respectively. In Figure 1 it also appears that the Manggu variety experienced a slower initial attack compared to the other two varieties, which occurred at 16 wap, while at the same time the other two varieties increased the development of the attack.

From the physical structure, the three plant varieties are not much different, as are the cyanide acid (HCN) content in plants. Laboratory test results of the Post-Harvest Agricultural Research and Development Center, Agricultural Research and Development Agency are known HCN levels of each variety below 50 ppm, Manggu varieties (31.20 mg / kg), Jimbul (32.06 mg / kg), and Roti (44.85 mg / kg). Cyanide acid compound is a secondary compound found in phloem from cassava plants, as it is known that phloem is the tissue where P. manihoti's white mites suck up liquid for consumption as nutrients. Cyanide acid compounds influence the development and reproduction of *P. manihoti*, so that with different rates of development and reproduction of these ticks in each cassava variety will affect the level of resistance of a cassava variety to *P. manihoti*. In this pest the secondary compound cyanide acid acts as a stimulant for its growth and development [18, 19]. Cassava varieties with higher HCN levels are preferred by P. manihoti for their growth and development. High levels of cassava HCN cause *P.manihoti* to develop better, causing the plant to suffer more damage due to a higher flea population. Roti on differences in the content of cyanide acid compounds from the three observed varieties, Manggu varieties with lower HCN content appear to be more able to survive, and Jimbul and Roti varieties are more susceptible to *P. manihoti* attacks.

Many factors affect the growth and development of an insect both physical and chemical factors. Physical factors include the structure of the host plant, temperature and humidity, chemical factors including nutrient content and secondary compounds present in plants [20]. Physical factors, such as leaf surface structure, do not play an important role in the selection or preference of P. manihoti on cassava plants. The presence or absence of hair on the leaf surface is not related to its preference for the cassava host plant [21]. The colonization of cassava plants by *P. manihoti* was more influenced by the content of cyanide acids found in leaves, stems and roots [22, 23, 24].

P. manihoti causes damage to the leaves, so that it will affect the resulting tubers due to leaf surface reduction and even leaf depletion causing reduced photosynthetic reactions needed for tuber formation [25], as a result can cause yield losses of up to 90% (cassava) [10].

Overall, it can be seen that the Manggu variety shows better endurance compared to the other two varieties, resulting in higher tubers. Jimbul varieties that experience the most severe attack symptoms produce fewer tubers. The severity of the damage that occurs in Jimbul varieties, in addition to higher HCN levels, is also due to the relatively slow planting of the other two varieties. Jimbul varieties are planted near the dry season (low rainfall) so that when a severe attack occurs (June), the plants are still younger.

There are differences in the level and pattern of attacks, resulting in differences in yield per tree and total yield per hectare. The results of the weighing showed that of the three cassava plantations that had been attacked by P. manihoti, the highest tuber production of Manggu variety was on average 3.16 kg / tree (31.6 tons / ha) and significantly different compared to Jimbul variety with an average of 0.94 kg / trees (9.4 tons / ha).

Effect of Attack Time on Plant Height and Weight of Bulbs

The average height of plants at harvest and the weight of tubers produced per tree are related to the time of the attack (Figure 3). Severe attacks result in plants experiencing disruption in its growth. In the three plant varieties the same tendency was seen, cassava plants which were attacked earlier showed more inhibited growth compared to plants that were attacked at a later age. Stunted growth causes production per tree is also different. Plants that are attacked early by *P. manihoti* at the age of 2–12 wap, the resulting tubers are much reduced by an average of 1.5 kg (+ 70%) from the average normal production without pests (reaching 5 kg / tree). This reduction continues, until the initial attack by pests occurs at the age of 24 wap, which produces an average of 2.9 kg / tree (+ 42%). The reduction in tuber production is relatively lower if the attack occurs after the plants are 24 wap with an average of 3.27 kg / tree (+ 30%).

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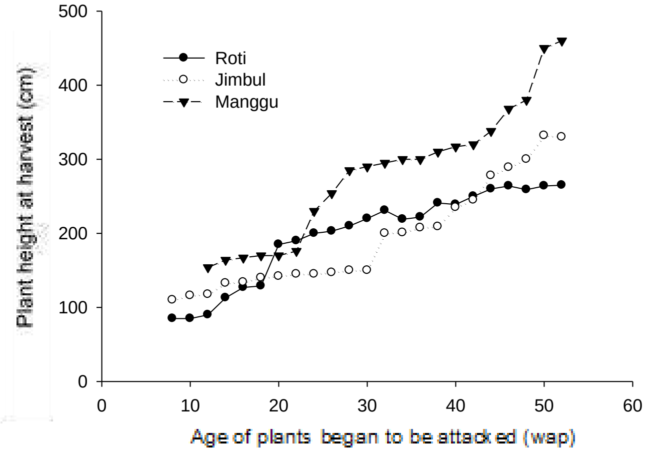
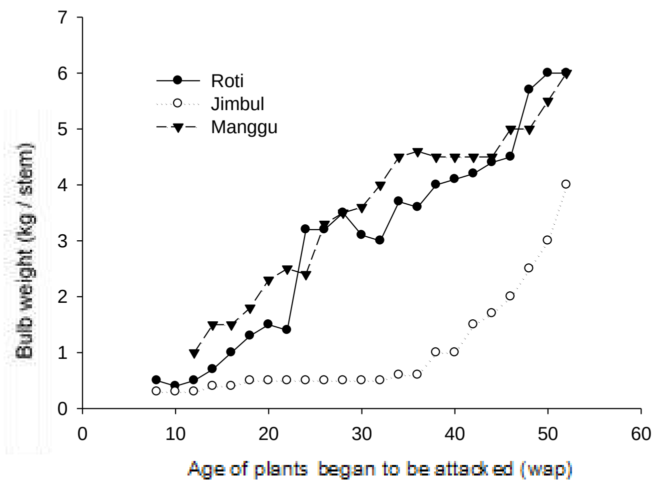


A)



B)

Fig.3: Relationship between time of attack with plant growth (A) and tuber weight (B)

The tuber weights per stem between plants that the initial attack occurred at a young age were significantly different from the tuber weights if the plants were attacked at a later age. The sharp decrease in production of plants attacked at the beginning of growth occurs because the initial attack occurs at a critical phase of the cassava plant (4-12 mst) [26]. In this phase, the plant undergoes an early phase of leaf growth and root system formation, and some photosynthates that are not used for growth are stored in thetuber. Thus, disturbance to the leaves will cause a significant decrease in photosynthate yield. The attack at the beginning of growth causes disruption to plant growth so that plants are shorter than normal plants. Age 12-24 wap is the phase of stem and leaf growth, at this time the maximum growth rate of leaves and stems, tubers continues to grow, and the most active vegetative growth occurs during this period [26]. *P. manihoti* attack in this phase will greatly affect plant growth and tuber production. There is a relationship between plant height at harvest from affected plants and yields (Figure 4). Shorter plants due to *P. manihoti* when they are young produce much lighter tuber weights. While tall plants, because the attacks occur later, produce normal tuber weights.

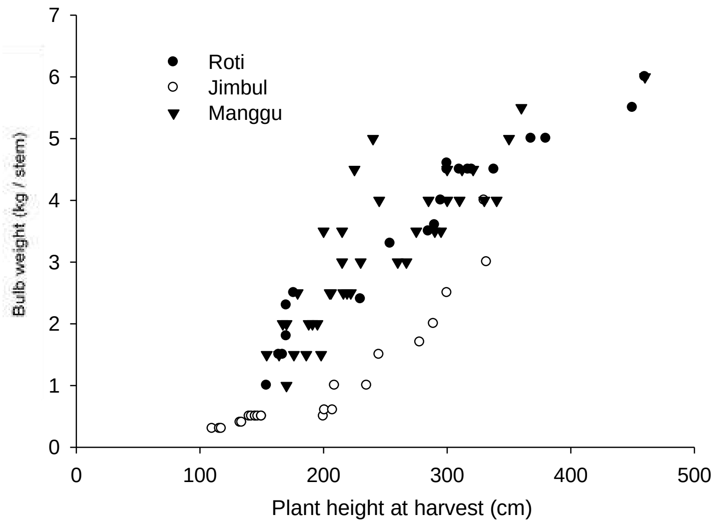


Fig.4: Relationship between plant height at harvest and tuber weights

The high yield reduction in cassava plants that are attacked at a young age, then to reduce yield losses due to *P. manihoti* attacks, planting time should be adjusted at the beginning of the rainy season so that cassava plants avoid pests at the beginning of its growth. Likewise with the existence of *P. manihoti's* primary feeding properties on cassava, then to break the food chain of pests, simultaneous planting on a stretch can suppress population development.

IV. CONCLUSION

Severe attacks of *P. manihoti* can cause symptoms of bunchy top, short and crooked books, deciduous leaves, and stunted plant growth. Attacks can occur since the plant is still young, and increase rapidly during the dry season with a peak occurring in September-October. The attack rate of *P. manihoti* in Jimbul varieties is heavier than in Roti and Manggu varieties. As a result, tuber weights produced by Jimbul varieties are lower. There is a pattern of the relationship between the initial attack occurs with the resulting tuber weights. Cassava plants that are attacked since young produce lower tuber weights, compared to when the attacks occur after the plants are older.

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Productivity of Small-Scale Yellowfin Tuna Fishing in West Region of Ceram District, Moluccas Province, Indonesia

Ruslan H.S Tawari[1*], Domu Simbolon[2], Haruna[1]

[1*]Fisheries and Marine Science Faculty of Pattimura University, Ambon,Indonesia
*Corresponding author : ruslan.tawari@fpik.unpatti.ac.id
[2]Department of Fisheries Resources Utilization, Faculty of Fisheries and Marine Sciences,
Bogor Agricultural University, Darmaga – Bogor, Indonesia,domusimbolon@gmail.com
[1]Fisheries and Marine Science Faculty of Pattimura University, Ambon,Indonesia,har_flash@yahoo.co.id

***Abstract*—*Analysis on factors which influence productivity of fishing businessis an essential matter to increase fisherman income. This research aims to: 1) Analyze factors which influence productivity of small-scale yellowfin tuna fishing business, 2) Establish structure model of small-scale Yellowfin tuna fishingbusiness productivity. The analysis on factors which influence development of yellowfin tuna fishing in West Region of Seram District uses Structural Equation Model (SEM). The result of the analysis shows that Fishing Operations Material (BOP) is the primary factor which contributes 88% influence. Furthermore, Fishing Operations Unit (UOP) factor has 26% influences. Yet, Fishermen Resources (SDN) does not have any influences on small-scale yellowfin tuna fishing business. Parameter of FishingGround (DRP) has 91% influences and Fishing Season (MSP) has 79% influences on yellowfin tuna fishing business productivity. Productivity structure of small-scale yellowfin tuna fishing has trust level of 99%. Hence, this model of small-scale yellowfin tuna fishing productivity has well accuracy and may become reference model for tuna fisheries management especially sustainable small-scale yellowfin tuna.*

***Keywords*— productivity, fishing business, Yellowfin tuna, small-scale fisheries, Ceram Sea.**

I. INTRODUCTION

Moluccas province is a potential area of Tuna-Cakalang which gives the second largest contribution in Indonesia (KKP, 2014) and located within area of "*Coral Triangle Tuna*" (Cabral et al, 2012; Bailey et al, 2012). Yellowfin Tuna (*Thunnus albacares*) is one of important fish commodities with high market demand. However, the fishing business immensely depends on the availability of fish resources and the aquatic environment. In other sides, national fisheries are still characterized by small-scale fisheries (Hermawan 2006; Tawari et al, 2014; Haruna et al, 2018).

Yellowfin tuna fishing is intensely determined by various factors either from internal factor of fish or environment factor. Production rate in fishing is determined by how big the effortof fishing which is done to utilize fish resources. Low production and productivity of yellowfin tuna fishing are generally caused by environment condition dynamics relating to fish distribution pattern, fish size, fishing season characteristic, and area of fishing (Tawari et al, 2014; Nelwanet al, 2016; Haruna et al, 2018; Haruna et al, 2019). In fact, technically production factor becomes a complicated issue in tuna fishing. The success of fishing operation is influenced by fishing tools, boat, supporting tools, and its human resources. Consequently, it needs knowledge between factor and output of production (Soekartawi, 2002; Tawariet al, 2013, Tawariet al, 2014; Sangadji, 2014).

Development of fisheries business including yellowfin tuna fishing gives social or economic impact on fishermen prosperity level if it is followed by the increasing of fishing result volume (Alhuda, 2016; Rahim and Hastuti, 2016; Maulana F, 2018). In consequences, analysis on factors which influence productivity of fishing business is an essential matter in increasing fishermen income. This research aims to: 1) Analyze factors which influence the productivity of small-scale yellowfin tuna fishing business, 2) Establish a structure model of small-scale yellowfin tuna fishingbusiness productivity.

II. METHODOLOGY

Time and Location of the Research

This research was performed on April 2017 – March 2018. It is located in Seram Ocean in which an operational area

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of fishermen who catch Yellowfin tuna. The sample of fishermen is spread in 9 urban villages in WestRegion of Seram District (**Fig 1**).

Data Collection

The collected data in this research consist of primary and secondary data which are acquired through literature review and field survey. Technique of sample collection (*expert survey*) is carried out with purposive sampling. The data collection is performed through questionnaire and structured interview to the fishermen (respondents) in 9 fishermen's urban villages. Questionnaire and interview assessment are carried out by using scale of Likert 1 until 5 with low until high category.

Extremely Low ***Extremely High***

Sample in this research is in the number of 150 respondents of yellowfin tuna fishermen in 9 urban villages. The obtained data numbers refer to *Maximum Likelihood Estimation* (MLE) technique.

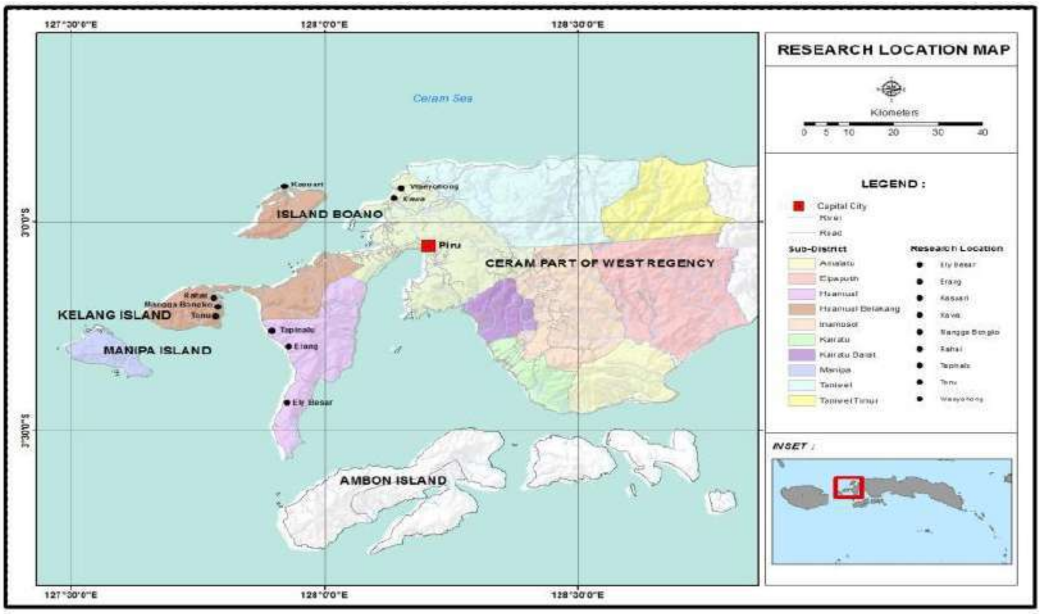

Fig 1. Maps of research location

Data Analysis

Analysis of factors which influence the development of yellowfin tuna fishing in West Ceram uses Structural Equation Model (SEM) approach. Teoretical model development is performed which involve initial design of path diagram, structural equation (structural model), and model interpretation. Afterwards, conformity test or compatibility model uses *GOF* (*Goodness of Fitt*) is conducted. Design of path diagram is presented in **Fig 2.**

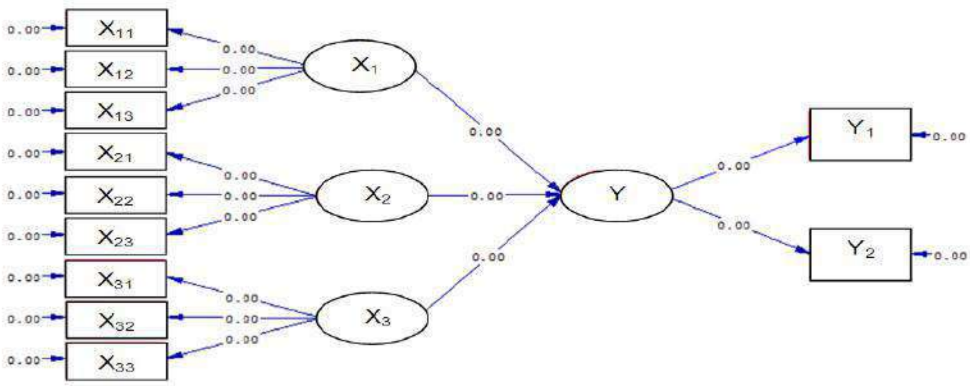

Fig 2. Design of path diagram on variable connectivity of yellowfin tuna (Thunnus albacares) fishing business

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Path diagram in this research is developed to see the interaction between the observed variables and know which interactions which have the biggest influence on productivity of small-scale yellowfin tuna fishing business in WestRegion of Seram District. After it is portrayed in a diagram, conversion into structural equaition series is performed. This equation represents causality relationship between various constructions with structur equation as follows:

$$\eta = \gamma_1\xi_1 + \gamma_2\xi_2 + \gamma_3\xi_3 + \zeta$$

Information:

η = latent endogenous variable (dependent)

ξ_n = latent exogenous variable, n = 1,..,3

ζ = latent errors in equations

γ_m = coefficient matrix for latent exogenous, m = 1,…, 3

Model compatibility test uses standard proposed by Wijanto (2008) with 15 sizes of GOF in Lisrel to assess compatibility of a SEM calculation model. Limitation and criteria to assess a model use goodness *of fit*.

Model Interpretation

After all of the previous steps have been conducted and the model is quite good, SEM performs interpretation. The usage of SEM is not to generate a theory, but to test model which has appropriate and well fundamental theory. Based on this idea, interpretation of the model can be accepted or prediction power from the model is not needed compared to the produced residual. The use of *standardized residual covariance* matrix will generate standard residual value. If interpretation towards produced residual through variable observation has bigger standard residual value from particular size, it means the model can be accepted and does not need model modification.

III. RESULT AND DISCUSSION

Business Productivity

Analysis result uses *Structural Equation Model (SEM).* There are nine (9) parameters which categorized as exogenous parameters with various connectivity levels towards three (3) latent independent variables. Furthermore, there are two (two) parameters which become endogenous variables and directly affect Yellowfin tuna fishing business productivity as the latent dependent variable in the research location. Analysis result of interaction and parameters connectivity level with latent variables is presented in form of analysis path diagram (**Fig 3**).

Three main factors are used as latent variable which influence yellowfin tuna fishing productivity. They are, fishing operations material (BOP), fishermen resources (SDN), and fishing operations unit (UOP). Each of those latent variables has parameter with various connectivity levels which shows the amount of influence towards independent latent variable as the determinant factor on yellowfin tuna fishing especially in the research location. In addition, there are two parameters/endogeneous variables which directly influence dependent laten variabel of yellowfin tuna productivity namely area of fishing parameter (DRP) and fishing season (MSP).

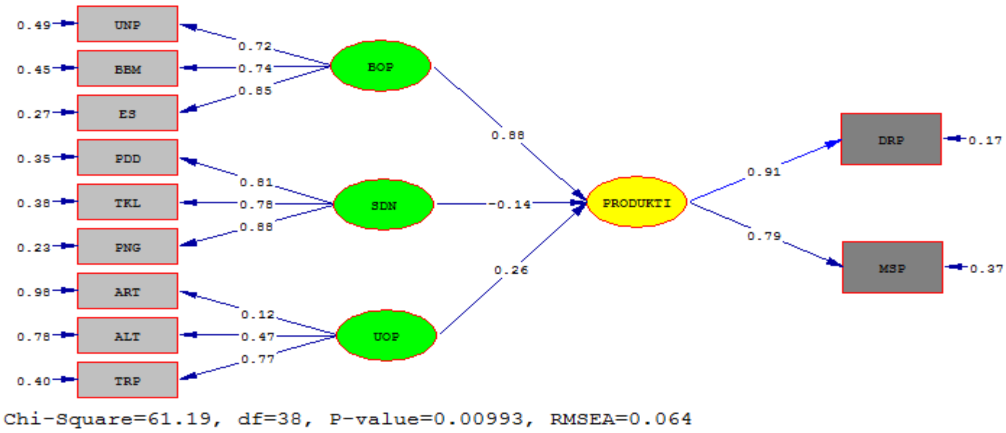

Fig 3. Connectivity and value of each element which influenceproductivity of yellowfin tuna(Thunnus albacares) fishing business

The amount of connectivity and the level of influence happenned between each factor either between exogeneous paramters with independent latent variable, independent latent variable towards dependent latent variable (productivity), or endogenous parameter towards productivivity (dependent latent variable) is a depiction of yellowfin tuna fisheries in the research location. More details are presented in Figure 3.

Fishing operations materal (BOP) is influenced by feed parameter (UMP) fuel oil (BBM), and ice cube (ES). Analysis result shows that feed is a parameter which has the highest error variance value in amount of 0.49. After that, fuel oil and ice cube follow with error variance value respectively in amount of 0.45 and 0.27. Error variance value posessed by each parameter is the value which depicts parameter that cannot influence independent latent variable particularly fishing operations material. The lower error variance value the better its parameter influence (observed variable) towards fishing operations material factor.

Those three assessed value have adequately big influence towards fishing operations material. Yet, ice parameter has bigger influence (0.85) towards fishing operations material and then followed by fuel oil (0.74) and feed (0.72). The amount of ice parameter influence shows that dependency of fishing material is to the value of 85% towards ice availability, compared to fuel oil dan feed parameters.

Fishermen resources (SDN) are influenced by parameter of education (PDD), technology (TKL), and experience (PNG). These three determinant parameters have various error variance values. Technology mastery is parameter which has the highest error variance value in amount of 0.38 and then followed by education parameter and experience parameter with respective error variance value of 0.35 and 0.23.

Those three parameters are deemed to have adequately big influence towards fishing operations material, but parameter of experience has the bigger influence of 88% towards fishermen resources and followed by parameter of education with 81% influence and parameter of technology with 78% influence. The amount of experience parameter influence shows dependency of fisherman resources factor towards such parameter to the value of 88%.

Fishing operation unit (UOP) is influenced by fishing fleets (ART), fishing tools (ALT), and fishing trip (TRP). Parameter of fishing fleet has error variance value bigger than fishing tool and fishing trip. Fishing fleet parameter has error variance value of 0.98, followed by fishing tool with error variance value of 0.78 and parameter of fishing trip with error variance of 0.40.The error variance value possessed by each parameter shows the number of parameters which are not observed beyond those three observed parameters. The high error variance value of fishing fleet shows that such factor has extremely low influence towards fishing operations unit.

Based on result of path diagram as in Figure 3, it shows that the three assessed parameters have various influences on fishing operations material. Yet, correlation connectivity of fishing trip parameter has bigger influence in which 77% towards fishing operation unit, followed by fishing tool with 47% influence and fishing fleet to the value of 12%. The influence amount of fishing trip parameter shows that dependency of fishing operation unit towards fishing trip parameter itself is more dominant than other parameters.

Endogeneous parameter is a parameter which has direct influence on yellowfin tuna fishing productivity (dependent variable) and can be measured as well as controlled. There are 2 endogenous parameters in which fishing ground (DRP) and fishing season (MSP). Based on analysis, fishing ground has error variance value of 0.17 and fishing season has error variance value of 0.37. Thus, it shows that fishing ground has dominant determinant factor towards yellowfin tuna fishing productivity compared to fishing season parameter. Fishing ground is a parameter which has dominant influence with factor loading value of 91% on yellowfin tuna fishing productivity (dependent latent variable). Meanwhile, fishing season parameter only has factor loading value of 79%.

The high influence of fishing ground and fishing season on yellowfin tuna fishing productivity in the research location is highly related to habitat and behavior of yellowfin tuna. Dynamics of yellowfin tuna fishing ground is temporary but it is highly influenced by environment condition and oceanograpic oceanic parameter such as sea surface temperature, chlorophyll-a, salinity, stream, depth, front, and upwelling (Zainuddin, 2013; Safruddin, 2018; Hidayat, 2019). Fishing ground influence is followed by fuel oil and ice usage on production because of tuna characteristics which always migrates so that fishing ground becomes uncertain and far away.

Business Productivity Structure Model

From basic model path diagram of the research, it shows clearly that yellowfin tuna fishing productivity is the latent variable which is influenced by two independent latent variable factors namely fishing operations material (BOP)and fishing operations unit (UOP). Among those two independent latent variables, it is found that fishing operations material has extremely big influence on Yellowfin tuna fishing productivity compared to factor of fishing operation unit. Based on result of the analysis, 88% yellowfin tuna fishing productivity is influenced by fishing operation material, followed by fishing operation unit with 26%, while fishermen resources factor does not give influence on the increasing of yellowfin tuna fishing productivity and even it has loading factor with value of -0.14%.

Furthermore, based on analysis result, correlation stucture of independent latent variable (BOP,UOP, SDN) on productivity (dependent latent variable) of yellowfin tuna fishing is in accordance with the used structure model, so that it is found structure of small-scale yellowfin tuna fishing with the following model equation structure :

PRODUCTIVITY = 0.88*BOP + 0.26*UOP - 0.14*SDN, Errorvar.= 0.015 , R² = 0.99
(0.47) (0.60) (0.78) (0.066)
1.87.1 0.43 -0.18 0.22

The equation above is productivity model of yellowfin tuna fishing with 9 exogeneous parameters, 2 endogeneous parameters, 3 independent latent variables, and 1 dependent latent variable. Structure model of yellowfin tuna fishing productivity in this research, as what has been presented, has determinant coefficient value (R^2) to the value of 99%.

It shows that parameters and factors used as benchmark in this research can describe the condition of yellowfin tuna fishing business productivity to the value of 99% and only 1% of description by other factors which are not observed in this research. Compatibility test of structure model of small-scale yellowfin tuna fishing business productivity is presented in **Table 1.**

Result of model compatibility test as in Table 1 shows that there are 4 GOF sizes that depict poor compatibility and 11 GOF sizes that depict good compatibility. It indicates that compatibility of all models is good. This condition concludes that the model of this research has good accuracy level and can be used as reference model for sustainable small-scale tuna fishery management.

Table 1. Result of compatibility test for all models with GOF size

GOF Size	Standard	Estimation Result	Accuracy
Chi-Square	Small value	Chi-Square = 61.19	*Poor*
P	p> 0.05	(P = 0.01)	
NCP Interval	Small value	23.19	*Poor*
	Narrow Interval	(5.68- 48.61)	
RMSEA	0.05<RMSEA ≤ 0.08	RMSEA = 0.064 p = 0.21	*good fit*
ECVI	Small value and close to ECVI saturated	M*=0.79 S* = 0.89 I* = 5.65	*good fit*
AIC	Small value and close to AIC saturated	M = 117.19 S = 132.00 I = 842.22	*good fit*
CAIC	Small value and close to CAIC saturated	M = 229.49 S = 396.70 I = 886.34	*good fit*
NFI	NFI ≥ 0.90	0.93	*good fit*
NNFI	NNFI ≥ 0.90	0.96	*good fit*
CFI	CFI ≥ 0.90	0.97	*good fit*
IFI	IFI ≥ 0.90	0.97	*good fit*
RFI	RFI ≥ 0.90	0.89	*Poor*
CN	CN ≥ 200	214.13	*good fit*
RMR	RMR ≤ 0.05	0.13	*Poor*
GFI	GFI ≥ 0.90	0.98	*good fit*
AGFI	AGFI ≥ 0.90	0.96	*good fit*

Information : M = model; S = saturated; I = Independence

IV. CONCLUSION

1. Two main factors in this model are Fishing Operation Material (BOP) and Fishing Operation Unit (UOP) with six parameters in which feed (UMP), fuel oil (BBM), ice cube (ES), fishing fleets (ART), fishing tools (ALT), and fishing trip (TRP). In addition, including two parameters of environment in which fishing ground (DRP) and fishing season (MSP) which

influence small-scale yellowfin tuna fishing business productivity in WestRegion of Seram District.

2. Correlation structure of independent latent variable Fishing Operation Material (BOP), Fishing Operation Unit (UOP), and fishermen resources (SDN) on productivity (dependent latent variable) satisfies requirements of structure model on small-scale tuna fishing business productivity.

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Correction of Vaginal Prolapse in a Pregnant EWE: A Case Report

Wahab Y. A.[1*], Olayinka S.O.[2], Olofintuyi O.K.[3], Raji L.O.[4], Tijani K.A.[4]

[1]Department of Veterinary medicine, University of Ibadan, Oyo State, Nigeria.
[2]Department of Biological Sciences, Eastern Illinois University, Charleston, IL, USA
[3]Department of Veterinary Microbiology, University of Ibadan, Oyo State, Nigeria.
[4]Department of Theriogenology and Production, University of Ilorin, Ilorin, Nigeria.
*****Corresponding author: wahabyunus.abiola@gmail.com

Abstract— *This case report describes a successful correction of vaginalprolapse in a pregnant ewe in her last trimester. The prolapse was noticed some days following abrupt change in feed. On physical examination, the sheep was depressed and recumbent with intermittent straining. Epidural anaesthesia was achieved via administration of lidocaine into the first intercoccygeal space. The swollen and edematous prolapse was cleaned and decontaminated using mild potassium permanganate, after which the prolapse was repositioned and purse string suture was applied on the vulva for retention purpose. The animal was placed on antibiotics for 5 consecutive days to prevent secondary bacterial infection and the suture material was removed on the 8th day with no signs of recurrence. The sheep delivered precisely a week after the suture was removed with no complications. In conclusion, this report has shown detail of a successful correction of vaginal prolapse in a pregnant ewe.*

Keywords— ***sheep, vaginal prolapse, pregnant, recurrence, epidural.***

I. INTRODUCTION

Genitalia prolapse is one of the major conditions of the reproductive tract with tendency to cause heavy economic loss to livestock farmers due to its negative influence on the productive and reproductive performances of female animals (El-wishy, 2007).Vaginal prolapse is one of the frequently encountered problems in late trimester of pregnancy in sheep and cattle. However, it has also been reported in non-gravid heifers (Yotov *et al.,* 2013). Numerous predisposing factors have been linked to the occurrence of vaginal prolapse. Some of the documented aetio-pathogenesis of vaginal prolapsed is the upsurge of hormones such as estrogen and relaxin during the last trimester of pregnancy. These hormones cause the relaxation of pelvic ligaments and other surrounding structures. This hormonal effect in combination with the increased abdominal pressure elicited by the gravid uterus is one of the predisposing factors of vaginal prolapsed (Wolve, 2009). Multiple fetuses, large fetus, intra-abdominal fat accumulation and ruminal distension are other causes of increased abdominal pressure which may contribute to the development of vaginal prolapse (Drost, 2007). Nutritional factors such as hypocalcemia, grazing on pastures with an abundance of clover and feed containing phytoestrogenic substances have also been documented as predisposing factors of this condition (Miesner and Anderson, 2008).Vagina prolapse often comes with devastating sequelae especially if not treated promptly. The swollen, edematous and congested vagina may result in vaginal rupture with herniation of the intestines, bladder or uterus. Additionally, the prolapsed tissue can trap the urinary bladder leading to urethral obstruction and subsequently urinary bladder rupture. Vaginal prolapse which contains the bladder within the prolapsed tissue will potentially alter the replacement (Veeraiah and Srinivas, 2010). Vascular disturbances, bacterial contamination and septicaemia leading to unfavourable prognosis are other potential complications (Hasan *et al.,* 2017).Abnormal clinical parameters such as an increased pulse, anorexia, an increased respiratory rate, signs of toxemia and congested mucous membranes may indicate fetal death and impending abortion, both known complications of vaginal prolapse in ewes (Scott and Gessert, 1998). Management of vaginal prolapse is often targeted towards eliminating systemic disturbances, vaginal replacement and prevention of recurrence (Bhattacharyya *et al.,* 2012).The condition has a significant economic implication, causing serious financial loss to the farmer as it assumes epizootic proportions, with its highest incidence during the later stages of pregnancy (Mclean, 1956). Therefore, for sheep production to be more economically sustainable, the incidence of vaginal prolapse must be greatly controlled and the negative influence of the condition be prevented through prompt medical attention to correct the condition.

In this article, we report a successful repositioning of vaginal prolapse in a ewe in late pregnancy.

II. CASE REPORT

History and clinical observation

A 2½-year-old Balami sheep in the third trimester of her second pregnancy was presented with prolapsed vagina which was earlier observed as a tiny eversion about 3cm two days prior to our visitation. The farm attendant observed that the everted tissue kept increasing in size and was almost 15cm when presented for treatment. History further revealed that, prior to the appearance of the prolapse, there was an abrupt change in feeding regimen as molasses was introduced to the herd in place of cassava peel. On physical examination, the ewe was restless and recumbent with intermittent straining (Plate 1&2).The prolapsed mass was swollen, edematous and soiled with dirt. The basic clinical parameters recorded were unremarkable (Table 1).

Plate 1: Recumbent ewe with vagina prolapse showing discomfort

Plate 2: The ewe straining when assisted to stand

Table1: Clinical parameters recorded for the ewe

Parameter	Patient's value
Rectal Temperature	38.7^0C
Heart rate	80bpm
Pulse rate	76bpm
Respiratory rate	44bpm
Capillary refill time	<2secs
Mucous membrane	Pink

Management and Outcome

In order to prevent further straining and to desensitize the perineal region for easy manipulation of the tail and the perineum, the animal was placed on epidural anaesthesia. This was achieved by administration of 2mls of 2% lidocaine into the first intercoccygeal space (Plate 3).The anaesthetic effect was confirmed after 7 minutes by needle prickling of perineal region, where failure to responds to pain and tail flaccidity indicates a loss of sensation. With gloved hands, the prolapsed tissue was carefully cleaned with moderately warm clean water to remove the debris and irrigated with potassium permanganate solution to further decontaminate and to reduce the edematous prolapse. The prolapse tissue was then gently repositioned. Thereafter, for retention purpose, a purse string suture was applied on the vulva mucosa using a nylon size 2 suture material, leaving 1-2 fingers space for urine to drain (Plate 4b). The animal was placed on Penicillin-Streptomycin 20/20 (Nanjing Vetop Pharma Co., Ltd) at 1mL/25kg intramuscularly for 5 consecutive days. Animal was closely observed for recurrence during the period and clinical parameters were also monitored.The suture material was removed on the 8^{th} day with no evidence of recurrence and the clinical parameters were found normal. The sheep lambed precisely two weeks after the correction of the vaginal prolapse.

Plate 3: Injection of epidural anaesthetic agent

a

b

Plate 4 a & b: Before and after correction of the prolapsed tissue, purse string suture on the vulva leaving enough space for urine to drain (arrow).

III. DISCUSSION

Vaginal prolapse is one of the frequently encountered gynaecological conditions in farm animals. It is seen mostly in cattle, buffaloes and sheep during late pregnancy (Hasan *et al.*, 2017; Patra *et al.*, 2015) as observed in this report. The incidence of vaginal prolapse especially during the last trimester of the gestation period has been linked to numerous predisposing factors. The most probable explanation at this stage could be due to the spike in hormone level in preparation for parturition. Increase in levels of hormones such as estrogen and relaxin at the latter stage of pregnancy have been found incriminating (Wolve, 2009).These hormones are responsible for the relaxation of the pelvic wall and the surrounding structures which enhances fetal expulsion. However, this hormonal effect in combination with other factors such as increased abdominal pressure from multiple fetuses or large fetus predisposes the animal to vaginal prolapse. Nutrition and genetic factors have also been linked to the occurrence (Veeraiah and Srinivas, 2010). Therefore, history of change in feed as reported in this article cannot be ruled out to the incidence, as the prolapse was first noticed some days following abrupt change in feed. This habit may have partly led to increase abdominal pressure which among other factors contributed to this condition. This is similar to a report of vaginal prolapsed in cowfollowing change in feeding habit earlier documented (Hasan *et al.*, 2017).The clinical parameters recordedin this casewere unremarkable, ruling out the likelihood of toxemia or fetal death. Vaginal prolapse can hinder fetal expulsion especially if it coincides with the animal's due date causing dystocia and possibly fetal death. Toxemia and fetal death or abortion is a known complication of vaginal prolapse in ewes (Scott and Gessert, 1998).The prognosis is also dependent on the type of case and degree of damage (Yotov *et al.*, 2013). More so, it may not be correctable due to excess edema or fibrosis especially if treatment is delayed (Hasan *et al.*, 2017). In this report, epidural anaesthesia prior to replacement of the prolapsed tissue was found rewarding as the straining ceased after induction of epidural anaesthesia. It also enhances easy manipulation of the perineal region (Patra *et al.*, 2015). Decontamination of prolapsed tissue using mild potassium permanganate reduces edema (Yotov *et al.*, 2013) and prevents vaginal or uterine infection, eliminating possibility of septicaemia (Hasan *et al.*, 2017). Therefore, the success recorded in this report could be ascribed to prompt intervention, proper management and the postoperative monitoring.

IV. CONCLUSION

Vagina prolapse is a common condition in sheep frequently seen in late pregnancy. It is an emergency condition and the outcome of its correction depends on early intervention. Therefore, livestock farmers should promptly call the attention of their veterinarians once this condition is noticed. This is necessary in order to prevent complications which may result in economic losses. More so, farmers should avoid practices that may increase the susceptibility of animals to this condition.

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Minapadi-Sri Pattern on Rice Cultivation with IR42 Varieties

Nalwida Rozen, Aswaldi Anwar, dan Nilla Kristina

Agrotechnology Study Program, Faculty of Agriculture Andalas University, Indonesia
Email: rozennalwida@agr.unand.ac.id
Agrotechnology Study Program, Faculty of Agriculture Andalas University, Indonesia
Email : alohaswaldi@gmail.com

Abstract— *Food needs of Indonesia's population is still dominated by rice. Meanwhile, the conversion of irrigated rice fields seems unstoppable. This fact should be addressed by policy and the application of technology for rice production so that it is still possible to meet the national food need while increasing the income of rice farmers. The experiment on Minapadi-SRI pattern of rice cultivation with variety IR42 conducted. SRI (the system of rice intensification) is the formation of phyllochron 12 times and many phytomers. The experiment was conducted from April to September 2017 at Sungai Bangek village, Balai Gadang, Koto Tangah, Padang, West Sumatera. The objective of the study was to get the best technology for food production which is profitable. A Randomized Block Design with split plots was used with two water levels (20 cm and 10 cm) and three trench widths (50 cm, 75 cm and 100 cm). Data were analyzed using the F test at the 5% level and the Honest Significant Difference test at the 5% level. Observation of the yield and yield components of rice crops; height of plants, number of tiller/clump, number of productive tiller/clump, panicle length, number of grains/panicle, number of pithy rice/panicles, weight of 1000 grains, and yield/plot were made. The result of this research is that 10 cm inundation with 50cm wide trenches are better for rice plant growth and yield/plot.*
***Keywords* — Appropriate technology, Minapadi, SRI, transformation of land, yield.**

I. INTRODUCTION

Rice is the staple diet of the Indonesina and most people in the world. National rice needs every year continue to increase as the population increases , on the other hand the area of rice fields cultivations continues to decrease for varous reasons. Various efforts to increase production have been implemented both through extensification and intensification. According to the Central Bureau of Statistics of rice production (kiloton) in Indonesia, was: 71,280 in 2013; 70,846 in 2014; 75,398 by 2015; 79,355 in 2016; and 81,382 by 2017.

Efforts to increase food production embodied in the Tujuh Gema Revitalitas Pertanian are: 1) Land Revitalization; 2) Revitalization of Seedlings; 3) Revitalization of Infrastructure and Facilities; 4) Revitalization of Human Resources; 5) Revitalization of Farmers Finance; 6) Revitalization of Farmer's Institutions; and 7) Revitalization of Downstream Technology and Industry (Nuryati *et al* ., 2015). Various efforts to increase production have been implemented both through intensification (which is the best cultivation of agricultural land by utilizing existing facilities, for example: the use of purple seeds, the use of fertilizers, the use of irrigation and so on) or extensification (opening up new farmland).

One of the efforts of the last decade to give hope is the application of SRI (*System of Rice Intensification*) in rice cultivation. Various research reports and farmers' cultivation results show an increase in yields of up three fold. SRI is a method in rice cultivation that has the potential to increase rice productivity by changing the planting system, plant spacing, water delivery, and fertilizer use. Barkelaar (2001) stated that the advantages of rice cultivation with the SRI method include: water-saving, being cost-effective, time-saving, saving seed, being environmentally friendly, and increased production. With the SRI method tillers production double, because with this method phyllocron is formed up to 12 times. Phyllochron is something circuit Phytomer formed for 35 days depends on temperature (Bakelaar, 2002). The phyllochron is affected by temperature, time of seedling transfer and nursery methods (Veeramani *et al.*, 2012). The most suitable rice fields for SRI development are irrigated rice fields whose water availability is more secure. The water does not need to flood the entire surface of the rice field, just enough to ensure the soil remains saturated (around field capacity).

The crops' microenvironment will be crucially influenced by the agronomic management practies, setting in motion important interactions. For example, the water management regime will affect soil aeration, the soil microbial communities, the organic matter mineraliation process, and as a result the dynamics and availability of soil nutrients. It will also affect the build-up of various insect pests, diseases and weeds (Stoop *et al.*, 2002). SRI will be defined technically by key practices (principles) mentioned above, but not a fixed package to be followed strictly. Even though only a part of key practices is adopted, it can be considered as SRI as far as SRI effects appear.

Kasli and Effendi's (2011) study provides hope for furture modofication of the SRI system. They reported that the best results with potted plants were obtained with water about 10 cm from the surface. Rice could planted in the surface layer part and enough water provided in a trench between the rice plots. This means it is sufficient for water to remains in the trench between plots rice from planting until close to harvest. If rice plants are about 100 days old of planting, then there would be a puddle for about 80 days. Sufficient time to growth fish in the waterh .

SRI among the methods has an edge over other water saving methods as water saving does not have a yield penalty in this system. Therefore, efforts are being made in many countries to popularize SRI to overcome the challenges of water shortages. System of rice intensification management purpose the use of single young seedling raised in raised bed under aerobic conditions, drastically reduced plant densities (16 hills/m^2), keeping fields unflooded and use of a mechanical weeder which aerates the soil and use of more organic manures, all the practices with the aim of providing optimal growth conditions for the plant, to get better performance in terms of yield and input productivity. SRI has been promoted for more than a decade as a set of organic management practices for rice cultivation that enhances the yield and reduce water requirements (Satyanarayana *et al.*, 2005 *cit* Kumar *et al.*, 2013).

Farmer's in some areas of Indonesia usually use water in their rice fields to keep fish, either directly among the flooded rice clumps or by making a special plot in the middle or on the edge of their rice fields, or by alternating fish farminge with rice planting. This cultivation method is known as minapadi.West Sumatra is one of the areas where farmers are used to doing this. Ramli (2010) reported that in Kabupaten Lima Puluh Kota about 150 ha of rice fields are used for minapadi.

Farmer's are in the habit of flooding the fields continuously from when the seed is planted until the plants close to harvest time, both in the rainy season and the dry season. To solve the problem, there needs to be technological improvements at the farm level to improve the efficiency of water use, such as an appropriate water management ystem. Generally, plant spacing and flooding time is known to have an effect on the growth and yield of rice.

Combining SRI with minapadi is possible. The merger is a form of product diversification. But some issues still need to answered so that application at the farm level is more practical, scientifically accountable and profitable not only for farmers' incomes, but also nationally beneficial because it can play a role in strengthening food security. Rozen *et al*., (2011) studied minapadi-SRI in paddy fields with tilapia fish and this can increase farmer's income, but they did have not study the best depth of water is profitable for fish life in the trench. Intensification of rice minapadi is one aspect of fish farming in rice fields. Rice farming is an agricultural activity that combines fish farming with rice field cultivation. According to Dio denha (2011), the minapadi farming system is not new since it has been used since the 1950-1960s but the profit earned is still low. This is because the cultivation technique is still simple (traditional) and diverse. Farming fish in the rice fields is one way to improve the efficiency of land use.

Some of these problems can be formulated as follows: if SRI rice cultivation is coupled with fish farming what depth of water in the trench is most advantageous for both, what is the most appropriate trench width, what type of fish is most profitable, what is good management, how should the products be processed and marketed. These questions need answers that can be justified. For this reason the research described below was conducted.

In general, this study aims to increase the value of irrigated rice fields, whose area is believed to be shrinking, while still producing rice as the main national food in addition to the producing products such as fish. Although some rice fields are used for fish maintenance, this will not interfere with the production of rice because the SRI system yields 2-3 times the national average, rather additional income from fish will benefit farmers. Even more so, if the fish produced are used to make processed foods like babyfish by adding a particular flavor, perhaps chili babyfish, babyfish, rendang flavoured and so on.

The most beneficial combination between water and trench width on SRI rice cultivation with minapadi, hereinafter abbreviated as MINAPADI-SRI was

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determined. Combinations of varieties and types of fish that are most profitablye cultivated/processed were examined. The results of this sort of study this sort of study are often not well explained to the target community so outcomes are often forgotten ends. Therefore, Banda Langik farmer's group was involved as a partner, to try and guarantee continue the application of MINAPADI-SRI and possibly its introduction to other areas.

II. MATERIAL AND METHOD

Field trials were conducted on land farmerd by members of the Banda Langik, Sungai Bangek, Koto Tangah, Padang from April- September 2017. The farmers were already using the SRI. A Split Plot Random Group divided plot design with two factors and three groups was used. Ditches were either 50 cm, 75 cm, or 100 cm wide (main factor) and contained either 10 cm (±2 cm), 20 cm (±2 cm) of water (subplot factor). Data were analyzed using the F test at the 5% level. Benchmarking value middle treatment use Median values were tested using Turkey's Honest Significant Difference test also at the 5% level..

Processing of land done by handtraktor 2 times then left for a week, made a plot of 18 plots with the size of each ran 1 m x 12 m = 12 m^2. After that made the channel as wide as 50 cm, 75 cm, or 100 cm then water is grabbed as high as 10 cm and 20 cm. IR42 rice seedlings planted 15 days after seedling 1 seeds per planting holes with spacing 25 cm x 25 cm. fertilization is done by giving urea 200 kg/ha, TSP 100 kg/ha, KCl 100 kg/ha. KCl and TSP are well matched at planting time along with a third-quarter urea. Giving urea second and third age 21 days and 42 days after planting.Weeding done in 10 days after planting and next done 21 days after panting with manually. Water regulation is done during vegetative not stagnant. Enter the generative phase of the land is flooded until 20 days before harvest.

For rice plants (variety IR42) the following data collected: high plant, number of tillers, number of productive tillers, length of the panicle, weight of 1000 grains, the number of grains per panicle, the number of unhulled grains per panicle, yield of grain per panicle and per plot from which the yield (dry weight was calculated). While for fish, the data collected was their initial and final weight.

III. RESULTS AND DISCUSSION

Plant height at 63 days after planting for different depths of water and channel widths are shown in Table 1. Only the depth of water showed a significant difference. The deeper water gave taller plants perhaps because of better soil moisture levels. The was no interaction between water depth and channel width, and no significant effect of channel width on plant height.

Table 1. Plant Height at 63 days after planting

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cm	66.56	66.06	64.78	65.80 b
2 0 cm	76.61	71.00	72.39	73.33 a
Average	71.59	68.53	68.59	

Numbers in the same column followed by the same lower case letters are not significantly different, Tukey's Honest Significant Difference test at the 5% level

In the SRI method, the humid state will make the soil aeration better so that sufficient oxygen will be available in the soil for root growth and development. The roots will be healthy so that the aerial parts of the plant will also be healthy. According to Kawano *et al.*, (2009) inundation will spur stem elongation as an escape strategy against flooding to help meet the need for oxygen and carbon dioxide to support aerobic respiration and photosynthesis

The total number of tillers is shown in Table 2. No significsnt differences were found.

Table 2. Number of tillers at 63 days after planting

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cm	45.39	47.61	55.00	49.33
2 0 cm	46.28	43.83	47.17	45.76
Average	45.84	45.72	51.09	

Numbers in the same row and column are not significantly different F test at the 5% level

The formation of tillers is influenced by the SRI method because in this method the seedlings are planted early to increase the number of tillers formed and phyllochron will be formed up to 12 times. In the third phyllochron will form a multiply tillers. Supported by Bakelaar (2002) report that with the SRI method the number of tillers will double, because the phyllocron formed up to 12 times.

Rozen *et al*., (2017) states that from rod main varieties of Batang Piaman SRI method formation puppies on phyllchron both of which amounted to 1 tillers. On puppies first formed again puppies start phyllochron to 4 to 9 with the number of 6 tillers. On puppies secondary formed puppies start phyllochron to 7 to 12 with amount tillers 14 cigarettes. On puppies third formed puppies start phyllochron to 8 to 12 with the number of 14 stems. While from puppies to four formed puppies star phyllochron to 10 to 12 amounted to 4 tillers. On puppies fifth appear puppies on phyllochron to 12 as many as 1 bar. Total tillers are erratic as many as 40 stems.

The number of productive tillers is related to the total number of tillers, the more tillers formed the more productive tillers. With respect to number of productive tillers no interaction and no significant differences were observed (Table 3).

Table 3. Number of productive tillers

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cm	28.67	34.11	31.56	31.45
20 cm	30.61	31.50	27.28	29.80
Average	29.64	32.80	29.42	

Numbers in the same row and column are not significantly different F test at the 5% level

As expected for the SRI system more productive tillers were formed compared with conventional methods (average 16-20 stems) because of the early transfer of seedlings. Increased number of tillers is, among others, due to increased nitrogen uptake during the vegetative phase (Ded atta, 1981). Flooding treatment in early rice growing increases the number of saplings. This is in the opinion of Vergara (1976), that the need for water for rice plants at the beginning of the vegetative phase is critical, where the vegetative phase is the phase of the formation of the active tiller and the maximum tiller.

No interaction and no significant differences in the length of the panicle were found (Table 4).

Table 4. Panicle length (cm)

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cm	28.17	27.38	27.69	27.75
20 cm	26.84	28.04	28.04	27.64
Average	27.51	27.71	27.87	

Numbers in the same row and column are not significantly different F test at the 5% level

No interactions and no significant differences in either the number of grains or the number of filled grains per panicle were found (Table 5 and 6).

Table 5. Number of grains per panicle

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cn	185.33	171.00	183.53	179.95
20 cm	187.80	190.73	170.40	182.98
Average	186.57	180.87	176.97	

Numbers in the same row and column are not significantly different F test at the 5% level

The absence of interactions and significant differences with respect to panicle length and the number of grains per panicle is because genetic factors are more influential than environmental factors. Uphoof *et al.*,(2002) states that SRI can improve results two fold or more, because of the planting distance (25 cm x 25 cm), the seedlings are replanted individually after only 7-15 days (Rozen *et al.*, 2009). Rozen *et al* ., (2011) states that the SRI method can be give dry weights harvest of 10 tons/ ha. While production rice in West Sumatera only reached 4,6 tons / ha.

Table 6. Number of filled grains per panicle

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cm	134.94	125.47	138.60	133.01
20 cm	134.20	144.07	128.53	135.6 0
Average	134.58	134.78	133.57	

Numbers in the same row and column are not significantly different F test at the 5% level

Penggenangan lahan sawah setelah proses pengolahan lahan akan memberikan konsekuensi perubahan fisikokimia tanah. Pada kondisi tanah tergenang maka kadar oksigen dalam tanah dapat menurun drastis sampai titik nol dalam waktu kurang dari sehari sehingga mikroorganisme anaerob menjadi aktif, bahan organik akan terdekomposisi lebih lambat dan kurang sempurna (Sanchez, 1993 *cit* Regazzoni *et al.*, 2013). Maka dari itu diperlukan pengeringan lahan agar oksigen dapat masuk kembali ke dalam pori tanah. Apabila tanah sawah mempunyai periode pengeringan maka mikroorganisme aerob akan aktif dalam mendekomposisi bahan organik sehingga laju dekomposisi bahan organik menjadi lebih tinggi dan mempunyai hasil yang lebih sempurna. Pengeringan selama 6 dan 9 hari pada 30 hari setelah tanam dapat meningkatkan hasil gabah sebesar dua sampai tiga kali lipat dibandingkan tanpa pengeringan (Hartatik *et al.*, 2014 *cit* Regazzoni *et al.*, 2013).

With respect to the weight of 1000 grains of rice no significant interaction or differences were observed Table 7.

Table 7. Weight of 1.000 grains (gram)

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
1 0 cm	22.18	21.42	22.01	21.87
2 0 cm	22.47	22.44	21.67	22.19
Average	22.32	21.93	21.84	

Numbers in the same row and column are not significantly different F test at the 5% level

Only the depth of water in the channel had a significant effect on the yield per plot (Table 8).

Table 8. Yield (kg/plot)

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cm	5.27	3.47	4.17	4.30 a
20 cm	3.40	3.43	3.43	3.42 b
Average	4.33	3.45	3.8 0	

Numbers in the same column followed by the same lower case letters are not significantly different, Tukey's Honest Significant Difference test at the 5% level

It turns out that the high puddle in the channel 10 cm heavier grain per plot compared to 20 cm high puddle. Plant height and yield may be inversely related. Lack of water during the flowering phase may result in the falling of flowers and empty grains, resulting in low yields (Rismaneswati, 2006).

The initial and final weights of fish are shown in Tables 9 and 10 respectively.

Table 9. Initial weight (kg) of Majalaya goldfish

Depth water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cm	0.10	0.08	0.09	0.09
20 cm	0.36	0.29	0.08	0.24
Average	0.23	0.19	0.09	

Numbers in the same column followed by the same lower case letters are not significantly different, Tukey's Honest Significant Difference test at the 5% level

Table 10. The final weight of Majalaya goldfish on the minapadi-SRI method

Depth of water in the channel	Water channel width			Average
	50 cm	75 cm	100 cm	
10 cm	0.37	0.30	0.21	0.29
20 cm	0.21	0.15	0.20	0.19
Average	0.29	0.23	0.21	

Numbers in the same column followed by the same lower case letters are not significantly different, Tukey's Honest Significant Difference test at the 5% level

Fish yields could not be followed because the fish were eaten by pests such as birds, frogs and other fish, so the fish harvest was very small. Although weight gain occurred (in 10 cm of water but not 20 cm) the result are not satisfactory.

IV. CONCLUSIONS

There is no interaction between the depths of water in the channel with the width of the channel. Ten cm of water in the channel is better for growth and yield of IR42 varieties of rice. The width of the ditch did not affect the growth and yield of IR42 varieties, but the 50 cm trench width is better.

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Integrated Effect of Rhizobium Inoculation and Phosphorus Application on Tissue Content, Symbiotic and Phosphorus use Efficiency in Soybean Production

Mulambula. S[1], Gathungu G. K[2], Ndukhu H.O[3], Ogolla F.O[4]

[1]Department of Plant Sciences Chuka University, Kenya
*Email:*siomulambula@yahoo.com
[2]Department of Plant Sciences Chuka University, Kenya
*Email:*gkgathungu@yahoo.com
[3]Department of Plant Sciences Chuka University, Kenya
*Email:*hndukhu@gmail.com
[4]Department of Biological Sciences, Chuka University, Kenya
*Email:*ogolla.fredy@gmail.com

***Abstract*— *Soybean (Glycine max) is an important legume crop that is cultivated all over the world as livestock feed, food for human consumption, soil fertility improvement and industrial products such as candles and paints. However, Nitrogen (N) and phosphorus (P) nutrient have been attributed to the decline in soybean yields. Furthermore, scanty information is documented on P-efficient soybean genotypes, which are a sustainable P management strategy for enhancing symbiotic efficiency (SEF) and phosphorus use efficiency (PUE). As a solution, field experiment was conducted at Chuka University farm to evaluate the integration effect of rhizobium inoculation (R) and P on tissue nutrient content, SEF and PUE in soybean production in Meru South Sub County. Two cultivations (Trial I and II) were done in 2018. Treatments included; three rates of R (0, 100 and 200 g ha^{-1}), three rates of P (0, 20 and 30 kg^{-} ha^{-1}), either applied alone or integrated and soybean genotypes (SB19 and SB24). Both Trials were laid out in a randomized complete block design in split-split plot arrangement with each treatment replicated thrice. Genotypes were assigned main plot, R subplots and P in sub-subplots. Data collected was subjected to analysis of variance using the Scientific Analysis System SAS and significantly different means separated using Tukey test at ($p \le 0.05$). The results showed significant difference in N and P tissue content, SEF and PUE for SB19 and SB24 genotypes in both Trials at ($p \le 0.05$). The highest N tissue content of between 1.73% and 9.10% was observed when integration of R and P were applied at the rate of 200 g and 30 kg for SB19 and SB24 in both Trials. While R and P at the rate of 200 g and 30 kg per ha showed the highest P content of between 849.6 ppm and 955.0 ppm in both Trials. The highest SEF recorded was 207% and 261%, and 201% and 227% in Trials I and II, respectively. The PUE was highest when R and P was applied at the rate of 200 g and 30 kg per ha for SB19 and SB24 soybean in both Trials. Integration of R and P at the rate of 200 g and 30 kg ha^{-1} and adoption of either SB19 or SB24 showed a potential in enhancing soybean cultivation.*
***Keywords*—Genotypes, phosphorus use efficiency, symbiotic, Tissue content.**

I. INTRODUCTION

Soybean (Glycine max) is a crop that is cultivated as a source of livestock feed, human food and soil fertility improvement. It is the world's largest source of protein feed, the second largest source of vegetable oil and the fourth leading crop produced globally [2][3]. Biological nitrogen fixation is crucial and efficient process in improving soil fertility [4]. Application of phosphorus in soybean cultivation enhanced biological nitrogen fixation BNF in soybean-rhizobium interaction [5] increasing nitrogen content in plant tissues [6]. Integration of rhizobium and phosphorus was reported to have improved uptake of N and P in soybean [7]. Elsewhere, intercrop of Soybean/maize resulted to a higher nitrogen content in maize grain, indicating enhanced nitrogen uptake [8]. Phosphorus content in grain and straw was influenced by R and P application [9]. Plant nitrogen concentration in response to increased soil phosphorus supply was observed, with

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phosphorus content in grain and straw being highest in integration of R and P [10]. Phosphorus and nitrogen play specific roles in symbiotic N_2-fixation efficiency through their effects on nodulation and N_2-fixation process [11]. High yields with subsequent profits, are related to symbiotic efficiency (SEF) of soybean with N-fixing bacteria [12]. Nitrogen fixation is very sensitive to P deficiency due to reduced nodule mass and decreased ureide production [7]. Symbiotic N-fixation has a high P demand for energy provision [13][14].

Nitrogen fixation varied among rhizobia isolates with SEF ranging between 27 and 112% when beans were inoculated with R [15] Isolates from beans had higher SEF compared to the commercial inoculants [15]. Koskey *et al*. [16] used plant shoot dry weight to estimate SEF which ranged between 86.7 - 123.72% in common beans. In seed potato tuber production, phosphorus use efficiency PUE increased from 0 kg/kg observed with 0 kg P per ha and 0 kg N per ha to a range of 75.9 kg/kg to 186.6 kg/kg when integration of P and N was used [17].

II. MATERIALS AND METHODS

The experiment was conducted at Chuka University farm, Meru South Sub-County in two cultivations (Trial I and II) in 2018. Chuka University lies at an altitude of 1399 m above sea level, at latitude of 0°20'0"S and longitude of 37°39'0"E (Fig. 1). Temperature range of 20.97 ºC - 27.25 ºC, rainfall of 1178 mm, and nitisol type of soils. Major crops in the area are; *Phaseolus vulgaris*, *Vigna unguiculate*, *Cajanus cajan*, *Glycine max*, *Sorghum spp*, *Musa spp*, *Mangifera indica*, *Coffea arabica* and *Camellia sinensis* [18].

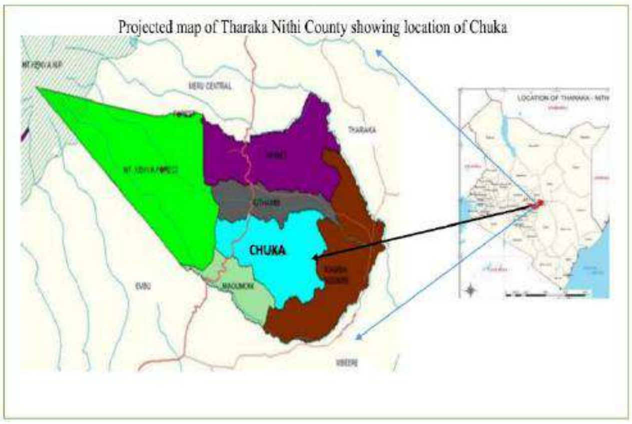

Fig.1: Location of Chuka in Tharaka Nithi County in Kenya

III. EXPERIMENTAL DESIGN

The experiment was laid out in a randomized complete block design in a split-split plot arrangement with each treatment replicated thrice. Treatments included; three rates of P (0, 20 and 30 $Kg^{-} ha^{-1}$), and three rates of R (0, 100 and 200 g ha^{-1}) either applied alone or integrated and two soybean genotypes (SB19 and SB24). The triple superphosphate (0:46:0) was used as the source of phosphorus. The SB19 and SB24 genotypes were assigned the main plot, P rates the sub-plot and R rates to sub-subplots. The size of experimental plot was 1.5 x 1.3 m with 1 m path between main plots and 0.5 m between subplots and sub-subplots.

A. Soil Sampling and Analysis

The soil was sampled across and diagonally in each site at a depth of 25 cm using a soil auger. A kilogram of a homogeneous composite soil sample from each site was packed independently into sterile bags for analysis. Soil analysis was done at Kenya Agricultural Research and Livestock Organization (KALRO) – Embu.

B. Planting Materials, Planting and Crop Management

Certified soybean seeds and inoculant were obtained from KALRO-Kakamega and MEA Limited-Nakuru respectively. The inoculation was done in Plant Science Laboratory of Chuka University. Where soybean seeds were moistened with 4% Gum Arabica solution in a basin and the inoculant was added at the rates of 10 g per Kg and 20 g per Kg of soybean seeds. The mixture was stirred until even coating was attained. The seeds were then spread on flat plywood under a shade and allowed to air dry for 30 minutes. Inoculated seeds were sown early in the morning to avoid its exposure to direct sun rays that could affect the efficacy of the inoculant.

A basal application of phosphorus at the rate of 0 Kg, 20 Kg, and 30 Kg P per ha which was equivalent to 0 g, 3.6 g, and 5.4 g per plot was done during planting to the assigned plots. Two seeds were sown at inter and intra row spacing of 0.5 m and 0.1 m respectively in a plot measuring 1.2 m x 1.5 m. Seedlings were thinned to one per hill one week after emergence giving a plant population of 200,000 plants per ha or 39 plants per plot. All cultural practices recommended for soybean cultivation were done equally to all the plots.

Data Collection

1) Integration Effect of Different Rates of Rhizobium and Phosphorus on Shoot and Grain Nitrogen and Phosphorus Content

Twenty grams of shoot and grain from experimental plants were randomly taken from every plot, placed in khaki papers and dried in the oven at 60 ºC for 72 hours. Dry shoot and grain samples were ground into powder using a blender. The powder was then sieved using a laboratory test sieve and packed in khaki paper bags ready for laboratory analysis. The plant shoots and grain powder were analyzed for nitrogen (% N) and P (ppm) content using Kjeldahl according to Bremner [19].

a) Integration Effect of Different Rates of Rhizobium and Phosphorus on Rhizobium Use Efficiency

The plant shoot dry weight (SDW) was used to estimate the symbiotic nitrogen-fixing efficiency (SEF) of the commercial R. Symbiotic efficiency in this study was determined according to Koskey *et al.* [16], using the formula below:

$$SEF = 100 \times \left[\frac{(SDW\ of\ inoculated\ (Kg))}{SDW\ of\ non\ inoculated\ (Kg)}\right]$$

b) Integration Effect of Different Rates of Rhizobium and Phosphorus on Phosphorus Use Efficiency

The phosphorus use efficiency (PUE) was computed according to Belete *et al*. [20] using the formula below:

$$PUE = \left[\frac{(Seed\ \ yield\ (kg)\ of\ plots\ with\ Fert. - yield\ (kg)control}{Quantity\ of\ phosphorus\ (P)\ applied\ in\ kg\ per\ plot}\right]$$

Data analysis

The data collected was subjected to analysis of variance using the statistical scientific analysis system for windows V8 1999-2001 by SAS Institute Inc., Cary, NC, USA and significantly different means separated using Tukey test at $p \leq 0.05$.

IV. RESULTS

1) Integration Effect of Different Rates of Rhizobium and Phosphorus on Shoot and Grain Nitrogen Content

There were no significant difference in shoot and grain N content between genotype SB19 and SB24 within and between Trial I and II ($p \leq 0.05$). However, there were significant respond of the integration of R and P in shoot and grain N content within genotypes SB19 and SB24 at ($p \leq 0.05$) in both Trials. Rhizobia application at the highest rate of 200 g per ha, increased the shoot N content from the control to 1.4% and 1.03%, and 1.46% and 1.09% for SB19 and SB24 genotypes in Trials I and II, respectively (Table 1). Application of P at the highest rate of 30 Kg per ha, shoot N content increased from the control treatment to 0.94% and 0.83%, and 0.96% and 0.86% for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 1). While, highest integration of R and P at the rate of 200 g and 30 Kg per ha, increased shoot N content to 2.98% and 1.73%, and 3.09% and 1.73% for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 1).

Integration of R and P significantly increased grain N content for SB19 and SB24 genotypes in both Trial I and II whether applied alone or integrated. Rhizobia at the highest rate of 200 g per ha, increased grain N content to 5.97% and 5.11%, and 6.02% and 5.07% for SB19 and SB24 genotypes in both Trial I and II, respectively. Compared to control, highest integration of R and P at the rate of 200 g and 30 Kg per ha, increased grain N content by 3.81% and 2.11%, and 3.53% and 1.93% for SB19 and SB24 genotypes in both Trial, respectively (Table 1).

Table 1: Effect of Rhizobia and Phosphorus on Soybean Shoot and Grain N Content.

Variety		Trial 1		Trial 2	
	Trt	Shoot N (%)	Grain N (%)	Shoot (N %)	Grain N (%)
SB19	T1	0.81^{g*}	5.12^{g}	0.7^{g}	4.4^{f}
	T2	0.88^{gf}	5.54^{f}	0.8^{fg}	4.5^{f}
	T3	0.94^{f}	5.69^{fe}	0.83^{fe}	4.73^{e}
	T4	0.98^{f}	5.8^{e}	0.92^{e}	4.83^{e}
	T5	1.53^{d}	6.8^{c}	1.06^{d}	5.20^{c}
	T6	2.2^{b}	7.69^{b}	1.6^{b}	5.41^{b}
	T7	1.4^{e}	5.97^{d}	1.03^{d}	5.11^{d}
	T8	2.15^{c}	6.82^{c}	1.44^{c}	5.31^{b}
	T9	2.98^{a}	8.93^{a}	1.73^{a}	6.51^{a}
SB24	T1	0.78^{g}	5.57^{g}	0.71^{g}	4.64^{f}
	T2	0.92^{gf}	5.62^{f}	0.81^{fg}	4.62^{f}
	T3	0.96^{f}	5.83^{fe}	0.86^{fe}	4.84^{e}
	T4	0.98^{f}	5.80^{e}	0.96^{e}	4.88^{e}
	T5	1.63^{d}	6.80^{c}	1.07^{d}	5.29^{c}
	T6	2.46^{b}	7.74^{b}	1.6^{b}	5.61^{b}
	T7	1.46^{e}	6.02^{d}	1.09^{d}	5.07^{d}
	T8	2.10^{c}	6.98^{c}	1.5^{c}	5.50^{b}
	T9	3.09^{a}	9.10^{a}	1.73^{a}	6.57^{a}
MSD		0.11	0.19	0.11	0.14
C.V.		6.75	2.78	9.16	2.65

**Means with the same letter along the column for the same variety are not significantly different at $p \leq 0.05$; MSD=Mean Significant Difference; Treatments: T1= Control (0 g R and 0 Kg P per ha); T2 and T3=20 Kg and 30 Kg P per ha respectively; T4 and T7=100 g R and 200 g R per ha respectively; T5=100 g R and 20 Kg P per ha, T6=100 g R and 30 Kg P per ha; T8= 200 g R and 20 Kg P per ha and T9= 200 g R and 30 Kg P per ha; R=Rhizobia; P=Phosphorus.*

Integration Effect of Different Rates of Rhizobium and Phosphorus on Shoot and Grain Phosphorus Content

There was no significant difference in shoot and grain P content between genotype SB19 and SB24 within and between the two Trials ($p \leq 0.05$). However, there were

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significant effect of the integration of R and P in shoot and grain P content within genotypes SB19 and SB24 at (*p≤0.05*) in Trials I and II. Rhizobia application increased shoot P content in SB19 and SB24 genotypes in both Trial I and II. Application of R at the rate of 100 g per ha, significantly increased the Shoot P content from 253.1 ppm and 248.2 ppm, and 256.9 ppm and 251.8 ppm observed with the control treatment to 334.3 ppm and 328.4 ppm, and 337.4 ppm and 332.1 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 1).

Application of P at the highest rate of 30 Kg per ha increased the Shoot P content from control treatment to 326.1 and 320.6 ppm, and 333.6 and 327.8 ppm for SB19 and SB24 genotypes in Trial I and II, respectively (Table 2). While, integration of R and P at the highest rate of 200 g and 30 Kg per ha, increased shoot P content to 849.6 ppm and 906 ppm, and 849.6 ppm and 913.8 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 2). Rhizobia application increased grain P content in SB19 and SB24 genotypes in both Trial I and II. For instance R application at the rate of 100 g per ha, significantly increased the grain P content from the control treatment to 509.9 ppm and 519.6 ppm, and 514.1 ppm and 524.5 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively. Compared to control, application of R at the rate of 200 g per ha significantly increased the grain P content from the control treatment to 430.1 ppm and 529.6 ppm and 434.2 and 534.6 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 2). Application of P at the highest rate of 30 Kg per ha, increased grain P content from the control treatment to 340.6 ppm and 350.8 ppm, and 346.4 ppm and 356.4 ppm for SB19 and SB24 genotypes in Trial I and II, respectively (Table 2). Further, integration of R and P at the highest rate of 200 g and 30 Kg per ha, significantly increased grain P content to 852.4 ppm and 950 ppm, and 853.3 ppm and 955 ppm for SB19 and SB24 genotypes in both Trials, respectively (Table 2).

Table 2: Effect of Rhizobia and Phosphorus on Soybean Shoot and Grain P Content.

Variety	Trt	Trial 1 Shoot P (ppm)	Trial 1 Grain P (ppm)	Trial 2 Shoot P (ppm)	Trial 2 Grain P (ppm)
SB19	T1	253.1^{h*}	225.6^{i}	248.2^{h}	235.6^{i}
	T2	290.2^{g}	323.3^{h}	285.0^{g}	333.3^{h}
	T3	326.1^{f}	340.6^{g}	320.6^{f}	350.8^{g}
	T4	334.3^{f}	509.9^{e}	328.4^{f}	519.6^{f}
	T5	488.4^{d}	579.8^{c}	497.9^{d}	589.8^{d}
	T6	671.6^{b}	734.4^{b}	737.2^{b}	834.3^{b}
	T7	361.7^{e}	430.1^{f}	385.4^{e}	529.6^{e}
	T8	542.0^{c}	524.6^{d}	649.9^{c}	624.6^{c}
	T9	849.6^{a}	852.4^{a}	906.0^{a}	950.0^{a}
SB24	T1	256.9^{h}	241.7^{i}	251.8^{h}	240.6^{i}
	T2	294.0^{g}	328.4	289.0^{g}	338.4^{h}
	T3	333.6^{f}	346.4^{g}	327.8^{f}	356.4^{g}
	T4	337.4^{f}	514.1^{e}	332.1^{f}	524.56^{f}
	T5	490.4^{d}	584.4^{c}	510.6^{d}	594.7^{d}
	T6	672.2^{b}	739.4^{b}	738.4^{b}	839.3^{b}
	T7	366.0^{e}	434.2	383.0^{e}	534.6^{e}
	T8	535.7^{c}	529.7^{d}	660.2^{c}	629.7^{c}
	T9	849.6^{a}	853.3^{a}	913.8^{a}	955.0^{a}
MSD		9.7	0.19	6.73	0.14
C.V.		2.01	1.8	1.31	0.85

**Means with the same letter along the column for the same variety are not significantly different at (p≤0.05); MSD=Mean Significant Difference; Treatments: T1= Control (0 g R and 0 Kg P per ha); T2 and T3=20 Kg and 30 Kg P per ha respectively; T4 and T7=100 g R and 200 g R per ha respectively; T5=100 g R and 20 Kg P per ha, T6=100 g R and 30 Kg P per ha; T8= 200 g R and 20 Kg P per ha and T9= 200 g R and 30 Kg P per ha; R=Rhizobia; P=Phosphorus.*

Integration Effect of Different Rates of Rhizobium and Phosphorus on Symbiotic Efficiency

There was no significant difference in symbiotic efficiency between genotype SB19 and SB24 within and between Trials I and II (*p≤0.05*). However, there was significant influence of the integration of R and P in symbiotic efficiency (SEF) within genotypes SB19 and SB24 at (*p≤0.05*) in Trials I and I. For instance, application of R at the rate of 100 g, increased SEF from 101% and 107%, and 101% and 100% observed with the control treatment to 129% and 165%, and 114% and 116% for SB19 and SB24 genotypes in Trial I and II, respectively (Table 3). When P was applied at the highest rate of 30 Kg per ha, SEF increased from the control treatment to 135% and 157%, and 126% and 131% (Table 3). Compared to control

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integration of R and P at the highest rate of 200 g and 30 Kg per ha, increased SEF by 106% and 154%, and 101 and 127% for SB19 and SB24 genotypes in Trial I and II, respectively (Table 2).

Table 3: Effect of Rhizobia and Phosphorus on Symbiotic Efficiency (%), Phosphorus (Kg/Kg) and Rhizobium (Kg/Kg)

Variety			Trial 1	Trial II	
	Trt	SEF (%)	PUE (Kg/Kg)	SEF (%)	PUE (Kg/Kg)
SB19	T1	101^{d*}	0^{d}	107^{d}	0^{d}
	T2	108^{d}	4.65^{c}	119^{d}	4.8^{c}
	T3	135^{cd}	3.9^{c}	157^{cd}	3.9^{c}
	T4	129^{cd}	0^{d}	165^{cd}	0^{d}
	T5	153^{bc}	8.15^{a}	182^{bc}	8.15^{b}
	T6	176^{ab}	6.49^{b}	149^{bc}	3.78^{c}
	T7	130^{cd}	0^{d}	152^{cd}	0^{d}
	T8	167^{ab}	8.6^{a}	200^{ab}	8.96^{a}
	T9	207^{a}	6.9^{a}	261^{a}	8.75^{a}
SB24	T1	101^{d}	0^{d}	100^{d}	0^{d}
	T2	103^{d}	4.2^{c}	104^{d}	4.3^{b}
	T3	126^{cd}	3.6^{c}	131^{cd}	3.6^{c}
	T4	114^{cd}	0^{d}	116^{cd}	0^{d}
	T5	145^{bc}	7.8^{a}	155^{bc}	3.8^{c}
	T6	187^{ab}	6.6^{bc}	209^{bc}	4.2^{b}
	T7	119^{cd}	0^{d}	122^{cd}	0^{d}
	T8	179^{ab}	8.9^{a}	199^{ab}	9^{a}
	T9	201^{a}	9.4^{a}	227^{a}	9.5^{a}
MSD		31	1.3	46	1.4
CV (%)		25	58	31	59

**Means with the same letter along the column for the same variety are not significantly different at ($p \le 0.05$); MSD=Mean Significant Difference; Treatments: T1 =Control (0 g R and 0 Kg P per ha); T2 and T3=20 Kg and 30 Kg P per ha respectively; T4 and T7=100 g R and 200 g R per ha respectively; T5=100 g R and 20 Kg P per ha, T6=100 g R and 30 Kg P per ha; T8 =200 g R and 20 Kg P per ha and T9 =200 g R and 30 Kg P per ha; PUE=Phosphorus Use Efficiency; SEF=Symbiosis efficiency*

Integration Effect of Different Rates of R and P on Phosphorus Use Efficiency

There were no significant difference in phosphorus use efficiency (PUE) between genotype SB19 and SB24 within and between Trials I and II (*$p \le 0.05$*). However, there were significant respond of the integration of R and P in PUE within genotypes SB19 and SB24 at (*$p \le 0.05$*) in Trials I and II. When P was applied at the rate of 20 Kg per ha, the soybean grain yield obtained increased to 4.65 Kg/Kg and 4.8 Kg/Kg, and 4.2 Kg/Kg and 4.28 Kg/Kg for SB19 and SB24 soybean genotypes in Trial I and II, respectively (Table 3). Furthermore, when P was applied at the rate of 30 Kg per ha the soybean grain yields increased from the control treatment to 3.93 Kg/Kg and 3.9 Kg/Kg, and 3.58 Kg/Kg and 3.6 Kg/Kg for SB19 and SB24 genotypes in Trial I and II, respectively. Integration of R and P at the highest rate of 200 g and 30 Kg per ha increased grain yield from the control treatment to 6.9 Kg/Kg and 8.75 Kg/Kg, and 9.37 Kg/Kg and 9.48 Kg/Kg for SB19 and SB24 genotypes in Trial I and II, respectively (Table 3).

V. DISCUSSIONS

There was significant increase of shoot and grain N uptake, these probably, could be associated with R and P. Which enhanced N fixation, root length and root mass resulting to absorption of mineral nutrients from the soil, particularly available N. Similarly, significant increase in root nodules due to integration of R and P, probably, increased N_2 fixation. This led to increase in N uptake which is in agreement with Bargaz *et al.* [21] who observed that, P plays a vital role in physiological and developmental process in plants. Similarly, significant increase in root nodulation due to integration of Rand P increased N_2 fixation that led to increase in N uptake by shoot and grain of soybean.

Higher P content in shoot and grain was attributed to the root length of soybean. A long root system might have created a greater root-soil contact which increased P uptake hence high P content in shoot and grain. These findings are in agreement with Mathenge [11] who observed that a larger root system enhanced by P provided greater root-soil contact hence higher uptake. Furthermore, high shoot and grain P content can be attributed to the presence of R applied which enhanced the solubilization of the P in the soil increasing available P for the uptake. This is in agreement with Adjei-Nsiah *et al.* [1] who observed that rhizobium has the ability to solubilize P increasing P concentration in the soil. This concurs with Abbasi *et al.* [22] who reported that soybean grain/straw P uptake was increased with increased integration of P and R.

High SEF observed in control treatment in both Trails, probably, suggest that native R were active and effective, which consequently increased SDW. Further, the good performance of control, could be associated with native strain adaptation to the ecological conditions of the study area. These results are in agreement with the findings by Kawaka *et al.* [23] who reported SEF ranging between 67 and 170% when common beans were inoculated with native rhizobia.

High performance of commercial R probably, could be associated with the commercial strains being more adapted to the study area compared to the native strains, leading to higher performance in SEF. This is contrary to Mungai and Karubiu [24] who observed native rhizobia isolated from common beans having higher SEF compared to commercial inoculants. Integration of R and P had the highest SEF, probably, this was associated with enhanced energy provision by P which improved the performance. This concurs with the findings by Bargaz *et al.*[21]who reported BNF having a high P energy demand.

Low PUE where P was applied alone in the present study, probably, may be associated with P fixation in the soil (beyond scope of this study) making P less available to the plants. This concurs with findings by Fageria and Barbosa [25] and Singh *et al.*[26] who observed higher P fixation decreasing PUE in lentil and rice enterprises, respectively. Overall, the present study observed low soybean grain produced for every Kg of P applied for soybean genotype SB19 and SB24 in trial I and II, respectively. This economic production was below findings by Abbasi *et al.* [22] who reported higher soybean grain yield produced with each Kg of phosphorus applied.

VI. CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that integration of R and P at the rate of 200 g and 30 kg per ha was the optimum rate of application compared to other treatment levels in soybean performance. It was observed that symbiotic and phosphorus use efficiency was influenced by the R and P for SB19 and SB24 soybean genotypes. The two genotypes, SB19 and SB24 performed equally well. Farmers can adopt integration of R and P at the rate of 200 g and 30 kg per ha and either of the genotypes for sustainable soybean cultivation. Further research by use of other sources of P such as phosphorus solubilizing bacteria to enhance symbiotic and phosphorus use efficiency is recommended.

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Assessment of Rhizobia Strains Isolates of Soils around Lake Victoria Basin for their Effectiveness in Nodulation and Symbiotic Efficiency on Soybeans and Bambara Groundnuts

Onyango O. Benson[1] and Ogolla O. Fredrick[2]

[1] Jaramogi Oginga Odinga University of Science and Technology
Department of Biological Sciences
P.O. Box 210-40601, Bondo, Kenya,
Phone: +254 724694613
Email: benboyih@gmail.com
[2] Chuka University, Department of Biological Sciences
P.O. Box 109-60400, Chuka, Kenya,
Phone: +254 708576198
Email: ogolla.fredy@gmail.com

***Abstract*— *The symbiotic Biological nitrogen fixation (SBNF) is a sustainable and low-cost alternative to expensive and inaccessible inorganic fertilizers. However, SBNF is underutilized in soils of Lake Victoria basin due to insufficient information on local rhizobial strains diversity and their N-fixation efficiency. This study was carried out to assess the effectiveness of rhizobium strains isolates of Kisumu, Port Victoria, Kendu bay and Karungu soils within Lake Victoria basin in nodulation and symbiotic efficiency on soybeans and bambara groundnuts. Two bambara seeds of groundnut landraces; Kakamega Cream (KAKC) and Busia Brown (BUSB) used in this study were collected from farmers in Kakamega and Busia counties respectively. Screen house experiment was performed at Kenya Forestry Research (KEFRI) in plastic pots with four seeds of each cultivar which was later thinned to two plants. Randomized Complete Block Design (RCBD) was used. Experiments data were subjected to analysis of variance (ANOVA) using Genstat 16th Edition and significant means separated using Least Significant Difference at [$LSD_{5\%}$] and Dancun Multiple Range Test (DMRT). Result indicated highly significant ($p<0.05$) effect of isolate inoculation on number of nodules per plant. Soybean Variety SB19 formed effective nodules with rhizobia in the genera Bradyrhizobium, Rhizobium and Agrobacterium. On the other hand, 'Safari' was quite selective and formed very few nodules with isolates identified as Bradyrhizobium. However, both varieties SB19 and 'Safari' had better growth under glasshouse inoculation with Bradyrhizobium spp., rhizobia isolates although one Rhizobium isolate (SoyKis1) resulted in good nodulation of both varieties. Seed treatment of the two legumes with some isolates resulted in improved nodulation and better plant growth; in some instances, outperforming the commercial strain Bradyrhizobium japonicum USDA110. In conclusion, Isolates BAMKis12, BAMKis8, BAMKis4, BAMKbay8 and SoyKar2 were found to be potential elite strains and are recommended for more host range tests as viable inoculants sources.*

***Keywords*—Rhizobia, Nodulation Effectiveness, Soybeans, Bambara Groundnuts.**

I. INTRODUCTION

Strategies to increase soil N-fertility as an alternative to expensive and inaccessible inorganic fertilizers lie in the use of symbiotic Biological nitrogen fixation (BNF). Symbiotic BNF is an important source of soil N. Legume crops have been shown to fix as much as 300 kg/ha/yr [1][2]. Globally, symbiotic N fixation has been estimated to amount to at least 70 million metric tonnes of nitrogen per year [3], with higher values of up to 90 million metric tonnes [4]. This is a proof that biological nitrogen fixation has significantly reduced the dependence of agriculture on N fertilizers [5]. The nitrogen in amino acids, purines, pyrimidines and other biomolecules ultimately comes from atmospheric nitrogen (N_2) and has to been converted into forms available for living organisms [6]. The biosynthetic process of conversion starts with reduction of N_2 into NH_3 (ammonia) in a process called nitrogen fixation by the rhizobia bacteria [7]. Majority of legumes have a distinctive symbiotic association with N-fixing rhizobia [8]. The symbiosis results in the plant roots being invaded by the bacteria to form organized plant root structures known as root nodules [9]. Effective use of this association requires knowledge of indigenous rhizobia that can effectively nodulate with legumes such as soybeans and bambara groundnuts cultivated in the region [10]. This information which is required for selection of competitive indigenous strains for use as bio-fertilizers in seed inoculation programmes is currently limited.

Commercial inoculants available in the market are made from exotic strains most of which may be less adapted to the local soils than indigenous strains. As a result, the potential of local strains to optimize BNF for improved soil N-fertility is not fully documented. The BNF is a sustainable and low-cost alternative to inorganic fertilizers in smallholder farming systems. Nonetheless, it is underutilized in soils of Lake Victoria basin partly due to insufficient information on the diverse populations of rhizobial strains and their N-fixation efficiency. To overcome these drawbacks, identifying resident and environmentally adaptable N-fixing rhizobia with superior symbiotic abilities and utilizing them in local cropping systems is necessary [11]. This is important because symbiotic efficiency of rhizobia is strongly influenced by edaphic and environmental factors [12][13].

II. MATERIALS AND METHODS

The study was conducted in glasshouse and screenhouse pot experiments at Kenya Forestry Research Institute's headquarters at Muguga, Nairobi. Soil samples used in pot experiments were collected from farmers' fields in Port Victoria in Busia County, Kisumu in Kisumu County, Kendu bay in Homabay County and Karungu in Migori County. A map of the sites is shown in Fig. 1 and site characteristics are shown on Table 1.

Table 1. Characteristics of soil sampling sites and soil types

Site	Site location	Agro-climatic zones	Soil type
Kisumu	0^0 6′0N 34^{0}45′ 0E	Sub-humid lower midland	Clay loam
Karungu	$0^0$51′ 0S 34^0 8′ 60E	Semi-humid lower midland	Clay loam
Kendu bay	$0^0$22′ 0S 34^{0}38′ 60E	Semi-humid lower midland	Sandy loam
P. Victoria	0^0 6′ 0N 33^0 58′ 0E	Sub-humid lower midland	Sandy loam

Source: Jaetzold *et al*.[14]

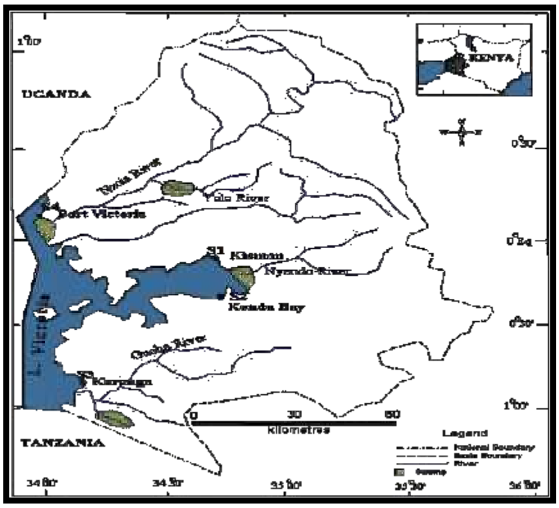

Fig.1. A map showing four sites within Lake Victoria basin where soil samples used to trap rhizobia were obtained.

Glass house inoculation experiments at KEFRI headquarters

Authentication and symbiotic efficiency glasshouse pot experiments were arranged in Completely Randomized Design (CRD) with the two soybean varieties or the two bambara groundnut landraces as treatments in four replicates. Sixty four and 42 rhizobial colonies isolated from bambara groundnuts and soybeans respectively were evaluated by 16S rRNA gene sequences and grouped according to the maximum identity of the genus with those at the NCBI gene bank.

Based on the results, 18 bambara isolates and 16 soybean isolates representative of all the groups identified were selected for determination of symbiotic status. One strain (*Bradyrhizobium spp*. KFR259) from Dr. David Odee's rhizobial collection at Kenya Forestry Research Institute's headquarters in Muguga and a commercial strain *Bradyrhizobium japonicum* USDA110 were chosen as reference strains based on previous reports that bambara groundnuts and soybeans nodulates better with *Bradyrhizobium spp*.[15].

Seeds were prepared as previously described and spread on water-agar (4:1) plates. The plates were inverted and incubated in dark cabinets at 25^{o}C for germination and were considered to be successfully germinated when their radicles reached the same length as the seed or longer. Improvised Leonard jars were prepared by sterilizing in 5% sodium hypochlorite solution for 15 minutes. Vermiculite obtained from a commercial supplier in Nairobi's industrial area was sterilized at 121^{o}C for 1 hr and the pH normalized to near neutral using 5% $CaCO_3$ solution and then filled in the jars.

Each filled jar bottom was covered with absorbent sterile cotton wool to allow free flow of solutions while limiting contamination.

Two seedlings of each variety/landrace were aseptically transferred into each vermiculite pot at a depth of 5 mm using sterile pipette tips. The inoculum was prepared by streaking each isolate on freshly prepared YEMA plates. 1 ml of the pure culture was aseptically picked, re-suspended into sterile bottles containing 10 ml of Yeast Extract Mannitol broth and left on a shaker for 2 days. Eight replicates per isolate were inoculated directly around seedling hypocotyls 3 days after establishment using sterile disposable pipette tips. On each occasion, 1 ml (about 10^8 cells) of bacterial suspension was used to inoculate each seedling. Immediately after inoculation, vermiculite surfaces were covered with steam sterilized sand to inhibit contamination.

Growing conditions, data collection and harvesting

The experiments to evaluate symbiotic status of selected isolates were conducted in glasshouse-controlled conditions at 16/8 hr light/dark cycle, 25/18 °C day/night temperature and 70% relative humidity. The test seedlings and the negative controls (-N) were watered twice each week using 20 ml of sterile N-free nutrient solution [16] but the solution was increased to 40 ml after 2 weeks of growth. The positive controls (+N) were supplied with 10 ml of 0.5 M KNO_3 solution each week to supply the plants with nitrogen. After 45 days, the growth media was gently shaken off the plants, the roots carefully washed and the number of well-formed nodules counted. Nodules (when available), roots and shoots from the same pot were considered to belong to the same unit and were put in separately marked 2×1.75 cm zip-lock polythene bags for laboratory analysis. Fresh weights in grams per plant was taken in four replicates and recorded on an analytical electronic balance (The Lab Depot Inc, USA). Nodules, shoots and roots were enclosed in aluminium foil and inserted in an oven set at 70ºC for 1-2 days and dry weights obtained on an analytical electronic balance (The Lab Depot Inc, USA).

Representative shoot samples were ground using a mortar and pestle and submitted to Kenya Industrial Research and Development laboratories in Nairobi for analysis of N-content using the Kjeldahl method. The procedure used was as follows; Approximately 1 g of the sample was placed in a digestion flask and 1.5 g of potassium sulfate, 0.04 g anhydrous copper sulfate and 1.0 g alundum granules was added followed by 2.0 ml of sulfuric acid. The flask was placed on preheated burner and heated for 90 min until white fumes appeared while swirling gently. The mixture was cooled to room temperature while adding 250 ml of distilled water. Three drops of tributyl citrate was added then 45% sodium hydroxide solution drop by drop. The flask was immediately connected to a distillation apparatus and distilled at 25ºC until at least 150 ml distillate was collected in titrating flask and N concentration determined.

III. RESULTS

Glasshouse Authentication Experiments of Rhizobial isolates

The population of rhizobia from the soils based on the MPN results were; 1.7×10^3, 2.0×10^3, 1.9×10^3 and 1.5×10^3 for Port Victoria, Kisumu, Kendu bay and Karungu respectively. Host-strain interaction test results are shown in table 2. Out of the 64 bambara groundnut isolates, only 24 formed nodules with landrace KAKC out of which 7 were highly effective, 9 were partly effective while 8 were ineffective. Eighteen isolates were able to form nodules on landrace BUSB, but only 6 highly effective nodules were observed. In particular, soybean Variety 'Safari' was quite selective in nodulation potential failing to form nodules with 36 out of the 42 isolates. Only 2 isolates formed highly effective nodules on 'Safari' while effective nodules were observed in plants inoculated with 4 isolates. A distinction was observed on Variety SB19 which produced nodules with 22 isolates in which 9 were effective and 4 formed highly effective nodules. Majority of effective isolates on bambara groundnuts and soybeans were derived from Kisumu and Karungu soils.

Symbiotic Status of Selected Rhizobial Isolates in Glasshouse Conditions

1. **Performance of Selected Isolates on Bambara Groundnuts**

Glasshouse inoculation experiments on two bambara groundnut landraces with 18 isolates from this study and two reference strains (*Bradyrhizobium spp*. strains KFR259 and USDA110) resulted in variation in nodule, shoot, root and total plant biomass factors evaluated as shown in Table 3. There was a highly significant ($p<0.05$) effect of isolate inoculation on *number of nodules per plant*, although no clear landrace difference ($p>0.05$) was observed on this factor. Plants inoculated with isolates BAMKis1, BAMKis8, BAMKis4 and BAMKar3 which were genetically identified as *Bradyrhizobium spp*. produced highly effective and significantly ($p<0.05$) the highest mean *nodule number per plant* (above 20) followed by two isolates BAMKbay8 and BAMsp3 identified as *Burkholderia spp*. also giving above 20 nodules per plant.

Results of mean *nodule number per plant* obtained from the six isolates compared favorably with those obtained from the reference strains *Bradyrhizobium spp*. strain KFR259 and *Bradyrhizobium japonicum* strain USDA110, which produced an average of above 30 nodules per plant on landrace KAKC with the highest value of 49 nodules occurring on plants inoculated with strain USDA110. One

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isolate from Kisumu soils BAMKis1 genetically identified as *Rhizobium spp*. produced highly effective nodules on both landraces which numbered above 30, outperforming some of the *Bradyrhizobia spp*. (BAMKis8 and BAMKar3) that produced below 25 nodules per plant. Nodules classified as effective based on internal nodule colour were mostly found in plants inoculated with isolates genetically identified as *Rhizobium* (BAMKar2, BAMKar1 and BAMKis2 – each giving below 20 nodules per plant).Inoculation with isolates identified as *Agrobacterium spp*. resulted in no nodules (BAMKar9, BAMsp5 and BAMKbay9) except one isolate BAMKbay1 which produced above 10 nodules per plant most of which were effective.

Results on mean *nodule fresh* and *dry weights* followed a similar trend and posted highly significant response due to isolate inoculation ($p<0.05$), but there was no significant landrace effect on the two variables ($p>0.05$). The greatest mean *nodule fresh weight* of 0.477 g per plant was obtained from plants inoculated with isolate BAMKis1, producing slightly higher values ($p<0.05$) than both the reference strains, followed by plants inoculated with isolates BAMKis4 and BAMKis12 giving 0.449 g. Nodules retrieved from plants inoculated with isolate BAMKis1 had the highest mean *dry weight* of 0.073 g per plant followed by plants inoculated with BAMKis4 and BAMKis12 which ranged between 0.064 g and 0.065 g, values which were nearly identical to the mean *dry weight* of 0.067 g obtained from landrace KAKC plants inoculated with the reference strain USDA110 ($p<0.05$).

The mean *root* and *shoot dry weights* did not give a significant ($p>0.05$) difference due to inoculation but numerically the highest root dry weight of 0.302 g per plant was surprisingly obtained from non-nodulated plants of landrace KAKC inoculated with BAMKar9 – (identified as *Agrobacterium* sp.), which was much higher than the results obtained from the +N un-inoculated positive control (averagely 0.25 g per plant for both landraces). Although not significantly different ($p>0.05$) numerically the highest shoot dry weights of 2.32 g and 1.59 g per plant were obtained from the +N control plants with all the plant appearing tall and exhibiting large leaves. The total plant biomass also showed no significant difference ($p>0.05$) due to inoculation having been obtained from both the root and shoot dry weights.

2. Performance of Selected Isolates on Soybeans

Glasshouse inoculation experiments of two soybean varieties (SB19 and 'Safari') with 16 isolates and two reference strains resulted in variation in plant growth as shown in Table 4. There was a significant ($p<0.05$) effect of isolate inoculation on the mean *number of nodules* obtained from both soybean varieties although there was no significant ($p>0.05$) inter-varietal difference for the same variable. Plants inoculated with isolates SoyKis3 and SoyKar2 identified as *Bradyrhizobium elkanii* had significantly ($p<0.05$) the highest nodule number per plant, giving over 20 highly effective nodules from SB19 and 'Safari'. Inoculation of SB19 with isolates SoyKar2 and SoyKar4 (*Bradyrhizobium spp*.) from Karungu soils resulted in effective nodules which ranged from 13.8 to 16.5 nodules per plant, significantly higher ($p<0.05$) than plants inoculated with isolates from Kendu bay soils which had less than 10 nodules per plant. It was evident that most isolates from Port Victoria soils (Soysp2, Soysp3 and Soysp4 – within *Rhizobium* and *Agrobacterium* genus) were incompatible with both soybean varieties used in this study as the isolates did not form any nodules; with only Soysp1 producing six nodules on SB19.

A comparison of mean *nodule fresh weight* revealed no significant ($p>0.05$) varietal differences between non-inoculated SB19 and 'Safari', but there was a significant response to inoculation in both varieties ($p<0.05$). The greatest mean nodule fresh weight of 0.350 g was obtained from varieties SB19 and 'Safari' inoculated with SoyKar1 while the least nodule fresh weight was obtained from plants inoculated with SoyKbay2 and SoyKbay1 both giving a similar value of 0.005 g for SB19. Plants inoculated with SoyKis1 and the reference strain KFR259 produced higher nodule dry weight ($p<0.05$) giving a similar value of 0.086 g.

A significant ($p<0.05$) variation in mean *shoot dry weight* occurred with +N positive control plants producing the highest value of 1.434 g on the specific 'Safari' variety, followed by the same treatment on Variety SB19 with a value of 1.159 g($p<0.05$). Inoculation with the reference strain USDA110 and one isolate SoyKis1 resulted in above 1 g shoot dry weight, significantly higher than the values obtained from all the other isolates. No significant difference ($p>0.05$) due to treatment effects occurred between the varieties with regard to root dry weight. The mean *total plant biomass* was significantly ($p<0.05$) affected by inoculation with the Variety 'Safari' posting values of above 1.0 g with most of the isolates which produced nodules. However, +N plants produced the highest mean plant biomass of 1.704 g.

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Table 2. Effectiveness tests of indigenous rhizobial isolates on two bambara groundnut landraces and two soybean varieties

Bambara groundnut isolates						**Soybean isolates**					
Isolate Identity	**Landrace**		**Isolate Identity**	**Landrace**		**Isolate Identity**	**Variety**		**Isolate Identity**	**Variety**	
	KAKC	**BUSB**		**KAKC**	**BUSB**		**SB19**	**SAF**		**SB19**	**SAF**
BAMKis1	HE	HE	BAMKbay12	I	I	SoyKis1	HE	E			
BAMKis2	E	PE	BAMKbay13	X	X	SoyKis2	HE	E	Soysp1	I	X
BAMKis3	PE	PE	BAMKbay14	X	X	SoyKis3	HE	HE	Soysp2	X	X
BAMKis4	HE	PE				SoyKis4	E	E	Soysp3	X	X
BAMKis5	X	X	BAMsp1	E	E	SoyKis5	I	X	Soysp4	X	X
BAMKis6	PE	PE	BAMsp2	E	E	SoyKis6	X	X			
BAMKis7	X	X	BAMsp3	HE	HE	SoyKis7	X	X	SoyKar1	E	E
BAMKis8	HE	H	BAMsp4	I	I	SoyKis8	I	X	SoyKar2	HE	HE
BAMKis9	X	X	BAMsp5	X	X	SoyKis9	X	X	SoyKar3	E	E
BAMKis10	PE	X	BAMsp6	X	X	SoyKis10	X	X	SoyKar4	E	E
BAMKis11	X	X	BAMsp7	E	X	SoyKis11	X	X	SoyKar5	X	X
BAMKis12	HE	HE	BAMsp8	I	X	SoyKis12	I	X	SoyKar6	X	X
BAMKis13	X	X	BAMsp9	I	X	SoyKis13	X	X	SoyKar7	X	X
BAMKis14	X	X	BAMsp10	X	X	SoyKis14	X	X	SoyKar8	I	X
BAMKis15	X	X	BAMsp11	X	X	SoyKis15	X	X	SoyKar9	X	X
BAMKis16	X	X	BAMsp12	PE	PE	SoyKis16	X	X	SoyKar10	X	X
BAMKis17	X	X				SoyKis17	X	X	SoyKar11	X	X
BAMKis18	I	X	BAMKar1	E	E	SoyKis18	X	X	SoyKar12	X	X

Bambara Groundnut Isolates						Soybean isolates					
Isolate	**Landrace**		**Isolate**	**Landrace**		**Isolate**	**Variety**			**Variety**	
Identity	**KAKC**	**BUSB**	**Identity**	**KAKC**	**BUSB**	**Identity**	**SB19**	**SAF**	**Isolate Identity**	**SB19**	**SAF**
BAMKis19	X	X	BAMKar2	E	E	SoyKis19	I	X			
BAMKis20	X	I	BAMKar3	HE	HE	SoyKis20	X	X			
BAMKis21	X	X	BAMKar4	X	X	SoyKis21	X	X			
BAMKis22	X	I	BAMKar5	X	I	SoyKis22	X	X			
			BAMKar6	I	X						
BAMKbay1	X	X	BAMKar7	X	X	SoyKbay1	I	X			
BAMKbay2	X	X	BAMKar8	PE	E	SoyKbay2	E	X			
BAMKbay3	X	X	BAMKar9	X	X	SoyKbay3	I	X			
BAMKbay4	PE	PE	BAMKar10	X	X	SoyKbay4	I	X			
BAMKbay5	X	I	BAMKar11	X	X						
BAMKbay6	I	I	BAMKar12	X	X						
BAMKbay7	X	I	BAMKar13	X	X						
BAMKbay8	HE	HE	BAMKar14	X	X						
BAMKbay9	X	X	BAMKar15	PE	PE						
BAMKbay10	X	X	BAMKar16	X	X						
BAMKbay11	PE	PE									

NOTE: Isolates from the same site have the same colour regime.

Sites of origin;
Kis– Kisumu isolates
Kbay – Kendu bay isolates
Sp – Port Victoria isolates
Kar– Karungu isolates

Host strain interactions
HE - Highly effective (>20 nodules /plant)
E– Effective (>10 and < 20 nodules /plant)
PE - Partly effective (<10 and >5 nodules /plant)
I– Ineffective (< 5 nodules /plant)
X - No nodulation (0 nodules /plant)

Table 3: Symbiotic status of 18 test rhizobial isolates, two reference strains and +N and –N controls on two bambara groundnut landraces (KAKC and BUSB) in glasshouse pot experiments

	Treatment /Isolate inoculant	NNO		NFW (g)		NDW (g)		RDW (g)		SDW (g)		TBM (g)	
		Landrace		Landrace		Landrace		Landrace		Landrace		Landrace	
		KAKC	BUSB	KAKC	BUSB	KAKC	BUSB	KAKC	BUSB	KAKC	BUSB	KAKC	BUSB
	+ N	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.200	0.263	2.121	1.591	2.321	1.854
	- N	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.142	0.154	0.328	0.390	0.470	0.544
	USDA110	49.00^{a}	32.00^{a}	0.387^{ab}	0.421^{ab}	0.064^{a}	0.067	0.168	0.288	1.005	1.143	1.236	1.498
	KFR 259	34.75^{b}	20.25^{b}	0.286^{b}	0.320^{bc}	0.044^{b}	0.044	0.186	0.239	0.661	0.889	0.891	1.171
	BAMKis12	30.50^{b}	26.75^{a}	0.203^{b}	0.444^{ab}	0.035^{bc}	0.065	0.149	0.292	0.896	1.152	1.081	1.510
	BAMKis8	22.71^{c}	20.50^{b}	0.319^{ab}	0.340^{ab}	0.041^{b}	0.049	0.172	0.289	1.062	1.031	1.275	1.370
	BAMKis4	37.23^{b}	27.75^{a}	0.306^{ab}	0.449^{ab}	0.053^{ab}	0.064	0.186	0.286	0.795	1.055	1.033	1.405
	BAMKbay8	21.25^{c}	22.31^{a}	0.314^{ab}	0.301^{bc}	0.044^{b}	0.047	0.159	0.198	0.842	0.791	1.145	1.036
	BAMsp3	22.02^{c}	15.75^{bc}	0.173^{b}	0.214^{c}	0.026^{bc}	0.031	0.208	0.212	0.759	0.695	0.993	0.938
	BAMKis1	35.77^{b}	31.75^{a}	0.293^{ab}	0.471^{a}	0.040^{b}	0.073	0.149	0.265	0.896	1.242	1.085	1.581
	BAMKar3	25.79^{c}	25.50^{a}	0.428^{a}	0.350^{ab}	0.059^{ab}	0.064	0.199	0.215	1.296	0.734	1.755	1.013
	BAMKar2	15.50^{cd}	22.01^{b}	0.288^{b}	0.173^{d}	0.038^{b}	0.026	0.110	0.208	0.767	0.759	1.016	0.993
	BAMKar1	17.50^{cd}	8.25^{c}	0.258^{b}	0.020^{e}	0.094^{a}	0.003	0.186	0.167	0.735	0.809	1.015	0.981
	BAMKis2	10.49^{d}	18.50^{b}	0.219^{b}	0.277^{cd}	0.032^{bc}	0.027	0.107	0.236	0.954	0.692	1.392	1.124
	BAMKbay1	11.25^{d}	17.50^{bc}	0.211^{b}	0.184^{cd}	0.039^{b}	0.022	0.185	0.225	0.581	0.914	0.766	1.139
	BAMsp2	1.51^{e}	4.51^{d}	0.016^{c}	0.017^{e}	0.021^{bc}	0.004	0.241	0.188	0.661	0.937	0.932	1.129
	BAMsp1	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.258	0.233	0.519	0.643	0.777	0.877
	BAMKbay2	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.231	0.184	0.838	0.678	1.369	0.863
	BAMKar9	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.302	0.210	0.663	0.669	0.965	0.879
	BAMKbay9	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.279	0.184	0.701	0.678	0.980	0.863
	BAMKbay3	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.198	0.192	0.791	0.621	0.989	0.813
	BAMsp5	0.00^{e}	0.00^{d}	0.000^{c}	0.000^{e}	0.000^{c}	0.000	0.179	0.264	0.609	0.779	0.789	1.043
	SED	**0.013**		**0.095**		**0.025**		**0.069**		**0.067**		**0.178**	
	Var.	**2.95^{ns}**		**0.042^{ns}**		**0.011^{ns}**		**0.041^{ns}**		**0.105^{ns}**		**0.133^{ns}**	
LSD (0.05)	**Isol.**	**9.78***		**0.139***		**0.036***		**0.138^{ns}**		**0.348^{ns}**		**0.441^{ns}**	
	CV (%)	**18.0**		**30.8**		**15.6**		**16.9**		**16.4**		**16.1**	

NOTE: *Means followed by the same letter in a column are not significantly different* (* - Significant at $p \leq 0.05$)

+N – Nitrogen positive control; **-N** Un-inoculated negative control; **NNO** – Nodule number per plant; **NFW** – Nodule fresh weight; **NDW** – Nodule dry weight; **SDW** – shoot dry weight; **RDW** – Root dry weight; **TBM** – Total biomass; ***ns*** – not significant; **Var** – Variety; **Isol**. – Isolate.

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Table 4. Symbiotic status of 16 test rhizobial isolates, two reference strains and +N and –N controls on two soybean varieties (SB19 and Safari) in glasshouse pot experiments

Treatment	NNO		NFW (g)		NDW (g)		SDW (g)		RDW (g)		TBM (g)	
	Variety		Variety		Variety		Variety		Variety		Variety	
	SB19	SAF	SB19	SAF	SB19	SAF	SB19	SAF	SB19	SAF	SB19	SAF
+N	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	1.159^{a}	1.434^{a}	0.305	0.271	1.463^{a}	1.704^{a}
- N	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	0.281^{e}	0.216^{e}	0.258	0.189	0.439^{e}	0.405^{e}
USDA 110	28.5^{a}	30.0^{a}	0.399^{a}	0.314^{a}	0.083^{a}	0.100^{a}	0.855^{b}	1.042^{b}	0.203	0.207	1.142^{b}	1.349^{b}
KFR259	13.5^{c}	17.0^{b}	0.286^{bc}	0.268^{b}	0.086^{a}	0.058^{bc}	1.007^{a}	0.664^{d}	0.218	0.223	1.311^{a}	0.945^{cd}
SoyKis3	25.5^{a}	20.5^{b}	0.322^{b}	0.296^{ab}	0.066^{abc}	0.077^{a}	0.790^{bc}	0.890^{bc}	0.185	0.161	1.040^{bc}	1.128^{b}
SoyKar2	25.3^{a}	20.0^{b}	0.310^{b}	0.169^{c}	0.076^{ab}	0.043^{c}	0.752^{bc}	0.618^{d}	0.169	0.154	0.997^{bc}	0.815^{d}
SoyKis4	19.3^{b}	8.5^{d}	0.296^{bc}	0.131^{c}	0.056^{bc}	0.031^{c}	0.675^{c}	0.808^{c}	0.142	0.228	0.874^{cd}	1.067^{c}
SoyKis2	18.0^{bc}	12.3^{c}	0.339^{a}	0.229^{b}	0.078^{ab}	0.058^{bc}	0.729^{bc}	0.880^{bc}	0.174	0.155	0.981^{bc}	1.093^{bc}
SoyKar1	15.5^{bc}	15.5^{bc}	0.350^{a}	0.350^{a}	0.075^{ab}	0.085^{a}	0.757^{bc}	0.997^{b}	0.177	0.164	1.008^{bc}	1.245^{b}
SoyKis1	13.0^{c}	11.0^{cd}	0.233^{c}	0.287^{ab}	0.051^{bc}	0.086^{a}	0.576^{cd}	1.007^{b}	0.217	0.218	0.845^{cd}	1.311^{b}
SoyKar3	16.5^{bc}	0.0^{e}	0.243^{c}	0.000^{d}	0.016^{d}	0.000^{d}	0.657^{cd}	0.790^{c}	0.215	0.185	0.988^{bc}	0.975^{cd}
SoyKar4	13.8^{c}	0.0^{e}	0.208^{c}	0.000^{d}	0.047^{c}	0.000^{d}	0.557^{cd}	0.808^{c}	0.194	0.228	0.799^{cd}	1.036^{c}
SoyKbay1	0.5^{e}	0.0^{e}	0.005^{e}	0.000^{d}	0.001^{d}	0.000^{d}	0.545^{cd}	0.602^{d}	0.181	0.158	0.927^{c}	0.760^{d}
SoyKbay2	2.5^{e}	0.0^{e}	0.031^{e}	0.000^{d}	0.005^{d}	0.000^{d}	0.575^{cd}	0.763^{c}	0.301	0.162	0.881^{cd}	0.925^{c}
SoyKbay4	1.3^{e}	0.0^{e}	0.005^{e}	0.000^{d}	0.002^{d}	0.000^{d}	0.489d	0.635^{d}	0.177	0.199	0.666^{cde}	0.834^{cd}
Soysp1	6.3^{d}	0.0^{e}	0.141^{d}	0.000^{d}	0.027^{cd}	0.000^{d}	0.563^{cd}	0.689^{cd}	0.198	0.175	0.788^{cd}	0.864^{cd}
SoyKbay3	1.5^{e}	0.0^{e}	0.010^{e}	0.000^{d}	0.002^{d}	0.000^{d}	0.600^{cd}	0.649^{d}	0.232	0.147	0.833^{cd}	0.796^{d}
Soysp2	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	0.521^{cd}	0.671^{d}	0.162	0.159	0.683^{cd}	0.830^{cd}
Soysp3	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	0.421^{de}	0.647^{d}	0.205	0.100	0.626^{de}	0.747^{d}
Soysp4	0.0^{e}	0.0^{e}	0.000^{e}	0.000^{d}	0.000^{d}	0.000^{d}	0.563^{cd}	0.772^{cd}	0.288	0.191	0.851^{cd}	0.963^{c}
SED	**0.737**		**0.044**		**0.004**		**0.113**		**0.048**		**0.032**	
LSD(0.05) Var	**1.458^{ns}**		**0.021^{ns}**		**0.007^{ns}**		**0.056^{**}**		**0.021^{ns}**		**0.064^{ns}**	
Isol.	**4.61***		**0.065***		**0.023***		**0.178***		**0.068^{ns}**		**0.266***	
CV (%)	**49.5**		**48. 1**		**66.6**		**23.1**		**34.2**		**20.2**	

NOTE: *Means followed by the same letter in a column are not significantly different* (* - Significant at $p \leq 0.05$)

KEY:+N – Nitrogen positive control; **-N** Un-inoculated negative control; **NNO** – Nodule number per plant; **NFW** – Nodule fresh weight; **NDW** – Nodule dry weight; **SDW** – shoot dry weight; RDW – Root dry weight; **TBM** – Total biomass; ***ns*** – not significant; **Var** – Variety; **Isol**. – Isolate.

IV. DISCUSSION

Inoculation of Bambara Groundnuts with Selected Rhizobial Isolates in the Greenhouse

Glasshouse inoculation of bambara groundnut plants resulted in significant variations in some plant growth parameters particularly in increased nodule formation. All treatments inoculated with *Bradyrhizobium spp*. isolates produced highly effective nodules with intensely pink internal nodule colours, with some strains (BAMKis12 and BAMKis4) outperforming *Bradyrhizobium japonicum* strain USDA110 in accumulation of plant biomass values. These findings imply a higher rate of symbiotic efficiency and N-fixation of strains BAMKis12 and BAMKis4 than the commercial strains indicating their elitist status as prospective sources of inoculants for local use as bio fertilizers.

Another remarkable outcome of this study was the occurrence of two *Burkholderia* spp. strains (BAMKbay8 and BAMsp3) as N-fixing associates of bambara groundnuts which was confirmed by highly effective and above 20 nodules per plant in the glasshouse experiments. Isolate BAMKbay8 outperformed the commercial strain USDA110 and nearly equalled the reference strain KFR259 in symbiotic performance. The N-fixation ability of some members of the genus *Burkholderia* was first proposed by Moulin *et al*. [17], confirmed by Chen *et al*.[18] and later symbiotic strains were distinctly established [19].

From the findings of Vandamme *et al*. [20], the Cape Fynbos in South Africa was listed as a major diversity centre of the N-fixing *Burkholderia* strains. The 16S rRNA gene phylogeny of the two isolates (BAMKbay8 and BAMsp3) from this study depicted their close relations to *Burkholderia tuberum* strain DUS833 and *B. phymatum* strain JVNU IL24 with the former having been isolated from the root nodules of *Aspalathus callosa* in South Africa. According to Ngugi *et al*. [21] bambara groundnuts landraces cultivated in Kenya are of West and South African descent and were introduced into East African farming systems through Uganda and Tanzania during the migration of Bantus, or later on during inter-border trading between the East African countries. It is therefore plausible to argue that the N-fixing *Burkholderia* strains found in this study may have been introduced together with the bambara groundnut germplasm during this period. Furthermore, the two isolates occurred in the soils of Port Victoria and Kendu bay which were active inter-border trading points through Lake Victoria. The absence of *Burkholderia* spp. isolates from Kisumu and Karungu soils may be due to its preference to less acidic soils.

Results of inoculation with isolates identified as *Agrobacterium* spp. resulted in one isolate (BAMKbay1) which produced effective nodules. This was unexpected since the N-fixation ability of this genus is still disputed as this process is mediated by *nif* and *nod* genes which are supposedly absent in this group [22]. Thus, the effective nodules produced by BAMKbay1 may have been as a result of acquisition of *nif* genes through lateral tsransfer of the *Sym* plasmid from other groups which possess the *nif* genes naturally [23]. Although *Agrobacterium* is known to be an effective endophytic inducer of tumors in plant roots [24], it is possible that the tumor inducing *Ti* plasmid responsible for the pathogenic structures were lost after it gained the genes for N_2-fixation. Indeed, the isolate may have acquired both *nif* and *nod* genes (within the *Sym* plasmid) which enabled it to produce the high number of nodules recorded. On the other hand, inoculation with isolate BAMKar9 also genetically identified as *Agrobacterium spp*. produced enlarged root tissues which appeared swollen that resulted in the higher root dry weight values than those obtained from plants under +N control treatment. Possibly, isolate BAMKar9 may have retained the tumor inducing *Ti* plasmid which resulted in larger roots that may have contributed to the observed difference in root biomass.

Some of the most promising strains for inoculation of bambara groundnuts established in this study were; BAMKis12, BAMKis8, BAMKis4 and BAMKar3 (*Bradryrhizobium spp*.) and BAMKbay8 and BAMsp3 (*Burkholderia spp*) which produced highly effective nodules and high plant biomass values. These strains have potential use as bio-fertilizers in the production of bambara groundnuts although they were relatively not well distributed in the four soils used for isolation unlike isolates identified as *Agrobacterium* and *Rhizobium* which occurred in all the soils. Possibly, stiff competition for infection and nodulation amongst different strains may have favoured the fast-growing genera (*Rhizobium* and *Agrobacterium*) which are known to have better rates of competition for nodule occupancy [25]. Thus, bambara groundnut is arguably more compatible with the genus *Bradyrhizobium* and *Burkholderia* but are seemingly out-competed for nodule occupancy in the local soils by the less/ineffective *Rhizobium* and *Agrobacterium* respectively. Conversely, since competitive ability and nodule occupancy is influenced by the prevailing status of the media [26]. The pH of the vermiculite used as the growing media was normalized to near neutral, this may have impacted negatively on some of the acid tolerant strains.

Although there were varying responses in nodulation of bambara groundnut plants due to inoculation with different isolates and the reference strains, it was surprising to find no significant difference in factors such as shoot and root dry weights. This may be attributed to the slow rate of N-nutrition of some bambara groundnut cultivars that which have previously been shown to convert only 33% of symbiotic N into the plant organic material [27].

Conversely, the period taken to harvest the test plants (45 days) for evaluation of dry weight may not have been sufficient for assimilation of fixed N that could result in significant difference in plant dry matter.

Inoculation of Soybeans with Selected Rhizobial Isolates

Results of glasshouse inoculation with 16 indigenous soybean rhizobia and the reference strains *Bradyrhizobium japonicum* USDA110 and *Bradyrhizobium spp*. KFR256 resulted in significant differences in all the growth attributes evaluated except in root dry weight. Variety 'Safari' was more selective forming nodules with only five of the isolates compared to SB19 which formed nodules with ten isolates. The best performing isolates were SoyKis3 and SoyKar2, genetically identified as *Bradyrhizobia* both comparing favorably with the reference strain *B. japonicum* strain USDA110, but outperforming *Bradyrhizobium spp.* strain KFR259. In terms of nodule formation, the two strains resulted in over 20 highly effective nodules for both SB19 and 'Safari', similar to the results obtained from strain USDA 110. According to Hirsch *et al*. [28], soybeans produce special isoflavonoids known as daidzein and genistein which are effective inducers of *B. japonicum nod* genes, but inhibit other rhizobial groups such as *S. meliloti nod* gene expression. This narrow host range was observed in this study as both SB19 and 'Safari' formed more nodules with indigenous *Bradyrhizobia* strains SoyKis3 and SoyKar2, as was previously observed by Broughton *et al*.[29].

Variety SB19 had a wider host range forming partially effective nodules with some non-*Bradyrhizobia* groups including SoyKis1 which was identified as *Rhizobium* sp. and SoyKar3 identified as *Mesorhizobium* spp. further confirming its promiscuous status. However, the symbiotic efficiency of the two strains (SoyKis1 and SoyKar3) was lower than that of strains identified as *Bradyrhizobia*. These findings indicate that SB19 may have a greater competition for nodule occupancy in cultivated soils with a wider range of rhizobial populations, which may negatively affect N-fixation. This challenge could be addressed by seed treatment of the promiscuous host with competent strains. This may provide a competitive advantage and result in higher nodulation [30].

V. CONCLUSIONS

Soybean Variety SB19 showed 'promiscuous' tendencies and formed effective nodules with rhizobia in the genera *Bradyrhizobium*, *Rhizobium* and *Agrobacterium* while 'Safari' was quite selective and formed very few nodules with isolates identified as *Bradyrhizobium*, which were mostly obtained from Kisumu soils. Both varieties SB19 and 'Safari' had better growth under glasshouse inoculation with isolates identified as *Bradyrhizobium spp*., although one *Rhizobium* isolate (SoyKis1) resulted in good nodulation of both varieties.

VI. RECOMMENDATIONS

Seed treatment of the two legumes with some isolates resulted in improved nodulation and better plant growth; in some instances, outperforming the commercial strain *Bradyrhizobium japonicum* USDA110. Isolates BAMKis12, BAMKis8, BAMKis4, BAMKbay8 and SoyKar2 are promising elite strains and are recommended for more host range tests as potential inoculants sources.

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Advanced Anaerobic Digestion and Associated Process for Zero Discharge Biowastes Treatment

V B Manilal*, B Chitrajit, R S Dipin Nath

Environmental Technology, CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram 695019, India
manilalvb@gmail.com

***Abstract*—*Anaerobic digestion is an attractive option for the treatment of organic wastes because of the net energy gain from the process to offset part of the operational cost. Invariably management of discharges from the anaerobic digester requires further processing and most of the known methods are either expensive or complex to practice. A complete solution to the problem of biowastes treatment is discussed in this paper through an improved anaerobic digestion process where no additional water is to be used in the operation, and the conversion of the digestate to solid manure. The tubular anaerobic digester employed in the study could remove nearly 90% of the volatile solids and produce biogas of around 550 litres/kg VS loading. The management of digester discharge was studied by following a method of evapouration drying by mixing with pre-dried discharged digester slurry to maintain combined moisture level below 60%. The average evapouration rate of moisture by this method was 211.6 ±16.6 g/kgTS/day under ambient conditions.*

Keywords*— *Anaerobic digestion, biowastes treatment, solid manure, tubular digester, evapouration, zero discharge.

I. INTRODUCTION

Anaerobic decomposition has been considered as a suitable method of wastes stabilization where generation of biogas is a valuable by-product. Currently it is applied widely not only to treat and dispose biowastes but for generating biogas fuel in bioenergy farms also (Wellinger, *et al*., 2013; Mao, *et al*., 2015; Scarlat, *et al*., 2018).

Generally the anaerobic digesters for bio-wastes and cattle manure treatment are operated by adding excess water to meet the design parameters (Mir, *et al*., 2016). This is done either by mixing freshwater or wastewater during grinding or smashing, and constituting to dilute slurries. Such preparations of slurry for the anaerobic digestion have certain immediate advantages because it could be handled like a liquid. Therefore such approaches demand the process design, treatment system design and operations similar to that in the anaerobic treatment of waste water. However, such cases of changing biowastes to dilute slurry prior to anaerobic treatment have serious disadvantages such as wasting of fresh water, converting of freshwater or less polluted water to high BOD wastewater, requirement to handle larger volume and thereby increases the digester volume, and the difficulty to manage increased volume of discharge.

There were some attempts to increase the solids content in the anaerobic digestion process by recycling the effluents and waste water (Smis, *et al*., 1995). The requirement of special facilities and their multi-step operations are turned out as major hurdles in such anaerobic digestion methods, especially when they are applied for smaller plants. Alternatively, dry anaerobic digestion of food wastes and other biowastes without water addition has also been reported where the process was affected due to the accumulation volatile fatty acids (Jansson, *et al*., 2019). Generally the microbial activity is expected to be affected seriously in the absence of minimal water medium because of the limitations for the microbes to access the substrates and nutrients in the system.

Most importantly in almost all anaerobic plants management of discharges from the process is a major problem that requires suitable methods of treatment to control further pollution (Møller, *et al*., 2009). Unless a suitable solution is developed the sustainability of anaerobic digestion becomes difficult despite of its merits including the production of biofuel during biowastes decomposition and treatment.

The present study is attempted to develop a complete solution to the problem of biowastes treatment through an improved anaerobic digestion process and augmented evapouration drying of the digested discharge without energy intensive operations.

II. MATERIALS AND METHODS

2.1. Advanced Anaerobic Tubular Digester

An anaerobic double tubular digester (ADTD) was designed based on the principle of plug flow slurry digestion developed previously for household biowastes treatment (Manilal, 2012).

2.1.1. ADTD Set up

The ADTD was fabricated in mild steel plate of 3 mm thickness and each tube had 1000 mm internal diameter and 2500 mm length. A centrally fixed shaft with spokes provided in each tubular digester was used to mix the content periodically with a geared motor assembly. Both the digesters were mounted on separate stands in parallel and had the ground clearance of 400 mm. At the end of one tube a feed port was fixed at the top for loading the biowastes. A connecting pipe of 200 mm diameter was fitted at the opposite side of the feed port to overflow the liquid from the first tube to the second tube as on feeding and filling the first tube. A schematic of the digester is shown in Fig. 1. Rusting of the tubular digester was prevented by coating epoxy paint outside and rubberised coating inside.

Biogas from the anaerobic digestion was collected in a 3 m^3 biogas balloon placed on a steel stand. Installation of the ADTD was done in the laboratory and leak was tested by filling water prior to the introduction of seeding material and commissioning of the plant.

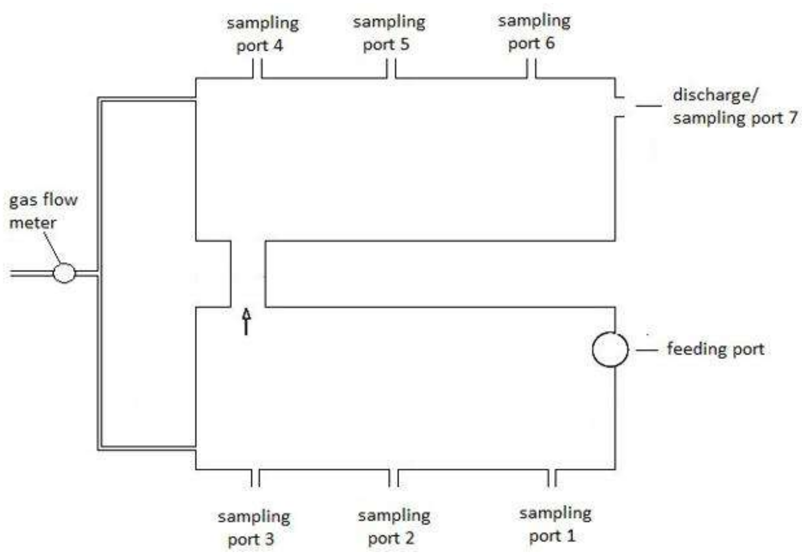

Fig. 1: Schematic diagram of anaerobic double tubular digester (ADTD)

2.1.2. Seeding Material and Biowastes

Initial seed for the ADTD was constituted by mixing anaerobic sludge from the laboratory biogas plant and cow dung slurry by adding water in the ratio 1:1. Total solids content of the seed was around 4.6 %.

Biowastes of the CSIR-NIIST canteen containing food wastes and the vegetable wastes of food preparation was the feed to the digester, which was collected, weighed and fed to the digester after grinding in a hammer mill every day except on Saturdays and Sundays. The ADTD was operated initially by feeding 5 kg biowastes on weekly basis for two weeks and changed to alternate days thereafter. The biowastes feeding of 5 kg was continued on daily basis from fourth week and the feeding rate was then increased to 10 kg/day from second month. Biowastes feeding was stepped up gradually by monitoring the volatile fatty acids in the ADTD and reached to the maximum of 50 kg/day from fourth month. At the experimental period the organic loading rate (OLR) of the reactor was 1.97±0.6 kgVS/m^3/day and 2.78±0.9 kg COD/m^3/day, at a hydraulic retention time (HRT) of 150 days. The digester was operated at ambient average temperature of 33 ºC.

2.1.3. Analysis of Biowastes

Samples were collected from all ports on a regular basis and measured the temperature and pH using a systronic pH system 362, and VFA and alkalinity were estimated by following the method of Anderson and Yang (1992). Analyses were also done for COD, total solids, total dissolved solids and total suspended solids as per the standard methods (APHA 1988). The COD and VS removal efficiency was derived using the formula described by Mohan and Bindu (2008). Average results of the duplicates/triplicates were accounted for each parameter on wet weight basis of the biowastes.

2.2. Slurry Drying

2.2.1. Preparation of Absorbent

The discharged digestate slurry from the ADTD was dried in sun light by spreading thinly in a metallic tray. During drying sticking and hardening of the material was prevented through periodical mixing prior to drying completely. The dried material prepared from the slurry was further shredded to breakdown the aggregates and used as the absorbent which had around 25% moisture.

2.2.2. Drying Bed

An absorbent bed of 50 mm thickness was prepared from the dried digestate. During drying fresh digestate of the ADTD was mixed with the absorbent bed in the ratio of 4:1 roughly so as to have maximum moisture not more than 60% in the mixture. The mixture was kept for evapouration drying under a roofed platform at the ambient temperature of around 33ºC. The moisture content was measured every day before and after fresh addition of digestate. Using the absorbent bed the evaporation drying was repeated for 17 days and the data was collected.

III. RESULTS AND DISCUSSION

Two important tasks were involved in this study of treating biowastes and recovery of by-products without environmental pollution; the first effort was designing and testing of a suitable anaerobic digester to function free of added moisture, and the second part was the energy free removal of moisture from the anaerobic digestate (treated slurry of the anaerobic digester) to produce solid manure. The experimental ADTD was fed with the ground biowastes without adding extra water and operated at the moisture content available in the biowastes.

3.1. Feed Character

The total solids (TS) of the biowastes were varied from 172 to 235 g/kg wet weight with an average of 202 g/kg and the volatile solids (VS) were in the range of 152 to 216 g/kg and an average of 181g/kg of wet sample. The chemical oxygen demand (COD) of the waste samples was ranged from 220 to 337 g/kg having an average value of 255 g COD/kg wet sample. The biowastes had the carbon nitrogen ratio of around 17. The analyses results of TS, VS and COD of the biowastes fed to the ADTD are presented in the Fig. 2.

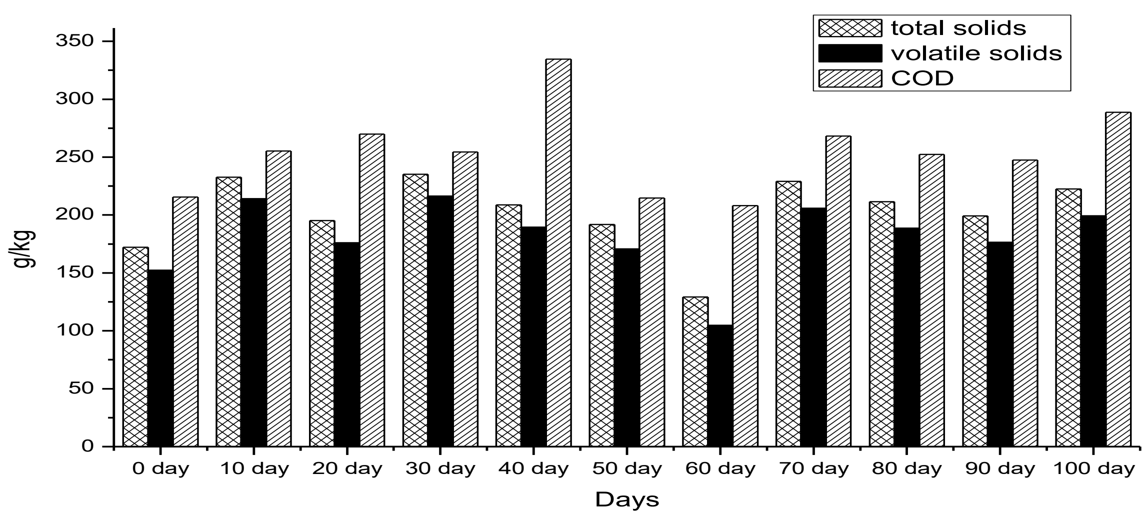

Fig. 2: Total solids, volatile solid and COD of the biowastes feed administered to the ADTD

3.2. Biogas production

The online reading of biogas production recorded every day by using a wet gas flow meter is presented in Fig. 3. The pattern of daily biogas production was more or less similar except on unfed days in the weekends. Biogas generation was almost instantaneous on feeding and an average production was more than 100 liters per kilogram of fresh biowastes. The average biogas production was nearly

550litres /kg VS loading which was higher than the earlier reports with the anaerobic digestion of similar biowastes (Zhang, *et al*.,2007; Ragazzia, *et al*., 2017).

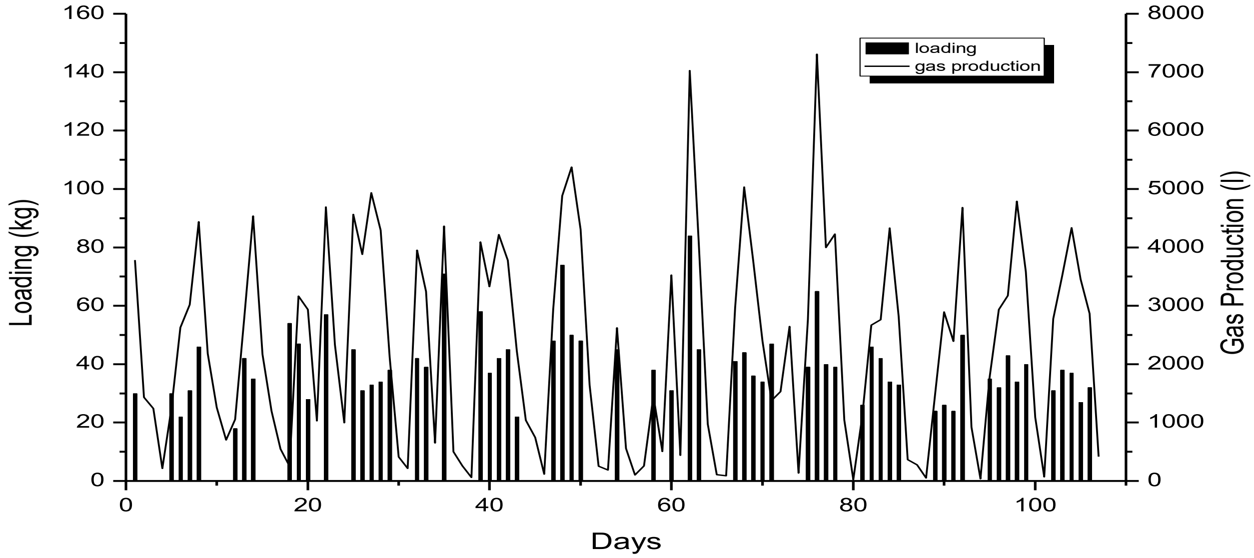

Fig. 3: Biowastes loading and biogas generation in the ADTD

3.3. Performance of the ADTD

The anaerobic digestion of the wastes was assessed by analyzing samples collected from 1 to 6 sample ports and from the final digestate discharge port 7 of the ADTD. The pH at the port 1 of ADTD was maintained above 6.8 automatically and therefore no additional buffering was required for the smooth operation of digester. While passing through every section of ADTD there was an increase of the pH further as evidenced by the analysis of samples drawn from 2nd port and beyond, and it was around 8.0 in the final discharge (Fig. 4*a*). This is an indication of stable anaerobic digestion ideally suitable for the biodegradation process (Buswell, et al.,1952, Liu, et al., 2008, Anthony, et al., 2019). The results of VFA analysis show variations at different sections of the ADTD as the anaerobic degradation and movement of the wastes proceeded from the feeding point to the discharge port (Fig. 4*b*). The material heterogeneity in the biowastes is expected to result varied pattern of biodegradation in the system and such patterns have been reported in the earlier studies on anaerobic digestion (Mohanan and Bindu, 2008).

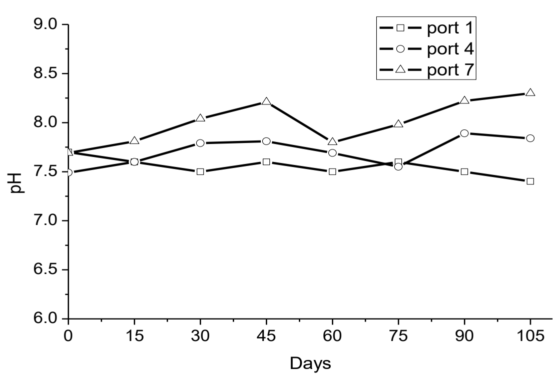

Fig.4a: pH measurements in different sample ports of ADTD during operation

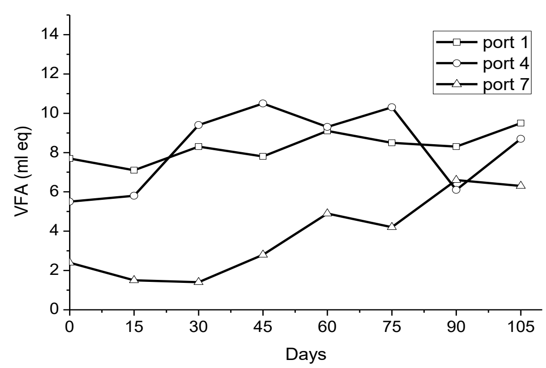

Fig.4b: VFA recorded in different sample ports of ADTD during operation

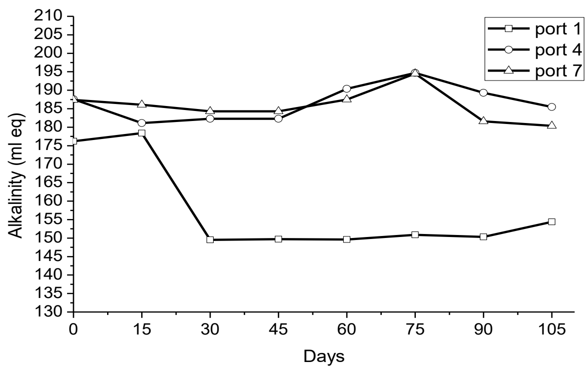

Fig.4c: Alkalinity readings in different sample ports of ADTD during the treatment period

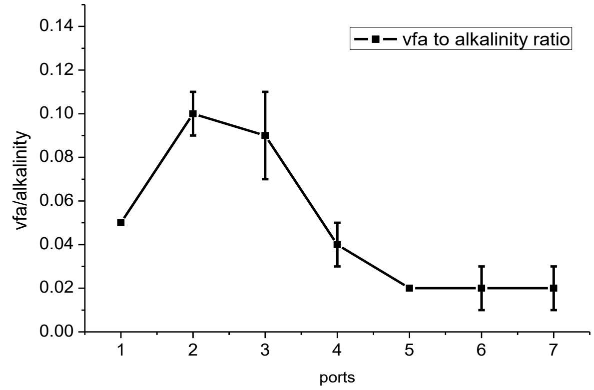

Fig.4d: VFA to alkalinity ratio calculated from the values measured at different sample ports of ADTD during the treatment period

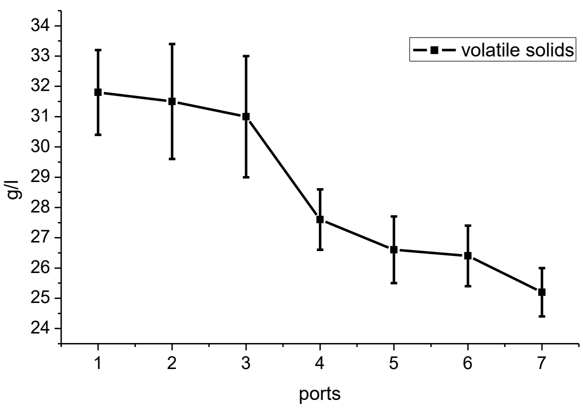

Fig. 4e: VS contents in different sample ports of ADTD during the treatment period

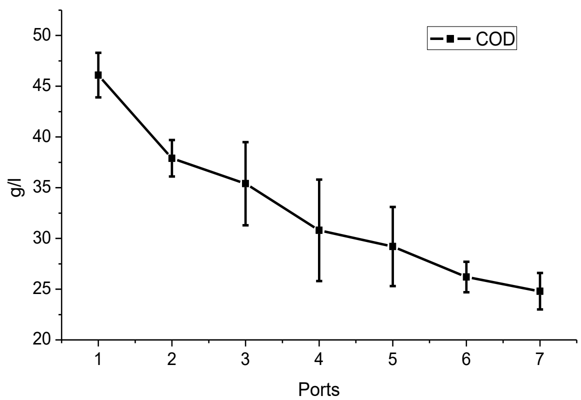

Fig. 4f: COD measurements in different sample ports of ADTD during the treatment period

Fig. 4a-f: Results of sample analysis collected from different sections of the ADTD on feeding biowastes

The build up of alkalinity in ADTD was measured and presented in Fig.4*c* for assessing the stability of anaerobic function at different sections. The ratio between VFA and alkalinity is an indicator of the digester performance, which was found desirable below 0.4 (Callaghan et al., 2002), and in ADTD the values were maintained constantly within that range (Fig.4*d*).

The reduction of volatile solids was more than 85% by the anaerobic digestion of biowastes in ADTD. The results were consistent throughout the digestion period and the pattern of VS removal is presented in Fig. 4*e*, which indicates the stable performance of ADTD.

COD removal was significantly high in the ADTD, and it was accounted to about 90%. The section wise results of COD removal obtained from sampling ports are presented in Fig.4*f* and it shows the effective degradation and treatment of biowastes in the ADTD. Because of the effective treatment of the biowastes the digestate from the anaerobic digester had low COD and volatile solids. The stabilization of wastes by the process thus demonstrates the possible use of the digestate directly for soil application safely.

3.4. Drying of digestate

Conversion of slurry discharges from the anaerobic digester to solid manure is the most desirable value recovery process for the sustainable operation and pollution free management of the process. However, the direct air drying of the digester slurry is extremely slow and difficult because of the limited contact surface of moisture evapouration as it has been reported for sewage sludge (Flaga, 2005). Results of this

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study demonstrated the removal of moisture from the digestate at the rate of 211.6 ±16.6 g/kgTS/day approximately (Fig. 5*a*). As a result solids were accumulated at a constant rate, which further enhanced the quantity of the absorption bed and the moisture absorption rate from the digestate to expedite the drying process (Fig. *5b*).

Restricting the total moisture to less than 60% in every addition of fresh digestate was favourable for easy passage of air as it has been reported as optimum in aerobic composting of biological materials (Diaz, et al., 2002). The usual limitation of slurry drying with respect to mass transfer could be improved in this method by extending the contact surface of moisture evapouration.

Atmospheric temperature and relative humidity are the governing factors that influence the rate of evapouration and drying at ambient conditions. However such limitations have little importance in this method as there is no costly energy input involved in the process.

This process of drying has several advantages which include; 1) solids are recovered from the digestate without energy intensive methods, 2) enables complete stabilization possible by aeration during the process, 3) produces organic manure without nutrients loss, 4) easy to handle the liquid discharge, 5) manure production from the treatment process, and thus the anaerobic treatment becomes more attractive.

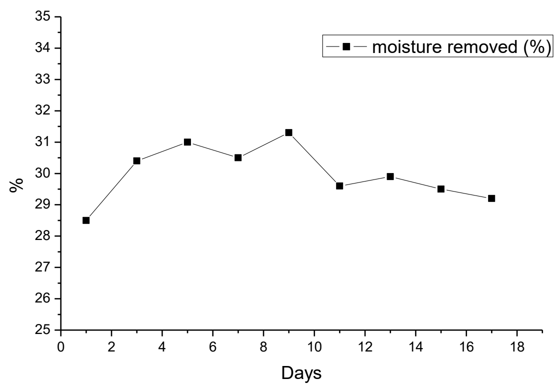

Fig. 5a: Moisture removed from the bed material during drying

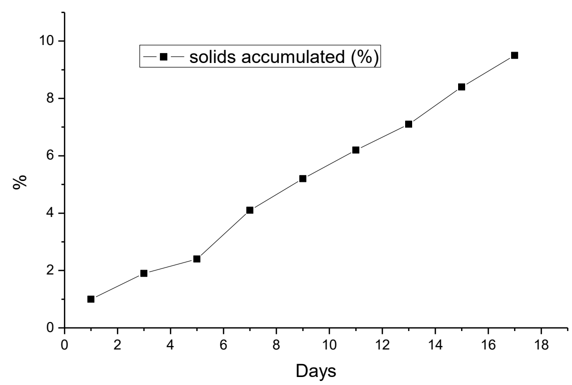

Fig. 5b: Solids accumulated in the bed during drying

Fig. 5a-b: Results of the evaporation drying with the extended contact surface using absorption bed.

IV. CONCLUSION

Biowastes treatment in the advanced anaerobic digester was found effective in removing the volatile solids to 85%, COD content to 90% and stable production of biogas to the tune of 550litrs/kg/VS. The performance of anaerobic double tubular digester system was better than many other existing models for biowastes treatment particularly for reducing water use and discharge volume, and concentrating solids in the discharge. This method to obtain higher concentration of solids in the discharge could reduce the problems of digestate drying and management.

The drying of anaerobic digestate by mixing with pre-dried absorbent bed and extending contact surface of evapouration was found appropriate, and it could demonstrate the repeated absorption and evapouration of water from the material to generate solid manure. The rate of evapouration drying is controlled mainly by humidity and temperature of the site of operation but has less significance on operation cost as the method could be applied without energy input.

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Introducing the genetic improvement of olive trees in Morocco by crossbreeding

EL Oualkadi A[1], Hajjaj B[1], El Hereich A[2], Sedra My H[2]

[1]INRA- Regional Agricultural Research Center of Tangier, Morocco
[2] INRA –Regional Agricultural Research Center of Marrakesh, Morocco
Corresponding author: ai.oualkadi@gmail.com

***Abstract*— The genetic improvement of the olive tree by crossing is a new work in Morocco and even in the olive countries of the Mediterranean. The objective of this work is to improve the performance of our selected Moroccan variety (Haouzia) by crossbreeding with European variety (Frontoio) with high-performance traits relating to the quality, quantity of fruit, oil and resistance to verticilliose. The result show Rate of fruit set is 63% as well as the fruiting rate of 50%.*

***Keywords*— Olive tree, Haouzia, Frontoio, genetic improvement, Morocco.**

I. INTRODUCTION

Olive tree is the main fruit species in most countries of the Mediterranean basin, both in terms of the number of trees grown and socioeconomic importance of its cultivation and its environmental role. In Morocco, this crop occupies a prominent place in the economic and social life of rural population. In Fact, olive tree plays an important role in helping to support most people in certain regions of the country where it is a main source of income. Genetic improvement of the olive tree by crossing is an essential way for the completion of a new and efficient genetic material. It is an alternative that preserves certain intrinsic traits to cultivars that have already been proven to adapt to environmental conditions or have intrinsic qualities that should be retained or to improve. In cultivated olive trees, knowledge of the genes involved in the variability of agronomic traits should make the best use of this variability for the selection of new varieties in response to new societal demands and environmental change. Disease resistance is one of the most important aspects currently studied. The olive tree is attacked by several fungal diseases such as the peacock's eye (*Cycloconium oleaginum*), rotting roots and wood (*Armillariella mellea*)...etc. However, the most important fungal disease is the Verticillium wilt, which can lead to the death of trees, and thus a reduction in production. This disease is found in several Mediterranean countries [1]. In Morocco, the disease was first noted in Meknes region [2]. Since then, it spread intensively in Morocco and is now widespread in the main growth area of the olive tree [3]. Control of *Verticillium dahliae* is difficult due to the absence of host specificity and extreme variability of the pathogen. Variety resistance offers the most effective and cost-effective ways to reduce the impact of the disease. Despite the economic importance of this plant, the genetic determinism of traits of agronomic interest is very poorly known. Unlike other species such as the genus *Vitis*, where only a few genomic regions involved in phenotypic variability have been mapped for major genes [4] and QTLs [5]. Seed germinability strongly depends on the parents used for crossing: germination rates can be very low for some breeding combinations [6]; [7]. There have been few crossbreeding programs for olive in different areas. Some of them are recent, while others have already reached their objectives, resulting in the release of new olive cultivars. Recently, resistance to pests and diseases, tree vigour and growth and grafting compatibility were considered as selective criteria [8]; [9]; [10], associated to indexing for viral infection [11]. Regional Agricultural Research Center of Marrakech has a wide range of genetic resources, including the global olive collection and the controlled croaking genotypes, as well as the molecular characterization of these genetics resource are necessary in order to select high-performance genotypes. The search for QTL related to the resistance to Verticiliosis will allow assistance in the selection of high-performance genotypes for these characters. In this work we crossed two varieties "Haouiza and Frontoio" for the search for QTL related to the resistance to Verticillium wilt which will allow assistance in the selection of high-performance genotypes for these traits.

II. MATERIAL AND METHODS

Vegetal material

We used too varieties of olive: Haouzia variety which is a new variety selected by INRA Marrakech productive and not altering, early maturity, average vigor, the tree has a rate of 50% -60 % perfect flowers, its olive production is 60Kg per tree and sensitive to Verticillium wilt. Frontoio variety which it is of Italian origin enters in production early and its high productivity improves in the presence of appropriate pollinator, its oil yield between 20-22%, high quality oil organoleptics, its production is 11.28Kg per tree, more or less self-fertile, good resistance to cold and drought and tolerance to *Verticillium dahliae.*

Crossover and technical plan

The cross is made between "Frontoio" and "Haouzia" whose "frontoio" is the female variety and the other is the male in 2013 at laboratory of genetic improvement of plant, Regional Agricultural Research Center of Marrakesh, Morocco. On the trees of the variety "Haouzia" chosen as pollinators, is carried out the bagging of twigs in order to collect pollen intended for the pollination of "Frontoio". To avoid any external pollination, bags previously put on the trees of "Frontoio" before opening the white button stage flowers. If there are flowers already open, unexploded anthers (castration) are eliminated, while those that are not dehiscent the entire flower is eliminated, with a daily passage for castration. After the anthesis of the pollen bags of the variety "Haouzia", bags are removed and their contents are sifted to rid pollen of unwanted parts (petals, sepals...) [12]; [13]. Finally, directed pollination is done using a brush.

Rate of fruit set and fruiting rate

After fecundation, ovaries begin to develop to young fruits that will continue their growth until the mature fruit is formed, these are harvested manually and then collected in bags. Afterwards, they are pitted at the end of obtaining nuclei and then they are rinsed and dried in the shade. After fertilization, flowers set are counted to calculate the rate of fruit set. Directly after fruit set, Urea 46% is sprayed every 15 days to promote fruit growth and reduce the percentage of falls. Rate of fruit set and Fruiting rate are calculated using the formulas: Rate of fruit set = [Number of flower set / Total number of Flower] x 100. Fruiting rate = [Number of fruit / Total number of flowers] x 100.

Genetic characterization of the descendants of the cross Haouzia and Frontoio by the molecular biology technique

Genomic DNA extraction

The extraction begins by grinding the freeze-dried leaves of each sample. It consists of drying the leaves, already wiped with cotton, cold by a freeze-drying system which must be set to a temperature that varies between -80 and -83˚C, and they are left three days before recovering them. Then, 40 mg of this grind is put in a 2 ml Eppendorf tube. The addition of the nuclear lyse solution to this grind allows the dispersal of lipid bilayers of membranes, and the denaturation of proteins, especially those associated with DNA. The mixture is incubated in water bath. The next step is to precipitate the proteins, which allows the isolation of the DNA by simple centrifugation. The addition of isopropanol to the isolated supernatant allows the DNA to be precipitated and collected in a nerve after centrifugation. The removal of traces of any contaminants that can influence the purity of DNA is ensured by ethanol. Finally, the DNA is solubilized by adding the rehydration solution.

Agarose gel electrophoresis 1%

To test the quality of the extracted DNA, an electrophoretic migration was performed on a 1% agarose gel (1g agarose in 100ml of TBE 1-1). The visualization of the gel is done through the addition of Ethedium Bromure (10mg/ml), which intersects between the nitrogen bases of the DNA strands and gives an orange-red inflorescence under ultraviolet radiation. After polymerization of the gel, 5l of DNA was mixed with 3l of agarose blue, then deposited in each well. To collect the size of the strips, the molecular marker (1 kb DNA Ladder) was deposited in another well. In an electrophoretic cell filled with TBE 1,000, the DNA migrates under a voltage of 160 Volts for 35 minutes.

III. RESULT AND DISCUSSION

Haouzia and Frantoio crossing

Obtained results from this cross are well representative, 577 of knotted flowers yielded 454 fruits. The Rate of fruit set is 63% as well as the fruiting rate of 50%. As of 10/11/2012, we harvested a total of 136 fruits that remained among the 454 fruits in July 2013. These fruits are then pitted and the olive stone are stored and dormant for a month. After one month, these olive stone will be germinated in petri dishes. Once sprouted these seeds will be transplanted into plastic bags and will be put in environmental conditions in the laboratory until the first leaves are developed. The rate of fruit set and fruiting rate we obtained for this crossing are shown in table 1.

Table 1: number of flowers, the rate of fruit set and fruiting rate

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Number of Flower bagged	Number of Flower set	Number of fruit	Rate of fruit set (%)	Rate of fruiting (%)
905	577	454	63	50

Genomic DNA extraction

We carried out genomic extraction using the CTAB extraction technique, to show the quality of our DNA extracted at the level of a 1% agarose gel. Thus DNA was quantified using the spectrophotometer (Eppendorf Biophotometer).

Checking the quality of DNA extracted

The quality verification of genomic DNA extracted from the various samples studied was carried out on 1% agarose gel. Fig. 4, fig.5 and fig.6 show results of extraction.

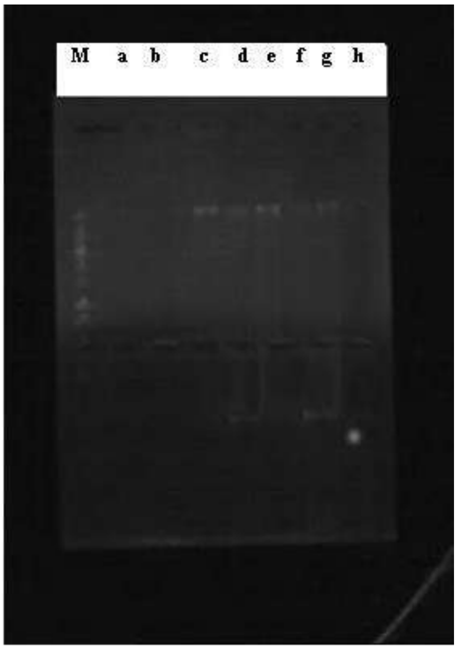

Fig. 4: Agarose gel 1% colored by Bromure Ethidium (plate 1)

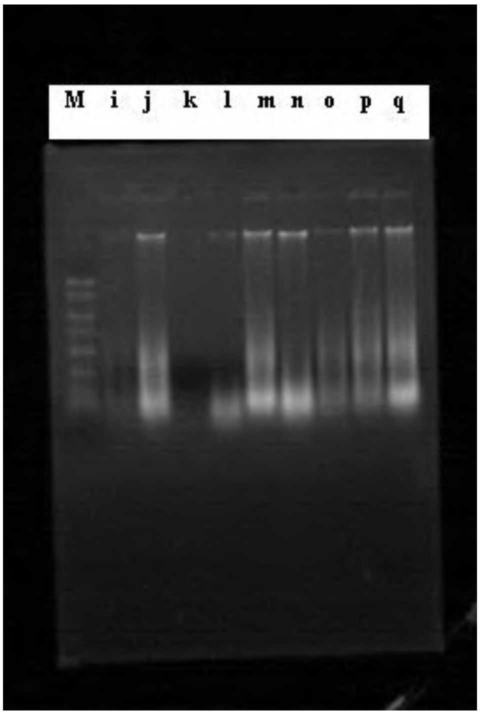

Fig. 5: Agarose gel 1% colored by Bromure Ethidium (plate 2)

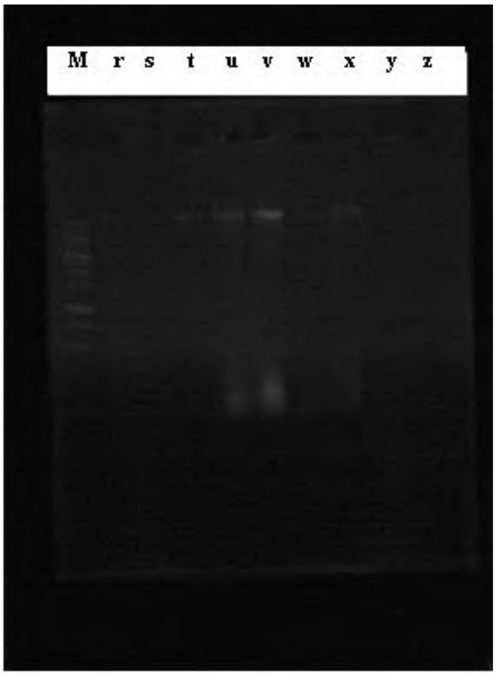

Fig. 6: Agarose gel 1% colored by Bromure Ethidium (plate 3)

Table 2: Intensity of DNA bands visualized on agarose gel 1% depending on the varieties

Plaque 1 :

Variety	a	b	c	D	E	f	g	H
Band Intensity	-	-	+++	+++	+++	+	++	-

Plaque 2 :

Variety	i	J	k	L	m	N	o	p	Q
Band Intensity	+	+++	-	++	+++	+++	++	+++	+++

Plaque 3 :

Variety	r	S	t	U	v	W	x	y	Z
Band Intensity	-	-	+	++	+++	-	+	-	-

The 1% agarose gel migration achieved to qualify and quantify the DNA obtained from the extraction of olive leaves by the method (CTAB), showed clearly visible bands on agarose gel in most of the individuals studied, indicating that our DNA extract is pretty good quality to good with little drag that shows there is no DNA degradation.

Checking the amount of DNA extracted

After measuring the optical density of the different olive genotypes at 260 and 280 nm, results are shown in Table 3. Result show that the ratio between Do at 260nm and Do at 280 nm of our extracted DNA solutions which varies between 2 and 0.33, this result allowed us to conclude that our DNA extracted are clean and of good quality and therefore good family. (Annex 4). From the amount of DNA extracted, DNA dilutions must be made for amplification reactions (PCR).

**Table 3: Measurements of the optical density of different olive tree genotypes*

Sample	DO to 260nm	DO to 280nm	Concentration in µg/ml	Report DO (260/280nm)
a	0.6	0.8	120	0.75
b	0.5	0.8	100	0.625
c	1.50	0.90	300	1.66
d	1.2	0.78	240	1.53
e	1.65	0.85	330	1.94
f	1	0.5	200	2
g	1.3	0.77	260	1.68
h	0.5	0.7	100	0.71
i	1.62	0.89	324	1.82
j	1.7	0.7	340	2.4
k	1.4	0.76	280	1.84
l	1.65	0.89	330	1.85
m	1.81	0.77	362	2.35
n	1.77	0.81	354	2.18
o	1.6	0.8	320	2
p	1.84	0.86	368	2.25
q	1.47	0.72	294	2.28
r	0.4	0.9	80	0.44
s	0.48	0.78	96	0.61
t	0.98	0.79	196	1.24
u	1.02	0.68	204	1.5
v	1.21	0.71	242	1.70
w	0.3	0.90	60	0.33
x	1.08	0.81	216	1.33
y	0.35	0.88	70	0.39
z	0.28	0.7	56	0.4

**Factor of dilution = 4*

IV. CONCLUSION

In conclusion, the obtained results show that the crossing with Frontoio and Haouizia generated 63% of Rate of fruit set. Stratification treatment and the female parent clearly influenced olive seed germination and seedling emergence. Early seedling growth is also affected by the female. An initial verification of the first descendants of this cross showed the good quality of the DNA of these descendants. Subsequent work continuing to crossbreed to enrich the list of descendants, and genetic analyses will then be carried out on these descendants for the search for molecular markers related to resistance to Verticillium wilt.

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Quality Improvement of Durian Waste and Tofu Waste Fermented with *Pleurotus ostreatus*

Victor Yaman Laoli[1], Nuraini[2], Mirzah[2]

[1]Department of Animal Science, Faculty of Animal Science, Andalas University, 25163, Indonesia
victorpwd@yahoo.co.id
[2]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, 25163, Indonesia

***Abstract*— *This study was conducted to determine the dose of inoculum and the duration of fermentation that is appropriate for the growth of Pleurotus ostreatus on a mixed substrate fermented of durian waste and tofu waste (DWTW) on crude fiber content, crude protein content and cellulase enzyme activity. This research was designed using Factorial Completely Randomized Design 3x3 with 2 replications. Factor A consisted of the dose of inoculum A1 (6%), A2 (8%), A3 (10%) and Factor B consisted of the duration of fermentation namely B1 (7 days), B2 (9 days), B3 (11 days). The variables observed were crude fiber content, crude protein content and cellulase enzyme activity. The results of the analysis showed that there was an interaction between the inoculum dose and the length of DWTW fermentation with Pleurotus ostreatus which had a significantly different effect (P <0.05) on cellulase enzyme activity, crude fiber content, and crude protein content. From the results of this study it can be concluded that the 8% inoculum dose and 9 day fermentation time are optimal and efficient conditions for increasing the quality of the mixture of durian fruit and fermented tofu waste with Pleurotus ostreatus. In this condition cellulase enzyme activity was 1.36 U / ml, crude fiber content was 14.69%, and crude protein content was 19.25%.*

***Keywords*— durian waste, fermented, Pleurotus ostreatus, tofu waste, quality.**

I. INTRODUCTION

The feed is a very important factor in determining the success of a livestock business, especially poultry farming. The availability of feed ingredients that are commonly used lately is increasingly difficult, due to the reduced land used for feed raw materials. The high cost of imported feed, such as corn, wheat, soybean meal, and fish meal is a significant problem faced by poultry farmers. Therefore, poultry feed must be diversified to maintain nutritional quality and reduce the use of imported feed ingredients, which in essence look for alternative feed sources that are easily available, low prices and have good nutritional value and do not compete with human needs.

Indonesia is a tropical country that is crossed by the equator so that it has a diversity of natural resources that can be used as food. One of the plants that have abundant byproducts is durian fruit (Durio zibethinus). Durian fruit production in Indonesia continues to increase every year. Indonesia has an area that stretches 5,000 km from 95 ° to 141 ° BT and has a varied agroecological zone (AEZ), combined with the distribution of durian plants in almost all regions of Indonesia will encourage the emergence of fruit in sequence. This situation provides the opportunity for a long harvest period supported by data in the field showing the average durian harvest period, in general, is around 8 months every year. Theoretically, if the durian's volume and distribution are evenly distributed along the 46 ° longitude, a uniform supply of durian will be obtained for most of the year. According to data from the Directorate General of Horticulture, Ministry of Agriculture in 2013, durian production in Indonesia was 759,055 tons and in 2017 it increased by 795,200 tons. The highest amount of production was in East Java Province, while West Sumatra was ranked 5th with a total production of 74,540 tons.

Durian processing will produce a lot of waste because the part that is generally consumed is fruit flesh which is around 20-25% and the remainder is the skin part 60-70% and the seeds 5-15% have not been maximally utilized (Untung, 2008). Durian skin contains essential oils, flavonoids, saponins, cellulose, lignin, and starch. According to Nuraini (2019), durian skin contains 4.25% crude protein, 29.50% crude fiber and 2,050 kcal/kg of metabolic energy. While the nutritional content of durian seeds according to Nuraini and Mahata (1998) is 9.79% crude protein, 2.41% crude fiber and 2,750 kcal/kg metabolic energy and durian seeds can be used up to the 24% level in broiler rations or can replace 42 % of corn.

The use of durian peel and seeds in poultry rations is limited by the high crude fiber content. The content of food substances durian waste (a mixture of 50% peel and 50% seeds), obtained crude protein that is 7.50%, 21.95% crude fiber and 2250 kcal/kg of metabolic energy (Guntoro, 2015). The high crude fiber in durian waste will affect the digestibility and absorption of food substances such as protein, vitamins, and minerals in poultry rations. Large amounts of crude fiber cannot be digested by poultry and are as a booster or bulky (Wahyu, 2004). Therefore, before it is given to poultry, durian waste needs to be processed to improve its nutritional quality. Methods that can be used to reduce high crude fiber and increase crude protein in durian waste can be done by the fermentation method.

Fermentation processing has the advantage of extending storage time, eliminating unpleasant odors, better nutritional value than its original ingredients, fermented food is easier to consume and increases digestibility, and increases flavor (Trisnadjaja and Subroto, 1996). The fermentation process is a process of chemical changes in an organic substrate through the activity of enzymes produced by microorganisms (Hidayanto, 2017). One way to reduce the content of crude fiber, especially cellulose and lignin, is to utilize microbial activity through a biodegradation process, where microbes can degrade fiber components more economically and the results can be more beneficial.

One source of microorganism that can increase protein and reduce crude fiber that has been done by previous researchers is the fungus *Pleurotus ostreatus* (oyster mushroom) which is lignocellulolytic because it can degrade cellulose and lignin which are components of crude fiber. *Pleurotus Ostreatus* is classified as white rot fungi from the Basidiomycetes group which can degrade lignin more extensively because it produces extracellular ligninolytic enzymes consisting of lignin peroxidase (LiP) manganese peroxidase (MnP) and laccase (Hatakka, 2001). Besides, the fungus *Pleurotus ostreatus* also produces amylase and cellulase enzymes (Sudiana and Rahmansyah, 2002) and protease enzymes (Shaba, 2012). According to Alarcon et al. (2003), the advantage of fermentation using the fungus *Pleurotus ostreatus* is that it can produce lovastatin compounds that can inhibit the formation of mevalonates, which ultimately inhibits the formation of cholesterol.

The presence of ligninase enzyme activity from *Pleurotus ostreatus* was reported by Badarina et al. (2013) that coffee waste fermented with *Pleurotus ostreatus* can increase crude protein content by 17.2% and reduce lignin by 31.12%. The substrate fermentation of a mixture of palm sludge and bran with a ratio (80: 20) incubated by *Pleurotus ostreatus* has been tried by Nuraini et al. (2017) can reduce crude fiber substrate by 41.10%, from 23.84% to 14.04%.

In the process of fermentation, pH, temperature, oxygen, and substrate composition are some of the factors that influence its success (Desroisier, 1998). Fermentation using *Pleurotus ostreatus* (oyster mushroom) requires a substrate that contains a source of carbon, nitrogen, and minerals to support the growth and development of mycelium. Durian peel and seeds can be used as a source of carbon (C) in the fermentation media but must be supplemented with a nitrogen source (N) to get a C: N balance suitable for the growth of *Pleurotus ostreatus*.

The source of nitrogen (N) that can be used is tofu waste. Tofu waste is an easily obtainable industrial waste, its availability is continuous and has good nutritional value, namely crude protein at 28.36%, fat 5.52%, crude fiber 7.06% and BETN 45.44% (Nuraini et al., 2012). High crude protein content in tofu waste can be used as a source of N for microbial growth. Mahfudz (2006) states that tofu waste contains amino acid lysine and methionine, as well as calcium which is quite high.

This study utilizes durian waste as a whole which consists of 70% durian waste (75% peel and 25% seeds) and 30% tofu waste obtained by crude protein 5.64%, crude fiber 22.73%, lignin 12.70 and cellulose 16.01% and metabolic energy 2,225 kcal/kg. To reduce crude fiber, fermentation using *Pleurotus ostreatus* was used in this research.

Nuraini (2006) states that the success of fermentation depends very much on the optimum conditions given, such as the composition of the substrate, thickness of the substrate, the inoculum and the duration of fermentation. According to Fardiaz (2005) the speed of fermentation greatly determines the number of enzymes produced, the longer the fermentation time used will be more and more ingredients are overhauled by enzymes, but with increasing time of fermentation the availability of nutrients in the media runs out so that the fungus eventually dies.

This study aims to determine other optimum conditions such as inoculum dosage and the correct fermentation time on a fermented mixture of 70% durian peel and seed fermentation and 30% tofu waste using *Pleurotus ostreatus* in reducing crude fiber (lignin and cellulose) and increasing protein content.

II. MATERIALS AND METHOD

2.1. Materials Research

The materials used in this study were 70% durian waste consisting of (75% peel and 25% seeds), 30% tofu waste and the fungus used as *Pleurotus ostreatus*. Durian waste is obtained from the fruit and processed fruit sales point at Iko Gantinyo Store, located in the Pondok City Region of Padang, West Sumatra Province. Tofu waste is obtained from the tofu factory in Rimba Datar area, Bandar Buat. *Pleurotus ostreatus* was obtained from LIPI, Cibinong, Bogor. Chemicals for proximate analysis and cellulase enzyme activity. The equipment used in this study is analytical scales, autoclaves, laminar airflow, ovens, a set of equipment for proximate analysis, cellulase enzyme activity.

2.2. Research Implementation

Activities in this research include fermentation of durian fruit waste mixture and tofu waste using *Pleurotus ostreatus* and the quality test of fermented products.

2.2.1. The preparation of the substrate

The processing of durian waste is carried out referring to Guntoro (2015) where the collected durian waste is cleaned of attached impurities, carried out washing or disposing of slime and remaining durian meat to get fresh durian seeds. After washing the durian seeds are chopped small with a maximum size of 0.5-1 cm. The durian waste used consists of 75% peels and 25% seeds, which is the overall utilization of waste from durian. Tofu waste that has been obtained, squeezed and placed in a container.

2.2.2. Fermentation of durian fruit waste and tofu pulp with *Pleurotus ostreatus*.

This experiment aims to obtain the best dosage of inoculum and fermentation time with *Pleurotus ostreatus* for the content and quality of nutrition of durian waste. A comparison of the composition of the substrate used is 70% durian waste and 30% tofu waste. The dose of inoculum used according to treatment was 6%, 8%, and 10% and the level of fermentation time was 7, 9 and 11 days. The steps of researching at this stage are as follows:

- Weigh the substrate with durian waste composition and tofu waste that is 70%: 30% as much as 200 gr.
- The substrate in a plastic bag added with 7 ml of mineral Brook et al., Stirred in a plastic bag until homogeneous.
- Then the substrate is sterilized using an autoclave (temperature 121ºC with 15 minutes), allow it to drop to room temperature, then remove and cool in a sterile room (laminar airflow).
- Sterile substrate is inoculated with *Pleurotus ostreatus* inoculum according to treatment (6, 8, 10% of the amount of substrate) in a sterile room (laminar airflow).
- Then incubated according to treatment (7, 9 and 11 days)

- After the fermentation process ends, the fermented product is then weighed in fresh weight, taken 10 g for determination of enzyme activity. The remainder is dried at 80ºC for 2 hours to kill the fungus, then continue drying at 60ºC for 10-12 hours, until dry. After that, stir evenly, ground, and taken samples for analysis.

2.3. Observed variables

2.3.1. Crude Protein (%)

Crude protein analysis is based on the Kehjdal method, AOAC (Association of Official Analytical Chemists, 1990).

2.3.2. Crude Fiber (%)

Crude fiber analysis is based on the AOAC method (Association of Official Analytical Chemists, 1990).

2.3.3. Cellulase (U / mL) Enzyme Activity

Measurement of cellulase enzyme activity based on the Nelson method (1944).

III. RESULT AND DISCUSSION

3.1. Effect of inoculum dose and duration of fermentation durian waste on cellulase enzyme activity (U / ml) from *Pleurotus ostreatus.*

The average activity of cellulase enzymes from fermented durian waste products and fermented tofu waste by *Pleurotus ostreatus* (DWTW) for each treatment can be seen in Table 1.

Table 1. Mean cellulase enzyme activity (U / ml) Pleurotus ostreatus based on different inoculum doses and fermentation time on DWTW.

Dosage inokulum	Fermentation time			Average
	B1 (7day)	B2 (9day)	B3 (11day)	
A1 (6%)	1,17 c	1,28 b	1,30 b	**1,25**
A2 (8%)	1,18 c	1,36 a	1,37 a	**1,30**
A3 (10%)	1,19 c	1,38 a	1,39 a	**1,32**
Average	**1,18**	**1,34**	**1,35**	

Table 1 shows the activity of cellulase enzymes from a mixture of durian waste and fermented tofu waste by *Pleurotus ostreatus* (DWTW) ranging from 1.17 to 1.39 U / ml.

Based on the variance test showed that there was an interaction that gave a significantly different effect (P <0.05) between factor A (inoculum dose) and factor B (fermentation time) on the cellulase enzyme activity of

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Pleurotus ostreatus on DWTW substrate. Likewise, the inoculum dose factor and fermentation time had a very significant effect (P <0.01) on the activity of cellulase enzymes.

DMRT test results showed that A2B2 treatment (8% inoculum dose and 9 days fermentation time), A2B3 (8% inoculum dose and 11 days fermentation time), A3B2 (10% inoculum dose and 9 days fermentation time) and A3B3 (10 % inoculum dose and 11 days fermentation time) had a significant effect (P <0.05) higher than other treatments.

The more inoculum doses (factor A) given into the substrate and the longer the fermentation (factor B) carried out is positively correlated to the high activity of cellulase enzymes produced, this is due to the more inoculum doses given in the substrate resulting in more *Pleurotus ostreatus* growing (fertile). This is supported by the opinion of Musnandar (2004) which states that the administration of doses with certain limits affects the increase in the number of microorganisms so that the substrate will be covered by mycelium, and result in increased activity of the enzyme.

The longer fermentation time affects the chance of mold to grow more optimally, this causes the activity of cellulase enzymes that occur higher. Setyawan (2005) states that fermentation is strongly influenced by the time used, the longer the time used by microbes to grow and develop properly, resulting in increased enzyme activity.

The high activity of cellulase enzymes in this treatment is caused by a combination of inoculum dosage and the right fermentation time so that molds grow better can remodel the substrate nutrition during fermentation. The substrate nutrition that is overhauled is cellulose, the more cellulose is regenerated into glucose as an energy source, the mold can produce cellulase enzymes with maximum activity. Following the opinion of Pujiati et al. (2014) states that the fermentation process with an appropriate dosage of inoculum and fermentation time can produce cellulase enzymes with maximum activity.

According to Santos et. al. (2012) states that cellulase enzymes are enzymes that can work synergistically remodel cellulose into glucose through a catalyst process. Furthermore, Murashima et al (2002) stated that cellulase enzymes consisted of 3 types of enzymes, namely endoglucanase (endo-1,4-β-D-glucanase), exoglucanase (ekso-1-4-D-glucanase) and cellobiose (β- D-glucosidase). These three enzymes work together to hydrolyze insoluble cellulose to be converted into glucose (Fikrinda, 2000).

The content of the best cellulase enzyme activity (which is efficient in terms of inoculum dosage and fermentation time) in this study is in the treatment of A2B2 (8% inoculum dose and 9 days fermentation time) which is 1.36 U / ml.

3.2. Effect of inoculum dose and duration of fermentation durian waste on crude fiber (%DM) from *Pleurotus ostreatus.*

The average crude fiber (%DM) from fermented durian waste products and fermented tofu waste by *Pleurotus ostreatus* (DWTW) for each treatment can be seen in Table 2.

Table 2. Mean crude fiber (%DM) Pleurotus ostreatus based on different inoculum doses and fermentation time on DWTW.

Dosage inokulum	Fermentation time			Average
	B1 (7day)	B2 (9day)	B3 (11day)	
A1 (6%)	18,45[a]	16,26[d]	15,99[d]	**16,90**
A2 (8%)	17,62[b]	14,69[e]	14,51[e]	**15,60**
A3 (10%)	17,28[c]	14,47[e]	14,35[e]	**15,36**
Average	**17,78**	**15,14**	**14,95**	

The results showed that the crude fiber content of DWTWF with *Pleurotus ostreatus* ranged from 14.35% to 18.45%. Based on the results of the variance test showed that there was an interaction that gave a significantly different effect (P <0.05) between factor A (inoculum dose) and factor B (fermentation time). Each factor, factor A (inoculum dose) and factor B (fermentation time) had a very significant effect (P <0.01) on the DWTW crude fiber content.

DMRT test results showed that A2B2 treatment (8% inoculum dose and 9 days fermentation time) had no significant effect (P> 0.05) with A2B3 treatment (8% inoculum dose and 11 days fermentation time), A3B2 (10% inoculum dose and 9 days fermentation time), A3B3 (10% inoculum dose and 11 days fermentation time), and significantly (P <0.05) lower than other treatments. The low content of DWTW crude fiber in A2B2, A2B3, A3B2, A3B3 treatments compared to other treatments due to cellulase enzyme activity in the 4 treatments was higher than other treatments. This is because the more doses are given and the longer the fermentation time is used resulting in the low fiber content of the substrate being low. The more doses of inoculum used in fermentation results in more ingredients that can be overhauled, to improve the nutritional content of a fermented product (Nurhaita et al, 2012). Furthermore, Musnandar (2004) states that the longer fermentation results in a longer chance of cellulase enzymes to be able to remodel crude fiber more optimally. Chesson (1993) also stated that the

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decrease in crude fiber was caused by the activity of extracellular enzymes produced by molds which caused the degradation of crude fiber cell wall components so that the crude fiber content decreased.

The longer fermentation time will cause the process of fungal metabolism to increase so that more energy is released by the fungus by degrading various energy sources contained in the fermentation durian waste extract, including crude fiber. Strengthening the results of this study Perez et. al., (2002) states that each microfungus has a different ability to decompose the substrate. The longer the incubation period, the more complex the compounds that are broken down by microbes into simpler compounds that can accumulate into energy.

In addition to producing cellulase enzymes that can break down cellulose to glucose (Belitz et al, 2008), *Pleurotus ostreatus* also produces extracellular ligninase enzymes consisting of Laccase, Manganese Peroxidase (MnP) and Lignin Peroxidase (LiP) which can remodel lignin (Hatakka, 1994).

In the treatment of A1B1, A1B2, A1B3, A2B1, and A3B1 the crude fiber content is still high. This is due to the small amount of inoculum used which is 6% and a short fermentation time which is 7 days. Short fermentation time causes a shorter chance of microbes in breaking down crude fiber components into simpler components (Fardiaz, 1989), in this case, less than the optimal activity of cellulose enzymes to remodel cellulose into glucose so that crude fiber is still high on the substrate.

The best crude fiber content (which is efficient in terms of inoculum dose and length of fermentation) in this study is in the treatment of A2B2 (DWTW with *Pleurotus ostreatus* at 8% inoculum dose and 9 days fermentation time) that is 14.69% (there was a decrease in crude fiber by 34, 44%).

3.3. Effect of inoculum dose and duration of fermentation durian waste on crude protein (%DM) from *Pleurotus ostreatus.*

The average crude protein (%DM) from fermented durian waste products and fermented tofu waste by *Pleurotus ostreatus* (DWTW) for each treatment can be seen in Table 2.

Table 2. Mean crude protein (%DM) Pleurotus ostreatus based on different inoculum doses and fermentation time on DWTW.

Dosage inokulum	Fermentation time			Average
	B1 (7day)	B2 (9day)	B3 (11day)	
A1 (6%)	$16,50^{c}$	$18,05^{b}$	$18,39^{b}$	**17,64**
A2 (8%)	$16,93^{c}$	$19,25^{a}$	$19,31^{a}$	**18,49**
A3 (10%)	$17,01^{c}$	$19,38^{a}$	$19,66^{a}$	**18,68**
Average	**16,81**	**18,89**	**19,12**	

Based on the results of the variance test showed that there were interactions and significantly different effects ($P < 0.05$) between factor A (inoculum dose) and factor B (fermentation time). Each factor, factor A (inoculum dose) and factor B (fermentation time) had a very significant effect ($P < 0.01$) on the DWTW crude protein content.

High crude protein content in A2B2 treatment (8% inoculum dose and 9 days fermentation time), A2B3 (8% inoculum dose and 11 days fermentation time), A3B2 (10% inoculum dose and 9 days fermentation time) and A3B3 (10 % inoculum dose and 11 days fermentation time) due to a large dose of inoculum and long fermentation time, causing microbial growth to increase and evenly distributed so that there is an opportunity for microbes to contribute a high enough protein, which causes crude protein to increase. The more inoculum doses used with optimum fermentation time, the more cell mass, so that the combination of optimum dosage of inoculum and fermentation time will increase the content and quality of food substances from fermentation products (Howard et al., 2003).

The increase in crude protein occurs because of the addition of protein donated by microbial cells due to growth that produces a single cell protein product (PST) or cell biomass that contains about 40-65% protein (Khrisna et al., 2005). *Pleurotus ostreatus* biomass which will contribute a lot of high crude protein in fermented products. Increased protein associated with additional protein from microbial cells that increase during the fermentation process (Bintang et al, 2009).The increase in crude protein is also caused by the presence of enzymes produced in the inoculum. The increasing dose of inoculum with long fermentation time, the increasing enzyme produced by *Pleurotus ostreatus* in the product so that the crude protein increases because the enzymes produced by microbes are also proteins (Noferdiman et. Al., 2008).

The low crude protein content in the treatment of A1B1, A1B2, A1B3, A2B1, and A3B1 is due to the inoculum dose given and the fermentation time is too short so that *Pleurotus ostreatus* grows little and the contribution of *Pleurotus Ostreatus* microbial body protein is small. The growth of molds that are infertile and uneven compared with A2B2, A2B3, A3B2 and A3B3 treatments is characterized by the low activity of cellulase enzymes acting on these treatments.

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The best crude protein content (which is efficient in terms of inoculum dose and length of fermentation) in this study is in the treatment of A2B2 (DWTW with *Pleurotus ostreatus* at 8% inoculum dose and 9 days fermentation time) is 19.25% (an increase in crude protein by 39, 12%).

IV. CONCLUSION

In this study, the best results were obtained on a mixture of durian fruit waste and tofu waste pulp with *Pleurotus ostreatus* with an inoculum dose of 8% and a fermentation time of 9 days. In this condition the crude fiber was 14.69%, the crude protein was 19.25% and the cellulase enzyme activity was 1.36 U / ml.

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Exploration and Characterization of Local Glutinous Rice Germplasm (*Oryza sativa* L. Var. *Glutinosa*) three Regencies in west Sumatra

Feri Hendriawan[1], Nasrez Akhir[2], Yusniwati[3]

[1]Department of Agriculture, Andalas University, Indonesia
Email: ferihendri1987@gmail.com
[2]Department of Agriculture, Andalas University, Indonesia
Email: nasrezaakhir@gmail.com
[3]Department of Agriculture, Andalas University, Indonesia
Email: yusniwatibismi@gmail.com

***Abstract*—*The purpose of this research was to identify and characterize the morphology of local glutinous rice germplasm three regencies in West Sumatera. This research was conducted in May – August 2019 in three regencies of West Sumatera. The result of this research indicate that glutinous rice plants can grow to a height of 1086 meters above sea level with a coordinate point 0°33'27" South Latitude and 100°32'28" East Longitude. The highest plants are found in white glutinous rice 'Puluik Tinggi' that is 150,03 cm and the lowest plants are found in black glutinous rice 'Puluk Itam' that is 90,6 cm. Observation of the highest amount of leaves found in white glutinous rice germplast 'Pulut Putih' as many as 60,4 strands/clumps while the smallest amount of leaves obtained in white glutinous rice germplasm 'Anak Ulek' as many as 51,3 strands/clumps. The fastest harvest period in white glutinous rice germplast 'Pulut Putih' during 120 days and the longest harvest period in white glutinous rice 'Kuku Balam', 'Puluik Tinggi', 'Puluk Putih' and red glutinous rice 'Puluk Sirah' during 165 days. There are 2 germplasm that has the highest similarity coefficient that is 42 % between white glutinous rice 'Anak Ulek' dan 'Pulut putih', this means that both germplasm have a close kinship.*
Keywords*— Morphology, Exploration, Characterization, Glutinous Rice.

I. INTRODUCTION

Indonesia is an agricultural country that has the most diversity of rice plants. Glutinous rice is one of the thousands of rice varieties. What distinguishes between glutinous rice with other rice plant varieties is a high amylase content and low amylopectin. Kadan *et al.* (1997), the carbohydrate content of glutinous rice is very high compared to protein, fat and vitamins. These carbohydrate have an important role in determining the characteristics of food ingredients such as taste, color, texture and others. Starch contained in glutinous rice amylopectin is very high around 97 % with low amylose.

The superiority of glutinous rice compared to rice varieties generally lies in the morphological characteristics of glutinous rice plants, where it have a rough leaf surface so that leaf insects do not like pests. Thus, it is necessary to identify and characterize a variety of glutinous rice germplasm so that it can be utilized by plant breeders in assembling superior varieties. IRRI (1995), identifying germplasm of local varieties that have superior genes makes it easier for plant breeders to obtain recombinant genotypes that have superior character in accordance with the target of improved varieties. In general, the use of local varieties as crossing parents produces derivatives with very diverse morphological and agronomic characteristics, so that a more intensive selection process is needed.

There are still many local glutinous rice germplasm that have not yet been identified and characterized. The community has begun to rarely cultivate it, there is no serious attention from the government to prevent it and fear of extinction of local glutinous rice germplasm is a source of problems for plant breeders in germplasm conservation programs. Efforts should be made to save the diversity of local glutinous rice germplasm by exploring and characterizing local glutinous rice germplasm in the regions. Daradjat, *et al*. (2008), the genetic diversity of a plant species can decrease, because of the planting and expansion of new superior species so that very diverse local species will be urged or even disappear. Scarcity of genetic resources can also occur due to the process of selection and refining of diverse forms of local varieties to form homogeneous landraces.

West Sumatra Province has several rice production centers, but for this sticky rice there are only a few regions. Not all rice production center areas, the people also plant glutinous rice plants. This is influenced by the community's traditions in the area and the community's need for glutinous rice.

Based on the above, identification and characterization of glutinous rice germplasm was carried out by observing morphological characterization. The purpose of this study was to determine, identify and characterize germplasm from local glutinous rice plants in three regencies in West Sumatra.

II. MATERIALS AND METHODS

This research was conducted from May to August 2019. The research took place in three different elevation districts in West Sumatra Province, namely regencies of Agam, Padang Pariaman and Tanah Datar. This research was conducted by survey method, using purposive sampling technique. Morphological characterization of glutinous rice plants refers to the Description of Varieties based on research by the Indonesian Center for Rice Research and Bioversity International, IRRI and WARDA. Qualitative data obtained from morphological observations carried out analysis of variability (variability) which aims to see the level of diversity of glutinous rice plants, using the formula Steel and Torrie.

Similarity analysis on morphological characters was carried out to see the pattern of grouping all of the glutinous rice germplasm. This analysis was carried out by calculating the similarity coefficient value with the output in the form of a dendogram using the Unweighted Pair Group Arithmatic Average (UPGMA) method using NTSYS (Numerical Taxonomy and Multivariate Analysis System) version 2.02.

III. RESULT

3.1 Identification and Characterization

Observations in three regencies that have been designated as research areas obtained 9 germplasm of glutinous rice plants. Details of glutinous rice germplasm per regencies, name regencies, coordinates and height can be seen in Table 1.

3.2 Morphological Characterization

Based on observations that have been made for the color of sticky rice plants there are three colors namely green, yellowish green and green with purple lines, while the color of the legs of the stem there are two colors namely green and green with purple lines.

From the observation of leaf morphology, high diversity in leaf ear color, leaf tongue color, leaf color, leaf position, flag leaf angle and number of leaves was found. However, no diversity was found in the shape of the leaf tongue and leaf surface, all glutinous rice plants were found to have the same shape of the leaf tongue which is 2-Cleft (split in two), while the leaf surface of the glutinous rice plant was classified as including the edges of the leaves.

Based on observations that have been made, there is a high diversity of sticky rice roots. The extensive and deep root system will affect the resistance of glutinous rice plants to vulnerability caused by wind. The quality of plant roots is influenced by the availability of nutrients and water, so farmers should pay attention to the time of fertilization and inundation of the fields carefully so that plants grow optimally. Kush (1995), roots of rice plants that are deep and thick, healthy, gripping the soil more broadly and stronger able to hold the plants from being overburdened, also allows more efficient absorption of water and nutrients, when the grain filling stage.

For panicle types there are three variations namely compact panicle types, panicle types between compact and medium, and panicle types between medium and open. While the observation of the shape of the grain there are three variations, namely the shape of the grain is rather rounded, the shape of the grain is medium and the shape of the grain is slim or long. The shape of this grain influences the process of processing grain into rice, not all rice mills are willing to process glutinous rice into glutinous rice, especially black glutinous rice. This is because the form of black glutinous rice grain is long, slim and light. So there is a slight difference in the arrangement of the wind valve and the filter device (filter) on the engine. According to Ramadhan (2006), each process of using a rice milling device must adjust the component of the tool which has different functions to avoid damage to rice and a reduction in the amount of rice. As the function of the wind valve is to separate peeled rice from the skin, this process uses a gust of wind.

The observation of grain color there are five variations, namely yellow straw rice, golden grain, brown yellow straw, brownish brown, yellow straw, brown stripes and yellowish brown grain. The color of this grain cannot be used as a benchmark to determine the color of glutinous rice, because there are grain colors that are both yellow straw brown stripes, but the color of the sticky rice is different, some are white and some are red.

3.3 Phenotypic Variability of Glutinous Rice Plants

In this study phenotypic variability of glutinous rice plants was calculated, both quantitative and qualitative characters. The phenotypic variability of 9 germplasm was found with 9 quantitative characters and 17 qualitative characters. Based on the measurement of observational variables for each germplasm by calculating the average value and standard deviation, it is shown in Table 2 and 3.

In table 2 above, it is known that there are three observational variables from nine quantitative characters that have broad criteria, namely plant height (cm), amylopectin content (%) and plant age (days). While six quantitative character observation variables have narrow criteria, namely the number of leaves, root length, number of tillers, number of productive tillers and average yield (tons). Fauza (2005), the broad phenotypic variability value means that the phenotypic appearance of the character is more influenced by environmental factors. Narrow phenotypic variability in morphological observation characters cannot be used as a basis for selection in plant breeding activities because selection will be successful or effective if the plant population to be selected has broad variability.

In table 3 above, it is known that there are one observational variables from 17 qualitative characters that have broad criteria, namely the color of grain. While 16 qualitative character observation variables have narrow criteria. Tedianto (2012), broad genetic variability of characters will provide broad phenotypic variability also if the interaction with the environment is high enough. Genetic variability occurs due to the influence of genes and different interactions with their environment. Narrow phenotypic variability cannot be used effectively in plant breeding, but can be expanded with hybridization, mutation and introduction of new germplasm.

3.4 Ethnobotany

The glutinous rice seeds used are local seeds that have been cultivated for decades. Giving a name to the local glutinous rice plants is obtained from the plant's unique character and the name of the area where glutinous rice plants are cultivated.

Different regencies have different community traditions and different types of sticky rice are utilized. Nevertheless, there are still some similarities in community traditions in these three regencies, namely the annual tradition called *Malamang*. This tradition has been carried out by the community for years. The time and type of evening traditions vary in these three regencies in West Sumatra Province.

3.5 Similarity Analysis

The pattern of similarity relationship between glutinous rice plants based on 27 morphological characters of 9 germplasm of glutinous rice plants in three districts with different altitude levels in West Sumatra Province shown in Figure 1, overall data processed using the NTSYS 2.02 program produced a dendogram pattern as shown in Figure 2. Classification of some germplasm occurred because there are similarities in the morphological characteristics of each glutinous rice plant. Swasti, et. al. (2007) states that similarity analysis is used to determine the distance or close similarity between plants by using the morphological properties of a plant.

The results of the similarity analysis are based on similarities in observations of 9 quantitative characters and 17 qualitative characters from 9 glutinous rice germplasm obtained by the similarity coefficient of 0.25 - 0.43. The results of the similarity analysis are divided into 2 main groups on the similarity coefficient of 0.25, namely groups 1 and 2. Group 1 consists of KA-KPKB, KTD-KPPP, KPP-KPPP, KPP-KPAU, KTD-KPK, KA-KPPT and KTD-KMPS meanwhile group 2 consisted of KA-KHPH and KTD-KHPI.

In group 1, KTD-KMPS separated at a similarity coefficient of 0.28 from the other group 1 germplasm. At the similarity coefficient of 0.31 KA-KPPT separates from other group 1 germplasm. In the similarity coefficient 0.33 group 1 germplasm is divided into 2 groups again, namely KA-KPKB and KTD-KPPP in group 1, both of which will be separated in the similarity coefficient of 0.39. While the other group 1 namely KPP-KPPP, KPP-KPAU and KTD-KPK, the similarity coefficient of 0.39 KTD-KPK will be separated from KPP-KPPP and KPP-KPAU. At the end of group 1 there was a similarity between the KPP-KPPP and KPP-KPAU on the similarity coefficient of 0.43. For group 2 between KA-KHPH and KTD-KHPI are separated at the similarity coefficient of 0.36. According to Syukur, et. al. (2012), the greater the similarity coefficient number, the greater the similarity level between the plants being compared and this shows the closer kinship level. Conversely, the smaller the coefficient of similarity, the smaller the level of similarity of plants and this shows the farther level of kinship.

IV. FIGURES AND TABLES

Table 1. Details of Germplasm per Regency, District, Coordinates and Altitude

Location		Name of Germplasm	Coordinates		Altitude (amsl)
Regency	District		South Latitude	East Longitude	
Agam	IV Koto	KA-KPKB	0°02’53”	100°32’18”	1086
Agam	Matur	KA-KPPT	0°01’45”	100°27’87”	1049
Agam	Palembayan	KA-KHPH	0°10’67”	100°15’47”	469
Padang Pariaman	Batang Anai	KPP-KPAU	0°50’30”	100°30’18”	6
Padang Pariaman	Lubuk Alung	KPP-KPPP	0°47’00”	100°27’42”	10
Tanah Datar	Batipuh	KTD-KHPI	0°30’00”	100°50’18”	794

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Tanah Datar	Batipuh	KTD-KPPP	0°31’70”	100°44’35”	706
Tanah Datar	Batipuh	KTD-KMPS	0°33’23”	100°43’99”	654
Tanah Datar	Pariangan	KTD-KPK	0°29’20”	100°51’86”	725

Table 2. Phenotypic Variability Based on Quantitative Characters of Glutinous Rice Plants in 3 different Regencies.

No	Quantitative Characters	Mean	S^2	SD	2 SD	Criteria
1	Culm Length (cm)	121,422	63,511	7,969	15,938	Large
2	Many Leaves	55,544	1,186	1,089	2,178	Tight
3	Root Length (cm)	31,301	1,338	1,157	2,313	Tight
4	Many Tillers	14,644	0,478	0,691	1,383	Tight
5	Many Productive Tillers	12,544	0,201	0,447	0,894	Tight
6	Weight of 1000 Grain (g)	31,222	1,661	1,289	2,578	Tight
7	Percentage *Amilopektin* (%)	81,044	7,684	2,772	5,544	Large
8	Age of Plants (days)	146,667	37,653	6,136	12,272	Large
9	Productivity (tons)	4,511	0,011	0,105	0,209	Tight

If S^2> 2 SD means that the phenotypic variant is broad and if $S^2 \leq$ 2 SD means the phenotypic variant is tight. (Pinaria, *et al*., 1995)

Table 3. Phenotypic Variability Based on Qualitative Characters of Glutinous Rice in 3 different Regencies (after scoring).

No	Qualitative Characters	Mean	S^2	SD	2 SD	Criteria
1	Shape Plant	2,000	0,666	0,816	1,632	Tight
2	Stem Color	1,667	0,429	0,655	1,309	Tight
3	Leg Stem Color	1,222	0,484	0,696	1,391	Tight
4	Auricle Color	1,667	0,221	0,149	0,297	Tight
5	Ligule Color	1,778	1,503	1,226	2,452	Tight
6	Leaf Color	1,778	0.258	0,508	1,016	Tight
7	Leaf Surface	2	0	0	0	Tight
8	Leaf Position	1,333	0,501	0,708	1,416	Tight
9	Flag Leaf Angle	1,444	0,553	0,744	1,487	Tight
10	Ligule Shape	3	0	0	0	Tight
11	Panicle Type	1,444	1,053	1,026	2,052	Tight
12	Collapse Plant	1,111	0,221	0,470	0,940	Tight
13	Category	1,778	0,388	0,623	1,246	Tight
14	Grain Loss	1	0	0	0	Tight
15	Grain Shape	3,111	1,220	1,104	2,209	Tight
16	Grain Color	2,889	5,720	2,392	4,783	Large
17	Glutinous Rice Color	1,556	1,553	1,246	2,492	Tight

If S^2 > 2 SD means that the phenotypic variant is broad and if $S^2 \leq$ 2 SD means the phenotypic variant is tight. (Pinaria, *et al*., 1995)

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Fig.1: Data on Germplasm of Glutinous Rice Plants in 3 Regencies.

Information:

A. White Glutinous Rice 'Kuku Balam', Nagari Balingka, Districts of IV Koto, Regencies of Agam.
B. Black Glutinous Rice 'Puluik Hitam', Nagari Tigo Koto Silungkang, Districts of Palembayan, Regencies of Agam.
C. White Glutinous Rice 'Puluik Tinggi', Nagari Tigo Balai, Districts of Matur, Kab. Agam.
D. White Glutinous Rice 'Pulut Putih', Nagari Punggung Kasiak, Districts of Lubuk Alung, Regencies of Padang Pariaman.
E. White Glutinous Rice 'Anak Ulek', Nagari Sungai Buluh Utara, Districts of Batang Anai, Regencies of Padang Pariaman.
F. Red Glutinous Rice 'Puluk Sirah', Nagari Batipuh Baruah, Districts of Batipuh, Regencies of Tanah Datar.
G. White Glutinous Rice 'Puluk Putiah', Nagari Batipuh Baruah, Districts of Batipuh, Regencies of Tanah Datar.
H. Black Glutinous Rice 'Puluk Itam', Nagari Batipuh Baruah, Districts of Batipuh, Regencies of Tanah Datar.
I. White Glutinous Rice 'Kunyit', Nagari Tabek, Districts of Pariangan, Regencies of Tanah Datar.

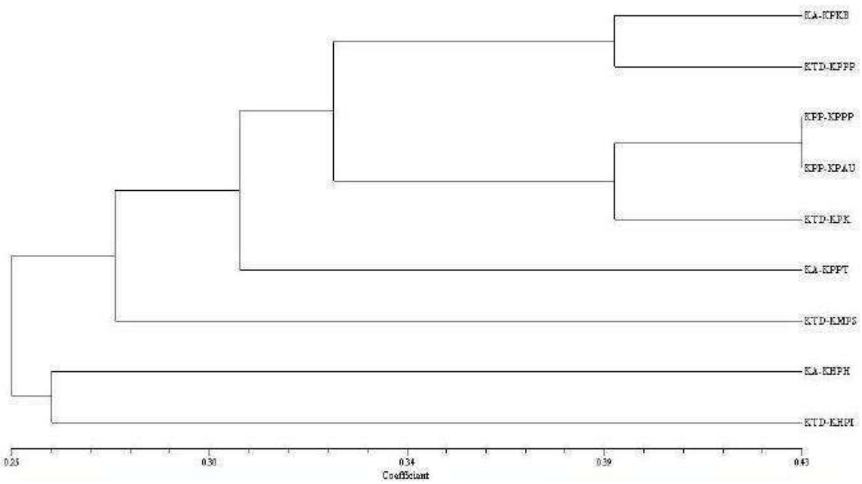

Fig.2: Similarity Analysis Dendogram Based on Quantitative and Qualitative Characters of 9 Glutinous Rice Germplasm in West Sumatra. Information : KA-KPKB = Kabupaten Agam - Ketan Putih Kuku Balam. KA-KPPT = Kabupaten Agam - Ketan Putih Puluik Tinggi. KA-KHPH = Kabupaten Agam - Ketan Hitam Puluik Hitam. KPP-KPPP = Kabupaten Padang Pariaman - Ketan Putih Pulut Putih. KPP-KPAU = Kabupaten Padang Pariaman - Ketan Putih Anak Ulek. KTD-KPPP = Kabupaten Tanah Datar - Ketan Putih Puluk Putiah. KTD-KMPS = Kabupaten Tanah Datar - Ketan Merah Puluk Sirah. KTD-KHPI = Kabupaten Tanah Datar - Ketan Hitam Puluk Itam. KTD-KPK = Kabupaten Tanah Datar - Ketan Putih Kunyit.

V. CONCLUSION

Based on the results of exploration of local glutinous rice plants in Agam Regency, Padang Pariaman Regency and Tanah Datar Regency 9 species of germplasm of local glutinous rice plants were found.

Similarity analysis on 9 germplasm of local glutinous rice plants, through observations of quantitative and qualitative characters obtained similarity coefficients with the numbers 0.25 - 0.43 or 25 - 43%.

The highest similarity coefficient is found in KPP-KPAU and KPP-KPPP which is 0.43 or 43%. The magnitude of the similarity coefficient between KPP-KPAU and KPP-KPPP shows the closer kinship level.

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Effectiveness of tuba root extract *(Derris elliptica L.)* against antifeedant of *Crocidolomia binotalis* caterpillar on mustard plant *(Brassica juncea L)*

Alfrits Komansilan[1], Ni Wayan Suriani[2], Helen Joan Lawalata[3]

[1] Department of Physics, Faculty of Mathematics and Natural Sciences, Manado State University, North Sulawesi, Indonesia
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Manado State University, North Sulawesi, Indonesia
[3] Department of Biology , Faculty of Mathematics and Natural Sciences, Manado State University, North Sulawesi, Indonesia
***Email:** alfritskomansilan@gmail.com*

***Abstract*— *The application of tuba root bioactive extract (Derris elliptica L.) as a natural insecticide on the Crocidolomia binotalis caterpillar on mustard plants (Brassica juncea L.) was carried out. The test results showed that there were significant differences in antifeedant activity of the Crocidolomia binotalis caterpillar on mustard (Brassica juncea L.) plants at various concentrations. The research was carried out in several stages, starting from the tubal root extraction stage, phytochemical testing, preparation of caterpillar tests, testing of antifeedant activity. As the treatment is the level of methanol concentration of 50 ppm, 100 ppm, 500 ppm and 1000 ppm. The parameter observed was the percentage of Feeding Reduction (FR). The test results showed that the best antifeedant activity of Crocidolomia binotalis was at concentrations of 500 ppm and 1000 ppm because it was able to inhibit feeding power or Feeding Reduction of test caterpillars by 15.35% and 32.33%, was able to inhibit the feeding activity of Crocidolomia binotalis.*

Keywords*— *Derris elliptica root, antifeedant, Natural Insecticide, Mustard, Feeding Reduction.

I. INTRODUCTION

Tuba root plant is a type of plant commonly used as fish poison. Plant roots tuba potential as biopesticide is in addition found in almost all regions in Indonesia also found in Africa, Southeast Asia and some islands in the Pacific (Novian, 2004).

The use of insecticides unwisely will have a bad influence on the environment and public health, especially farmers. Farmers in general overcome pest caterpillars by using synthetic chemical pesticides. In terms of pest population suppression, the results of chemical control with pesticides are quickly felt, especially in large areas. Until now, pest control of mustard greens which is commonly done by farmers is chemically using synthetic pesticides. Soewadi (2002) suggested that the application of synthetic chemical pesticides which are not wise and not in accordance with Integrated Pest Management (IPM) can have various negative impacts such as the occurrence of pest resistance, the emergence of secondary pests, the killing of non-target organisms, the presence of insecticide residues on food ingredients, pollution environment, and dangerous for consumers. As an alternative, the use of plant material to be used as a vegetable pesticide is now being developed.

To get an effective, efficient and safe insecticide, it is necessary to have a comprehensive and directed study so that a formulation that is ready to be used by agricultural actors will be produced. Making a simple plant insecticide formulation is expected to be the forerunner to the development of an environmentally friendly plant-based insecticide industry on a large scale and will be able to compete with insecticidal formulations made from synthetic active conditions provided that the plant-based insecticides have efficacy and competitive prices, practical in use, and the most important is safe for human health users. Plants that have been isolated by researchers contain active compounds of plant-based insecticides on Aedes aegypti mosquito larvae are soursop seeds (Annona muricata) with Lethal Concentration LC_{50} = 117. 27 ppm (Komansilan *et al.* 2012), and Hutun seeds (Barringtonia asiatica Kurz) with Lethal Concentration LC_{50} = 35.72 ppm (Komansilan and Suriani. 2016).

Tuba root plant is a type of plant commonly used as fish poison. Plant roots tuba potential as biopesticide is in addition found in almost all regions in Indonesia also found in Africa, Southeast Asia and some islands in the Pacific (Novian, 2004).

Crocidolomia binotalis is an important pest in cabbage plants. This caterpillar attacks plants from the *Brassica* family such as mustard greens, radish cabbage and radish, these pests attack mainly on the inside of the plant until it reaches the point of growth (Pracaya, 1999; Kalshoven, 1981). Control measures that can be applied to these pests are regulation of cropping patterns, farming techniques, utilization of natural enemies, physical, mechanical control and use of vegetable pesticides. The use of tuba root extract as a vegetable pesticide to kill insect pests has not been widely reported, therefore it needs to be studied and examined how the effect of extracts from the plant *Derris elliptica L.* on *Crocidolomia binotalis* which is one of the important pests in mustard plants.

Antifeedant is a substance that can stop eating insects or other animals permanently or temporarily depending on the strength of the substance (Garson, 2010). The potential of antifeedant substances has long been known, because it has become one of the alternatives in food crop protection.

The process of food crop production is often hampered due to insect pests that cause crop failure. The development and spread of insect pests that disturb agricultural crops, now require serious attention. To overcome this, farmers generally use synthetic pesticides in pest control. The use of synthetic pesticides in the process of agricultural production can result in the presence of pesticide residues in agricultural products (Untung, 1996). Residues of a number of chemicals such as pesticides can be left through various cycles directly or indirectly, so that it reaches humans and enters the digestive tract with food and drinking water (Tjokronegoro, 1987).

Seeing the negative impact of pesticides, the development of a biorational pest control agent needs to be done. This antifeedant substance has good prospects to be developed into a biopesticide agent (Mayanti, *et al.*, 2005). This research will examine the potential of food activity inhibition for pest insects with different inhibitory activities.

II. RESEARCH METHODS

A. Research Location and Time

This research was conducted at the Laboratory of Integrated Sciences, Laboratory of Physics and Chemistry Laboratory, Faculty of Mathematics and Natural Sciences ,Manado State University, in Tondano. The study was conducted from May to August 2019, starting from the sampling phase, phytochemical screening extraction and testing of *antifeedant* activity .

B. Materials and tools

The material used is tuba roots sampled from Bulo plantation in Bulo village, Mandolang sub-districts, Minahasa Regency. The materials used are 70% ethanol and 95% for maceration of tubal root samplings, technical methanol acetic acid, sulfuric acid, chloroform, 5% $FeCl_3$ % solution, Dragendorf reagent, Meyer reagent, tissue, cotton, whattman filter paper no. 42, aluminum foil, plastic samples, mustard leaves, and caterpillars *Crocidolomia binotalis.* The tools used are analytical scales, petri dishes, vial tubes, Erlenmeyers, goblets, measuring cups, volumetric pipettes, fillers, test tubes and tube racks, drop pipettes, 50 mL and 100 mL measuring flasks and *rotary vaccum evaporators (Heidolph-Laborota 4000/4001 efficient).*

C. Experiment Design and Data Analysis

This study uses a Completely Randomized Design (CRD) as a treatment that is the concentration level of methanol solvent 50 ppm, 100 ppm, 500 ppm and 1000 ppm and negative control 0 ppm. Each treatment was repeated 3 times. The parameters observed were percentage of *Feeding Reduction* (FR) or % *antifeedant* and phytochemical screening / screening test for tubal root ethanol extract. The data obtained were analyzed using one-way analysis of variance (ANOVA). If the treatment has a significant effect on the inhibition of eating *Crocidolomia binotalis* on mustard plants, then further testing of LSD or LSD at 5% significance level.

D. Research procedure

Tuba Root Extraction (*Derris Elliptica L.*)

Tuba root samples obtained from the Bulo plantation in the village of Bulo, Mandolang sub-district, Minahasa Regency. The extract material used in this study was the roots of the tuba plant which grew around Bulo, the village of Bulo, Mandolang sub-district, Minahasa Regency. Making the root extract tuba done by weighing 1,2 Kg of tuba root dried at room temperature, then soaked in ethanol (maceration) for 1 x 24 hours in maserator. Maceration is done several times until the extracts run out. The maceration solution is then filtered using Whattman filter paper No. 42. The filtrate obtained was then put into a *vaccum evaporator* at 40^0C until the ethanol solvent evaporated to obtain a thick ethanol extract. Furthermore, the extraction results obtained were weighed using analytical scales. To make the test solution, a dilution was carried out using technical

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methanol solvents. The concentration of the tuba root extract used in this study was 50; 100; 500 and 1000 ppm. While the control (0 ppm) is in the form of methanol solution. Each treatment was repeated three times.

E. Preparation of Test Larvae / Caterpillars

Pupa *Crocidolomia binotalis* instar to III obtained by way of setting up a mustard that has not been sprayed synthetic pesticides. A 1 x 2 m dark culture box is prepared. Larvae prepared by way of taking pupae of agricultural acreage of mustard incorporated into the plastic bottle and then hung in the breeding box. At the top of the culture box will be hung cotton that is tied to a rope and has been dipped in a mixture of 1 mL of honey with 10 mL of water. Honey solution serves as a food source for the *Crocidolomia binotalis* imago . The next pupa hatches and becomes a moth after two days. Moths will reproduce and lay their eggs on mustard plants. The eggs will hatch into larvae instar I to instar III. Furthermore, third instar larvae will be used in *antifeedant* activity testing.

F. *Antifeedant* Activity Testing

The test was carried out using the leaf disc method according to (Atta, Choundary, & Thomson, 2001). On sterile petri dishes are placed wet filter paper / tissue and gauze and the filter paper is coated with transparent plastic that has been perforated. Leaf discs are made with a circle the size of a petri dish on mustard leaves that have not been given synthetic pesticides. Leaf discs to be made are the same in size, shape and thickness. Leaf discs were dipped in each extract sample and compared with positive control. The study was conducted with three repetitions. Leaf discs are dipped / applied for 5 minutes then aired for 5 minutes. After aerating, the leaf disc will be weighed and put into a prepared petri dish.

Caterpillars *Crocidolomia binotalis* included 1 caterpillar on each petri dish, the petri dish that already contains leaves and caterpillars test discs will be observed caterpillars avoidance response to the leaf discs that had been given each extract concentration. Observations are made after 24 hours. Antifeedant activity testing is done by looking at the nature of the *Feeding Reduction* of the sample. The parameter to be observed is the weight of the remaining leaves that are not eaten by the larvae or *Feeding Reduction* (FR). Leaf discs were then weighed, to find out the weight of mustard leaf discs eaten by the *Crocidolomia binotalis caterpillar*, the percentage of *Feeding Reduction* (% FR) was used. The percentage value of *Feeding Reduction is* measured by the formula (Atta *et al.*, 2001):

$$\%\,FR = \left\{1 - \frac{\text{Weight of the treated leaf eaten}}{\text{Control weight of the leaf eaten}}\right\} x\ 100\%$$

G. Phytochemical Screening

Phytochemical Test Work Procedures (Ayoola, *et al.*, 2008 & Farnsworth, 1966)

A certain amount of viscous extract was carried out by phytochemical tests which aimed to determine the class of compounds contained in the roots of the tuba. Phytochemical tests were carried out on the group of Alkaloids, Flavonoids, Phenols, Saponins, Triterpenoids, Steroids, Terpenoids, and Tannins.

a. Alkaloid Test

One gram of ammonia extract was added to 10% and then extracted with chloroform and added 1 N hydrochloric acid. The extraction results will be divided into two layers. The upper layer (acid layer) is divided into two tubes. In one tube Meyer reagent was added, while in the other tube Dragendorf reagent was added. Yellow indicates a positive alkaloid.

b. Flavonoid Test

Two methods are used to test Flavonoids.

1. Dilute ammonia (5 mL) is added to the aqueous filtrate portion of the extract. Then concentrated sulfuric acid (1 mL) is added. A missing yellow indicates flavonoids.
2. A portion of the extract is heated with 10 mL ethyl acetate which has been evaporated for 3 minutes. The mixture is then filtered and 4 mL of the filtrate is shaken with the addition of 1 mL of aqueous ammonia solution, the formation of a yellow color indicates the presence of flavonoids.

c. Phenol Test

To one gram of extract was added 1% iron (III) chloride. Green / red / purple / blue / black colors indicate positive phenols.

d. Saponin Test

One gram of extract is added to water then boil in a water bath for 5 minutes, after which it is shaken vigorously. Saponin is positive if foam forms stable for ± 30 minutes.

e. Triterpenoid and Steroid Test

Anhydrous acetic acid was added to the extract until it was submerged; leave for ± 15 minutes. After that, add 1 drop of concentrated sulfuric acid. Green / blue deposits indicate steroids, while red / orange deposits indicate triterpenoids.

f. Terpenoid Test

A number of extracts were added with 2 mL chloroform. Then carefully added concentrated H_2SO_4 (3 mL) to form a layer. The formation of a brownish red color indicates terpenoids.

III. RESULTS AND DISCUSSION

Insects will face two things to start eating activities, first there are stimuli to initiate feeding activities (*feeding stimulants*) in plants that provide input signals for the introduction of food types and maintain eating activities. The second is the detection of the presence of foreign substances (*foreign compounds*) which act as inhibitors to eat so can shorten or terminate the feeding activity feeding activity at all. Based on the results of interviews with farmers spraying mustard area where pest control caterpillars *Crocidolomia binotalis* still relies on the use of chemical pesticides. Spraying intervals with chemical pesticides are carried out for 5-6 days, while the recommended use of pesticides should ideally be once a month. This results in faster selection of insect resistant to insecticides. The use of botanical insecticides was also not carried out because given the vast land area making it less practical to apply. According to Danar Dono *et al,* insect resistance to synthetic insecticides can be broken using botanical insecticides, due to the different mechanism of action of the two insecticides. In addition, one of the advantages of botanical insecticides is that it is difficult to cause an immune (*resistant*) reaction on the target pest so it is safe for the balance of the ecosystem.

Based on research results the influence of tuba root ethanol extract produces data that is food activity / food inhibitors (*Feeding Reduction*). The results of barriers to eating *Crocidolomia binotalis caterpillars* can be seen in Table 1.

Table 1. Effect of tuba root extract on decreased feeding activity of Crocidolomia binotalis caterpillar on mustard plants.

Treatment	U	Area of leaves eaten (gr) within 24 hours	Percentage of Food Obstacles (%)	Average
P1 (50 ppm)	1	2.21	8	6.66
	2	2.17	10	
	3	2.37	2	
P2 (100 ppm)	1	1.79	26	27.73
	2	1.70	29.2	
	3	1.75	28	
P3 (500 ppm)	1	1.68	30	30.16
	2	1.80	25	
	3	1.55	35.5	
P4 (1000 ppm)	1	1.11	54	44.0
	2	1.50	38	
	3	1.45	40	
control	1	2.40	0	0

Based on table 1 , eating activity data can be seen from the percentage of food barriers at a concentration of 50 ppm for replications 1, 2, 3 in succession of 8, 10 and 2 while for 100 ppm concentrations of 26, 29.2 and 28 . At concentrations of 500 ppm and 1000 ppm the percentage of food barriers increased by 30, 25 and 35.5, while for the concentration of 1000 ppm amounted to 54, 38 and 40 . The higher the value of eating barriers means a decrease in the eating activity of the *Crocidolomia binotalis caterpillar* on mustard plants. The average percentage of food resistance (*Feeding Reduction, %*) results in the following diagram:

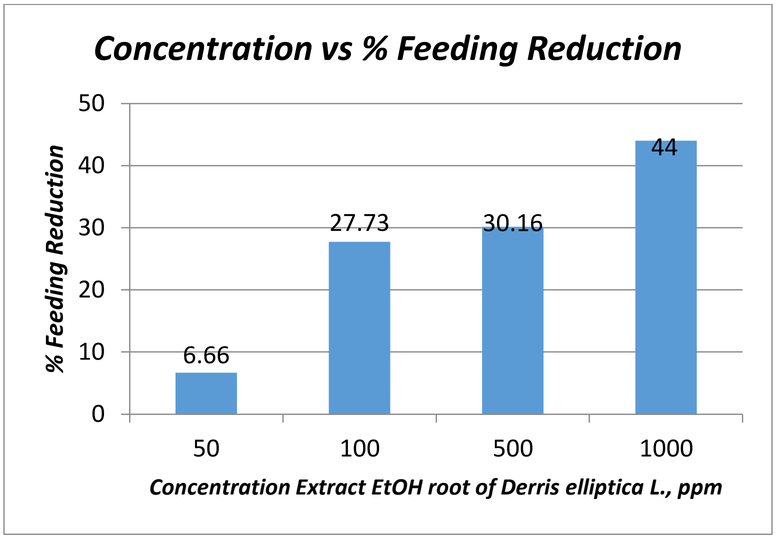

Fig.4.1 Diagram of average decrease in feeding activity (% Feeding Reduction) of caterpillar Crocidolomia binotalis

Based on the results of the test the influence of tuba root ethanol extract with three repetitions gives a value of eating resistance that is different from each concentration. The higher the concentration, the higher the percentage of eating obstacles will be and this means a decrease in eating activity. The highest value of eating resistance is at a concentration of 500 ppm and 1000 ppm.

Normality test is a test used to determine the distribution of data obtained is normal or not. The normality test is a prerequisite for the one-way ANOVA test. If the number of samples >50 used is Kolmogorov -Smirnov, whereas if the number of samples <50 then what is used is Shapiro-Wilk. The results in table 2 are then tested for normality as follows:

Table 2. Test for normality of feeding activity of Crocidolomia binotalis caterpillars on mustard plants.

Tests of Normality

		Kolmogorov-Smirnov [a]			Shapiro-Wilk		
	Treatment	Statistics	df	Sig.	Statistics	df	Sig.
Antifeedant	1.00	292	3	.	.923	3	.463
	2.00	.232	3	.	.980	3	.726
	3.00	.179	3	.	.999	3	.948
	4.00	.343	3	.	.842	3	220

a. Lilliefors Significance Correction

If in the Shapiro-Wilk column the value of Sig.> 0.05 then the data for each treatment is normally distributed, whereas if the value of Sig .< 0.05, then each treatment data is not normally distributed. The conclusion of the normality test on the above eating activity data meets the normal requirements because the Sig. > 0.05 for each treatment.

Table 3. Homogeneity test of feeding activity of Crocidolomia binotalis caterpillars on mustard plants

Test of Homogeneity of Variances

Antifeedant

Levene Statistics	df1	df2	Sig.
2,922	3	8	.100

Data for each treatment is said to be homogeneous if the Sig value > 0.05 and vice versa the treatment data is said to be homogeneous if the Sig value <0.05. Based on the homogeneity test table above the activity data of each treatment was declared homogeneous because the Sig value > 0.05 so that the ANOVA test could be performed. ANOVA test table can be seen in Table 4.

After ANOVA test (variance analysis) at a 5 % confidence level , the results showed that the treatment had a significant influence on the antifeedant of the *Crocidolomia binotalis caterpillar* on mustard plants. can be seen in the Table 4 below:

Table 4. ANOVA test of feeding activity of the Crocidolomia binotalis caterpillar on mustard plants

ANOVA

Antifeedant

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2138,789	3	712,930	23,085	.000
Within Groups	247,060	8	30,883		
Total	2385,849	11			

If the Sig . Value < 0.05, the treatment was stated to have a significant effect. Based on the ANOVA test table above shows that there is a significant effect of tuba root ethanol extract on the feeding activity of the *Crocidolomia binotalis caterpillar* on mustard plants.

Based on the ANOVA test results above, the treatment data can be further tested to find out more specific effects. Further tests used were those with the smallest significant difference (LSD) or LSD (Least Significant Different) to show differences between each treatment individual.

Table 5. Average% of leaves eaten and decreased feeding activity of tubal roots against the Crocidolomia binotalis caterpillar on mustard greens Brasicca junceae.

Concentration of tuba root ethanol extract , ppm	Average food resistance (%), *Feeding Reduction (%)*	Average food resistance at 24 hours after application (ppm) ± SD	Average food resistance at 24 hours after application (ppm) ± SEM
P1 (50)	6.66	6.66 ± 4.16	6.66 ± 2.40 [a]
P2 (100)	27.73	27.73 ± 1.61	27.73 ± 0.93 [a]
P3 (500)	30.16	30.16 ± 5.25	30.16 ± 3.03 [b]
P4 (1000)	44.00	44 ± 8.71	44 ± 5.03 [c]

Note: the treatment followed by the same letter shows no significant difference and the treatment followed by different letters shows significantly different

Phytochemical Test Results

Table 6. Phytochemical screening test results of ethanol extract (EtOH) from tuba root plants

No.	Group	Observation result	
1	Alkaloids	Hager (-)	Meyer (+)
2	Flavonoids	(-)	
3	Phenol	(++)	
4	Saponin	(+++)	
5	Steroids / triterpenoids	(++)	
6	Terpenoids	(+)	

Note: +++ = Compounds that are contained a lot
++ = Medium contained compound
+ = The compound contained is small
- = The compound contained does not exist

Table 7. Phytochemical Testing of ethanol extract (EtOH) of tubal roots (Derris elliptica L.)

Phytochemical Test	Results (color)	Standard Result (color)
Saponin	Shaped foam (+++)	Formed foam ± 15 minutes stable
Phenol	Purple color (++)	Bluish purple
Steroids / triterpenoids	Green or blue (++)	Brown sediment
Terpenoids	Chocolate (+)	Reddish brown deposits
Flavonoids	Yellow (-)	Chocolate
Alkaloids	Light Brown (+)	Brown sediment

Based on the results of phytochemical tests, tuba root ethanol extract belongs to the saponin, steroid, phenol, and alkaloid classes. Saponins are generally bitter and also toxic to some cold-blooded animals such as fish and amphibians. The use of saponins as an antidote to predator attacks, the media to fight over the scope, and help the process of reproduction (Liang & Guo, 2013). The bitter taste issued by saponins is thought to inhibit the feeding activity of test larvae.

The content of triterpenoid compounds in ethanol extract was characterized by the formation of reddish brown deposits in the extracts tested. The terpenoid compounds contained in the tuba roots function as fish poisons to fight predators that threaten their survival (Handayani *et al.*, 1997). Alkaloids can inhibit the response of cyanogenic glycoside sugars, which are sugars formed from bonds between sugar and toxic compounds stored in plants so that the toxic compounds are lost in toxicity.

IV. CONCLUSIONS AND SUGGESTIONS

Based on the results of further tests BNT at 5% level showed significantly different from the administration of ethanol extract to the feeding activity of the *Crocidolomia binotalis caterpillar* on mustard greens *Brasicca junceae L.* In the treatment P1 was not significantly different from the treatment P2, but significantly different from P3 and P4. In the P2 treatment also not significantly different from P1 but significantly different from P3 and P4. In the treatment of P3 significantly different from P4, also significantly different from P1 and P2. While the P4 treatment was significantly different from P1 and P2 also significantly different from P3. The best antifeedant activity against Crocidolomia binotalis is at concentrations of 500 ppm and 1000 ppm because it is able to inhibit feeding power or Feeding Reduction of caterpillars test 30.16% and 44.00%.

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Assessing the quality of water used by the community of Madlangamphisi in the Hhohho region of Eswatini

Dlamini, M. V.*; Manyatsi, A. M. and Dlamini, K. A.

Dept. of Agricultural and Biosystems Engineering, University of Eswatini, Faculty of Agriculture, Private Bag, Luyengo, Eswatini.
*Corresponding Author: musavd@uniswa.sz or musavd@gmail.com.

Abstract— *Clean water is necessary for communities. However, in some communities water is obtained from various sources which potentially can cause a health hazard. The research was conducted to assess the quality of domestic water used by the community of Madlangamphisi area in the Hhohho district of Eswatini. A questionnaire and physical water sampling were used to collect both the qualitative and quantitative data reported in this paper. Water samples to determine the quality of water used for domestic purposes in the area were taken from the common sources, the community borehole and the Nkomazi river. The samples were tested for microbial water quality and the Eswatini Water Services Corporation drinking water guidelines used as the bench mark. A total of 169 households out of 300 households were randomly selected to participate in the survey. The majority (51.5%) of the households used water from the river, 24.9% used water from the borehole, 18.3% depended on rainfall and 5.3% used water from seasonal streams. Water analysis results revealed that water from the river was polluted, as it contained 8/100 ml of faecal coliforms (E. coli) which was higher than 0/100 ml recommended in the benchmark. The water from the borehole did not contain E. coli. The majority (56.2%) of the households did not purify the water before use, 27.2% added bleach, 12.4% used boiling and 4.1% used treatment sachets for purifying the water. The study concluded that Madlangamphisi community faced a serious risk of water borne diseasese specially faecal coliforms (E. coli).The study observed that there is a need to introduce a rural water supply scheme in the area to solve the water quality problemsto avoid a potential outbreak of diseases.*

Keywords*— *Community, water, rural schemes, faecal coliforms, water quality.

I. INTRODUCTION

Water is a valuable life commodity (Chaplin, 2001)that supports numerous ecosystems. It is absolutely essential not only for survival of human beings, but also for animals, plants and all other living things (Razo *et al.*, 2004).It is however becoming a scarce resource (Khalifa and Bidaisee, 2018) in most parts of the world, partly due to global warming which results to drought conditions and mismanagement by humans (Srinivasan, *et.al.*, 2012). Water pollution and wasteful use of freshwater are threatening development projects, making water treatment an indispensable requirement to produce safe drinking-water (Helmer, 1994).Water of good quality is a crucial key to sustainable socio-economic development.

In a model of water quality selection described by Carrie and Genevieve (2007) the protection of source water quality for domestic use (drinking water, abstraction etc.) was identified by most experts' group as a priority for assessment.

Eswatini is one of the countries that have an average number of water sources which includes dams, rivers, groundwater, wetlands, springs and streams (Manyatsi and Brown, 2009) to name a few, yet the supply of water is insufficient. It has been observed that the most important challenge facing the South African Food Energy Water Nexus is water quality (Oberholster and Botha, 2014). Most domestic water is obtained from rivers that have been reported to contain high levels of microbial pollutants (especially *E. coli*) which can act as a vehicle for food contamination (Genthe et al. 2013).

Meals (2001) suggested that a combination of vegetated buffer strips and riparian zones shows a significant decline in microbial counts. Riparian zones are naturally occurring within the flood area of rivers or streams and need to be protected to improve downstream ecosystem services. River water quality is a key concern as it is used for drinking and domestic purpose, irrigation and aquatic life including fish and fisheries (Uddin, et al., 2014).

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On the other hand, polluted water is the greatest source of disease and besides debasing the land also becomes unfit to sustain life (Khalifaand Bidaisee, 2018). Natural waters are afflicted with a wide variety of inorganic, organic, and biological pollutants. The increased application of commercial fertilizer and widespread use of a variety of new pesticides (insecticides, nematicides, herbicides and weed killers) in agricultural practices are resulting in a host of new pollution problems from land drainage. This type of agricultural pollution has severe impact on water Pollution, as most of pollutants are resistant to natural degradation. Although concentration of the pollutants are still rather low, many of this compounds are toxic to human or animal life; some of them are carcinogenic or have serious ecological contamination (Davis and Cornwell,1998; Peavy, *et al,* 1985; Sawyer, *et al*., 1994).To add to the contamination from humans and animals, nature itself can contaminate the water and make it unusable, however many populations are forced to use these unsafe water sources (Lenntech, 2016). The two main factors that hold communities back from their bright futures are illnesses from drinking unsafe water and the time required in fetching water due to access being an issue (The Water Project, 2016). If clean water becomes more accessible for populations, it can be safe to assume that health will also increase; this in turn will decrease the time lost to sickness.

More than five million people die each year from diseases caused by unsafe drinking water, lack of sanitation, and insufficient water for hygiene. In fact, over two million deaths occur each year from water-related diarrhoea alone. At any given time, almost half of the people in developing countries suffer from water-related diseases (Gleick, 2002). Every dayit is estimated that one thousand lives of children are taken from illnesses like diarrhoea, dysentery, and cholera because of unclean water and unhygienic living conditions.

II. MATERIALS AND METHODS

Madlangamphisi is an area in the Hhohho district of Eswatini located at 26º05′22.70″S and 31º32′59.52″E at an elevation of 397 m above sea level. It is a community of about 300 households.A questionnaire was administered to members of the community to help in the collection of both qualitative and quantitative data,169 households were selected with 95% confidence level and 5% margin of error. Water samples from a community borehole and the Nkomazi River (used as sources of water)were collected and then tested in a certified laboratory to obtain water quality results, mainly *Escherichia coli*. The Eswatini Water Services Corporationand the World Health Organization (WHO) drinking water guidelines were used as a benchmark for the water quality tests.

The daily water consumption for the household was estimated using the following equation.

$$Daily\ Water\ Consumption = \frac{Total\ amount\ used\ in\ household\ per\ day}{Number\ of\ people\ in\ the\ household} \qquad (1)$$

III. RESULTS AND DISCUSSIONS

Demographic information

The number of people per household (Table 1) were categorized into four groups: 1 to 3 people, 4 to 7 people, 8 to 10 people and 11 to 14 people. A majority of the households 46.2% had 4 to 7 family members. This showed a high population density, and this indicated that the area had a high domestic water demand. According to Jaeger(2012) when the population is high water scarcity arises as the demand grows beyond the available supply and is mainly limited by physical availability of water. The number of people per household was used to calculate the daily water requirements of each of the households.

Table 1: The number, frequency and percentage of people per household in the sample surveyed (N = 169).

Number of people per household	Frequency	Percent (%)
1-3	52	30.8
4-7	78	46.2
8-10	29	17.2
11-14	10	5.9
Total	169	100.0

A presentation of the length of stay for the households in the Madlangamphisiarea is illustrated in Figure 1. A majority (74%) of the households had been staying in the area for more than ten years, and only less than three percent had been in the area for more than 50 years. This shows that the information provided about the status of water availability or scarcity was reliable, as the people are well versed and have experienced on all water related issues in the area.

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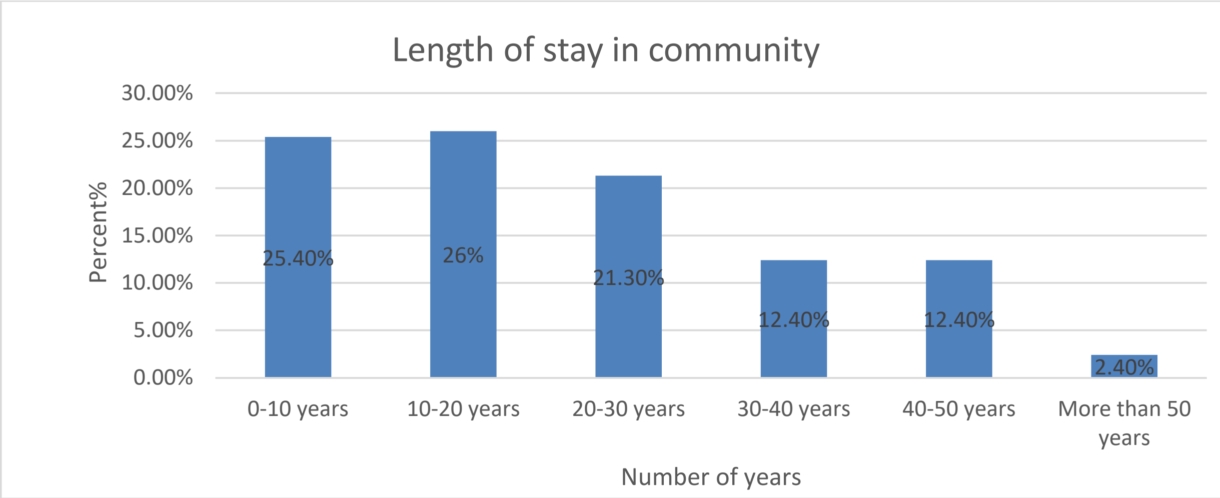

Fig.1: Residence time of the households in Madlangamphisi community

Domestic water sources

The various sources of domestic water at Madlangamphisi are shown in Table 2.The majority 51.5% of the households in the community used the Nkomazi river as their main source of water for domestic use, while 24.9% of the households use a community borehole and 18.3% use harvested rainwater and only 5.3% used seasonal streams. The Nkomazi river and community boreholes were found to be the main water sources. The Nkomazi river was preferred by most of the households since it does not dry up even during dry seasons, hence it is a reliable source of water. Other minor alternative sources of water included buying water from shops specifically used for drinking since it was bought in small quantities.

Table 2: Sources of domestic water used by Madlangamphisi households (N = 169)

Water sources	Frequency	Percent (%)
Borehole	42	24.9
River	87	51.5
Seasonal streams	9	5.3
Rainwater	31	18.3
Total	**169**	**100.0**

Although some of the households used harvested rainwater and streams, they still depended on the community borehole and the Nkomazi River since they could not depend on the unreliable rainfall. The results showed that a majority(56.8%) of the households relied on unprotected water sources, as their main water sources were the river and streams. Water from these sources particularly the river and seasonal streams was exposed to contamination owing to the fact that surface water sources were prone to being polluted. This means that the households were more vulnerable to infection by waterborne diseases.

Water quality results

The outcome of a survey of the residence on the perception of the quality of the water is shown in Table 3. A majority, 63.9% of the population perceived the water to contain some soil sediments and solid waste. Only 36.1% of the population used clean water which they obtained from the borehole and rainwater. As the majority of the households used unsafe, dirty water when asked if they purified the water, 56.2% responded negatively.

Those households that treated the water use either one of the following methods: boiling (12.4%), adding bleach (27.2%) and use the P&G water treatment sachets is 4.1%. The 56.2% of the residents which did not treat the water are more vulnerable to waterborne disease because the water they used was contaminated.

Table 3: Perception of households on the water quality and method of treatment (N = 169)

Water quality		Frequency	Percent (%)
Is water clean	Yes	61	36.1
	No	108	63.9
	Total	169	100.0

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Treatment used to clean water	Boiling	21	12.4
	Adding bleach	46	27.2
	Use P&G water treatment sachets	7	4.1
	None	95	56.2
	Total	169	100.0

Faecal coliforms in groundwater

Table 4 shows the level of *E.coli* bacteria found in borehole and river water. No E.coli was detected in borehole water at Madlangemphisi, meaning that it was safe for drinking.

Table 4: E. colicounts in groundwater and surface water

Source of water sample	Samples	E.coli (counts/100 ml)
	1	0
Borehole	2	0
	Mean	0
Nkomazi	1	7
	2	9
Mean 8		

The water sourced from the Nkomazi river was contaminated with *E. coli.* The first sample results indicated (7/100 ml) while the second results reflected (9/100 ml) *E. coli* presence. This meant that water sourced from Nkomazi River was not suitable for domestic use as it was more than the WHO water standards guidelines. However, the values were within the standards set by the Government of Eswatini for rural water (Government of Swaziland, 1998). The Nkomazi river is a surface water source which is unprotected, making the water vulnerable to contamination from human waste through open defecation and animal waste. The community has some farmers who kept livestock, that also used water from the Nkomazi river. The faecal coliform counts detected from the water of the Nkomazi river showed that the majority of the residents used water that was contaminated thus of poor quality making them potentially vulnerable to waterborne diseases.

Incidence of water borne diseases at Madlangamphisi

When responding to the incidences of water borne diseases (Table 5), 60.9% reported that they had no incidences of waterborne diseases for the past year (2017) and 39.1% households have had incidences of water borne diseases. This was due to the use of untreated water, and storing of rainwater for a long time. The rainwater after sometime, is said to contain microbes which may cause cholera as reported by18.9% and rashes 11.2% of the households. Those using untreated water from the river usually suffered from bilharzia 8.3% with 0.6% suffering from bladder infections.

Table 5: Water borne diseases experienced by households in the area (N = 169)

Waterborne diseases		Frequency	Percent
Incidences	Yes	66	39.1
	No	103	60.9
	Total	169	100.0
Types of diseases	Bilharzia	14	8.3
	Rash	19	11.2
	Cholera	32	18.9
	Bladder infection	1	0.6
	Total	66	39.1
	None	103	60.9
	Total	169	100.0

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IV. CONCLUSION

From the study, it was noted that the majority 63.9% of the households relied on unsafe drinking water for domestic use and that 56.2% of the households did not purify the water before use. The water quality tests indicated that water sourced from the Nkomazi River was contaminated with feacal coliforms (8/100 ml), making its water unsuitable for domestic use. The community therefore faced a serious risk of water borne diseases. 39.1% of the households reported to have had an incidence of disease, mostly cholera, rash, and bilharzia.It was concluded that there was a need to introduce a rural water supply scheme in the area to help solve the water quality problems.

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Mechanism of Indigenous Rhizobacteria Isolate Growth Inhibition of *Ganoderma boninense*

Imam Rifai[1], Yulmira Yanti[2], Nurbailis[2]

[1]PostGradauated Pests and Plant Diseases of Faculty of Agriculture, Andalas University, Padang, West Sumatera, Indonesia
Email: imamrifai1396@gmail.com
[2]Department of Plant Protection, Faculty of Agriculture, Andalas University, Padang, West Sumatera, Indonesia

***Abstract*— *Basal stem rot (Ganoderma boninense) is the main disease of oil palm. Previous research results obtained 7 isolates of rhizobacteria indigenous which have the best ability to control G. boninense in oil palm seedlings and increase growth. The purpose of this study was to characterize the ability of PGPR and biocontrol of indigenous rhizobacteria isolates and inhibitory testing of G. boninense in vitro. Characterization methods include (Phosphate solubility, siderophore production, chitinase activity, hemolytic activity and dual culture test). The results showed four isolates of RZ1A 2.1, RZ2E 2.1, RZ1E 2.1, and RZ2B 1.1 were able to dissolve phosphate. only isolate RZ2B 1.1 produced siderophore. Two isolates produced chitinase RZ1E 2.1 and RZ2E 2.1. All isolates did not produce hemolysis. The best three isolates were obtained from RZ1E 2.1, RZ2E 2.1 and RZ2E 1.2 which have inhibitory properties against G.boninense*.

Keywords*— characterization, indigenous rhizobacteria, *G. boninense.

I. INTRODUCTION

Oil palm (Elaeis guineensis Jacq.) is a monocotyledon from the family Arecaceae (formerly Palmae) within the subfamily Cocosoideae It is a major crop that grows in the tropical areas mainly in Southeast Asia. Palm oil is used worldwide for processing food, cosmetics, phar- maceuticals, biodiesel and in oleo chemical industry The high economic value and its role in the plantation sector has caused the oil palm commodity to be widely cultivated in various regions in Indonesia. The national productivity of oil palm is 3.6 tons / ha / year (Direktorat Jendral Perkebunan, 2017). The productivity is still very low when compared to the optimal productivity of oil palm which should be able to reach 7 - 8 tons / ha / year (Fauzi *et al*., 2008).

One of the low productivity of oil palm plants is caused by plant pathogen attack. Basal Stem Rot (BSR) caused by *Ganoderma boninense* is a major disease in oil palm plantations in Southeast Asia including Indonesia (Chong *et al*., 2011). This disease is reported to cause losses of around 50-80% per ha Indonesia and Malaysia are the countries with the largest losses due to BSR estimated at 500 million USD / year (Rees, Flood, Hasan, Wills, & Cooper, 2012). BSR is difficult to control, because *G. boninense* is a soil-borne pathogen that has a wide range of hosts and has a special structure in the form of klamidospora and pseudosclerotia that have an impact on the ability to survive and infect target plant (Sanderson, 2005; Susanto *et al.*, 2013).

Control efforts that have been made to *G. boninense* namely physical control through sanitation techniques, and using synthetic functions have not shown maximum results because they can cause negative impacts on the environment such as the killing of non-pathogenic organisms, causing human health problems, animals, and the occurrence of resistance to pathogens (Puspita *et al*., 2013). Alternative control of *G. boninense* that is safe for the environment is to use biocontrol agents from groups of microorganisms (Bivi *et al.*, 2010). Microorganisms that have been widely reported as biocontrol agents are rizobacteria from the group Plant Growth Promothing Rhizobacteria (PGPR) (Beneduzi *et al*., 2012).

Rhizobacteria are a group of bacteria that can improve the quality of plant growth both directly and indirectly (Vishwakarma *et al.*, 2018). Rhizobacteria exert antagonistic effects on plant pathogens in several ways, namely siderophore production, chitinase enzymes, parasitism, competition for nutritional sources, inducing systemic plant resistance and being able to dissolve phosphates which can increase the availability of nutrients for plant growth (Verma *et al.*, 2010). Indigenous bacteria are better introduced to plants, because indigenous bacteria are more adaptable to the environment and more competitive than non-indigenous bacteria (Burelle *et al.*, 2006:Yanti *et al*., 2013).

Control of soil borne pathogens with rhizobacteria has been reported to be effective, some rhizobacteria that have been reported to have the ability as

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biocontrol agents against *G. boninense* include *Pseudomonas*, *Bacillus* spp, and *Burkholderia* (Suryanto *et al*., 2012; Buana *et al*., 2014). *Achromobacter, Bacillus, Burkholderia, Enterobacter, Ochrobactrum* and Providencia genera were able to be both non-pathogenic and possessed the ability to colonize the host plant (Gowtham *et al*., 2018).

Previous research (Rifai, 2018) obtained 7 indigenous rhizobacteria isolates isolated from the roots of PTPN IV oil palm plantations in Simalungun Regency as a result of in planta selection has the best ability to increase growth and resistance of oil palm seedlings to BSR disease. These isolates still need to be characterized for their ability to control BSR disease caused by *G. boninense*. This study aims to characterize the ability of PGPR and biocontrol of indigenous rhizobacteria isolates and inhibitory testing of *G. boninense* in vitro.

II. MATERIALS AND METHODS

This research has been done as an experiment at Laboratory of Microbiology, Department of Plant Protection, Faculty of Agriculture, and Biomedical Laboratory Faculty of Medicine University of Andalas, Padang, Indonesia from Januari to April 2019.

In Vitro Characterization of PGPR and Biocontrol of Rhizobacteria Isolates

a. Phosphate solubilization

The isolates' ability to solubilize tri-calcium phosphate was assayed using methods of Wahyudi *et al.*, (2011). The isolates was inoculated to Pikovskaya's Agar (Compositons per litre glucose 10g, Ca3 (PO4)2 5g, (NH4)2SO4 0.5g, KCl 0.2g,

b. Siderophore Production

Siderophore productions was determined using Chrome Azurol Sulphonate (CAS) agar medium described by (Husen, 2003). Each isolate was streaked on the surface of CAS agar medium and incubated at room temperature for 3 days. Siderophore production was indicated by orange halos around the colonies after the incubation.

c. Chitinase Test

Testing is done by dipping a 6 mm filter paper in indigenos rhizobacteria isolate suspension, then filter paper is placed on the surface of the media so that chitin is solid (15 g Bacto agar, 5 g glucose, 2 g peptone, 10 g colloidal chitin, 0.5 g K2HPO4, 0.5 g MgSO4, 0.5 g NaCl in 1 liter of distilled water (Cattelan *et al*., 1996).

d. Hemolytic assay

Hemolytic activity was determined using agar diffusion technique by Monteiro *et al.*, (2005) namely by using Blood Agar (TSA enriched with 5% of sheep blood pH of 7.3) where halo zone (hemolysis) around the colony observed as hemolytic activity.

e. Antagonist in vitro test

Rhizobacteria inhibition testing of *G.boninense* was carried out by the dual culture method by cutting *G.boninense* on a solid 5 mm diameter PDA using cork borer and placed on petridish containing mixed media (NA: PDA). Calculation of the radius of the colony was carried out at 7 days, until the Petri dish in the control treatment was fulfilled by *G. boninense*. The percentage of growth suppression of *G. boninense* is calculated by (Bivi *et al.*, 2010).

$$\text{PIRG} = \frac{R1 - R2}{R1} \; x\, 100\%$$

Where

R1: radius of the *G. boninense* colony in the direction towards the antagonist colony

R2: radius growth of *G. boninense* in the control plate

III. RESULT

Characteristics of PGPR and Biocontrol Indigenous Rhizobacteria Isolates

Rhizobacteria isolates of indigenous oil palm roots are character to determine their ability as Plant Growth Promothing Rhizobacteria (PGPR) and biocontrol in vitro. In this study, four rhizobacteria isolates of RZ1A 2.1, RZ2E 2.1, RZ1E 2.1, and RZ2B 1.1 were able to dissolve phosphate (table 1). This is indicated by the presence of clear zones produced by rhizobacteria isolates in *Pikovskaya agar* medium (Figure 1A). In this study, isolates that were able to dissolve adhesives were the *Bacillus* spp group. The *Bacillus* spp was group reported to have an advantage over other types of bacteria because it can excrete organic acids, such as formic acid, acetate and lactate which function to dissolve phosphate forms that are difficult to dissolve into forms available to plants so as to increase plant growth (Khan *et al.*, 2009; Mehrab *et al.*, 2010). *Bacillus* and *Arthrobacter* have ability solubilized phosphate (Vanissa *et al*., 2018).

Siderophore production showed that only isolate RZ2B 1.1 was able to produce siderophore with an orange zone around the disc paper on CAS media (Figure 1B). Siderophore production by rhizobacteria is one of the characters and a direct mechanism in suppressing the growth of pathogenic fungi. Siderophore directly stimulates the biosynthesis of antimicrobial compounds for the availability of minerals for bacteria that will suppress the growth of pathogens of *R. solani* and *F. oxysporum* which will induce the resistance of host plants (Wahyudi *et al.*, 2011). The ability of bacteria to produce siderophore also an important component in PGPR, because

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siderophore are able to bind iron ($Fe3$ +) into siderophor-iron bonds that become available to plant (Prihatiningsih *et al*., 2017; Ferreira *et al.*, 2019). Bacterial siderophores have a higher affinity for Fe than phytosiderophores and are able to remove Fe from Fe3+-phytosiderophore complexes (Aguado-Santacruz et al., 2012).

Testing the activity of indigenous rizhobacteria chitinase isolates obtained two isolates of RZ1E 2.1 and RZ2C 2.1 (Table 2). Isolates are able to produce chitinase enzymes with clear zones on chitin agar media (Figure 1C). Chitinase enzymes produced by indigenous rhizobacteria isolates play a role in the degradation or lysis of chitin which is the structure of *G. boninense* fungal cell wall. In line with the research of Wibowo *et al.*, (2017) which stated that 3 of 63 isolates of chitinase producing bacteria isolated from oil palm plantations showed three isolates TB04-05, SW0111, and SW02-08 were able to suppress the growth of *G. boninense* fungi. Azizah *et al.*, (2015) also stated that *Serratia marcescens* KAHN 15.12 and *B. amyloliquefaciens* SAHA 12.07 are able to produce chitinase so that it can suppress the development of *G. boninense* fungal hyphae. Hemolysis activity showed that all isolates of negative activity did not form a hemilysis zone (table 1), meaning that it was not pathogenic in humans and animals, so it was safe to be used as a candidate for biologycal control agents (Figure 1D) Figueroa-Lopez *et al.*, (2016) stated that pathogenic bacteria able to produce hemolysis so that it can cause disease for humans

Antagonist in vitro test

In vitro inhibition testing of rhizobacteria isolates against *G.boninense* in dual culture test showed six isolates were able to inhibit the growth of *G. boniense* with various inhibitory percentages at 7 days after inoculation. Three best rhizobacteria isolates were obtained, namely isolates RZ1E 2.1, RZ2E 2.1 and RZ2E 1.2.

Isolate RZ1E 2.1 has a high percentage of inhibition on the growth of *G. boninense* (Figure 2). Inhibition of growth of *G. boniense* with rhizobacteria in a dual culture test is thought to be influenced by the ability of rhizobacteria isolates to produce secondary metabolite compounds that can inhibit the growth of antifungal *G. boninense* fungi which is antifungal in accordance with the reported research (Bivi *et al.*, 2010; Suryanto *et al.*, 2012; Buana *et al*., 2014).

The mechanism responsible for the biocontrol activity of plant pathogenic fungi is through the production of antifungal compounds (Lee *et al*., 2015). The results of research Bakhtiar *et al.*, (2012) reported that *B. subtilis* B10 isolated from oil palm roots was able to produce active compounds that are antifungal in order to suppress the growth of *G. boninense* in vitro. is antifungal so that it can suppress the growth of *G. boninense* in vitro. Other research results Parvin *et al.*, (2016) confirmed that *Pseudomonas aeruginosa* isolated from palm oil rhizosphere produces metabolite products such as phenazine so that it can inhibit the growth of *G. boninense. P. aeruginosa* were able to inhibit G. boninense growth with the percentage of inhibition radial growth (PIRG) values of 71.42% (Muniroh *et al*., 2019).

IV. FIGURES AND TABLES

Table 1. Phosphate solubility, siderophore production, chitinase activity, and hemolytic activity of indigenous rhizobacteria isolates

Isolates	Phosphate solubility	Siderophore Production	Chitinase activity	Hemolytic activity
RZ1A 2.1	+	-	-	-
RZ2E 2.1	+	-	+	-
RZ1E 2.1	+	-	+	-
RZ2E 1.2	-	-	-	-
RZ2C 2.1	-	-	-	-
RZ2B 1.1	+	+	-	-
RZIE 1.2	-	-	-	-

Table 2. Percentage of inhibition ratio from rhizobacteria isolates against G.boninense growth in vitro

Isolates	**PIRG (%)**
RZ1E 2.1	70.22
RZ2E 2.1	62.00
RZ2E 1.2	45.50
RZ2C 1.2	40.56
RZ2B 1.1	40.00
RZ1A 2.1	35.00
RZ1E 1.2	0
Control	0

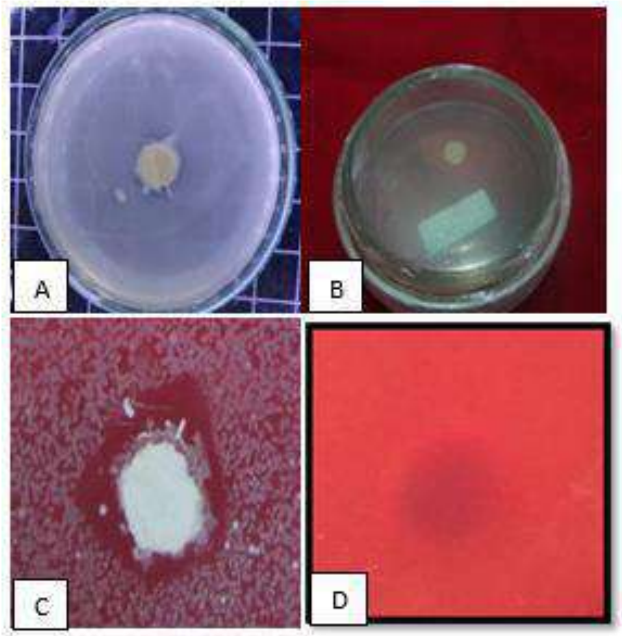

Fig.1: Character of indigenous rhizobacteria isolates (A) phosphate solubility (B) siderophore production (C) chitinase activity (D) hemolytic activity.

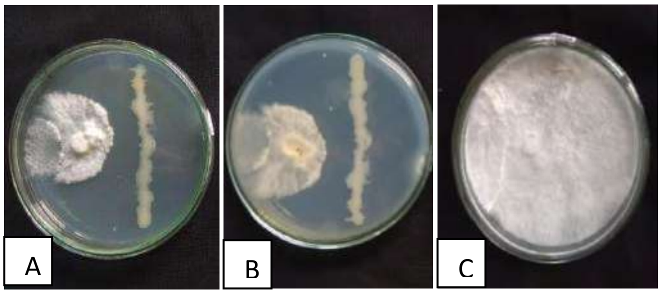

Fig.2: Effect of rhizobacteria isolates on the radial growth of G. boninense in the dual culture test (after 7 days of incubation) (A) Top, (B) bottom side of isolate RZ1E 2.1 and (C) G. boninense in control plate.

V. CONCLUSION

The results showed four isolates of RZ1A 2.1, RZ2E 2.1, RZ1E 2.1, and RZ2B 1.1 were able to dissolve phosphate. only isolate RZ2B 1.1 produced siderophore. Two isolates produced chitinase RZ1E 2.1 and RZ2E 2.1. All isolates did not produce hemolysis The best three isolates were obtained from RZ1E 2.1, RZ2E 2.1 and RZ2E 1.2 which have inhibitory properties against *G.boninense*

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The Effect of Time Shoulder (Topping) toward Growth and Results of Chocolate Seeds in Shadowing Conditions

Nursamsidar*, Kisman, I Wayan Sudika

Dry Land Study Program, University of Mataram, Indonesia

***Abstract*— This study aims to determine the effect of shoots of some brown soybean strains on shading stress conditions on growth and yield. The study was conducted in December 2018 - March 2019 Sekarbela District, Mataram. The experiment used a completely randomized two-factor design (CRD). Factor 1 is the strain (G) consisting of G1 = KH9, G2 = KH14, G3 = KH1 G4 = Dena, G5 = Anjasmoro. Factor 2 is the prune time (T) consisting of T1 = without pruning, T2 = pruning 3 MST, T3 = pruning 4 MST, T5 = pruning 5 MST and T5 = pruning 6 MST. The results showed that the KH14 brown soybean strain had better growth characters compared to the KH9, KH1 and yellow Dena and Anjasmoro varieties of soybean. The brown KH1 soybean strain showed high yields and components such as the weight of 100 seeds and the weight of the seeds of the plantings which was higher than other strains. Prune time affects the character of growth and yield of soybean plants. Prune time 3 MST showed the highest character of growth (plant height, number of productive branches, number of books), as well as yields and the highest yield components did not differ with the age of pruning 5 MST. There was no interaction between the prune time factor and soybean strain on the growth character and soybean yield*

***Keywords*— Chocolate soybean, pruning time, shade, growth, yield.**

I. INTRODUCTION

Soybean (Glycine max (L.) Merrill) is an important food commodity in Indonesia because it is a producer of protein, it is safe to consume and the price is relatively cheap compared to animal protein sources. BPS data (2019) shows that domestic soybean demand is still high, indicated by the number of soybean imports until 2017 of 2 671 914.1 tons

Soybean cultivation as intercropping under plantation stands, industrial timber estates (HTI), or intercropping with other annual food crops is a strategy to increase national soybean production. However, the presence of canopy shade from higher plants causes light to be a major obstacle or limiting factor for the growth and development of soybean plants. According to Asadi and Arsyad (1995); Asadi et al (1997), light intensity was reduced by 75% under the stand of plantation crops and 33% under intercropping with corn and sorghum

Decreasing the intensity of light will affect the growth and yield of plants. Reduction of light interception by 22% at the end of vegetative to reproductive start suppresses seed yield by 23% (Singer, 2001; Sopandie et al. 2003), affecting the pod filling phase after passing through the flowering phase (Mathew et el., 2000), causing elongation internod and plant vulnerability (Ephrath et el., 1993) and reduce seed weight (Sundari and Susanto, 2011). Some similar research results on the effect of low light intensity on growth and results have also been reported by Anderson and Osmond, 1987; Baharsjah, 1985; Chozin et al., 1999

The growth of soybean plants requires optimum solar radiation, (about 0.3-0.8 cal / cm2 / minute equivalent to 431-1152 cal / cm2 / day with light spectrum or wavelengths ranging from 400-700 nm to get high yields (Kassam 1978; Salisburry and Ross 1992) Free R / FR light and very low blue light intensity with a value of only 6.3 Wm-2 or 0.01 cal / cm2 / minute have been able to stimulate stem elongation in soybean plants (Wheeler et al., 1991) Board (2000) explains that the quality of light is determined by the ratio between red light with far red (FR) and blue light radiation which in this case affects the elongation process of the rod

The ability of plants to overcome shade stress depends on the ability of plants to carry out photosynthesis in light deficit conditions. This form of plant adaptation to low light intensity can be studied through specific responses at various levels such as anatomic, morphological, physiological, biochemical and molecular changes, this has been widely reported (Soepandie et al. 2001; Khumaida 2002; Murchie 2002; Alves 2002) . Anderson (2000) explains that plants that grow in such a dense environment are difficult to express their kinetic potential for maximum

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growth and development. Therefore a technology is needed to increase growth and yields in low light conditions. One such effort is pruning (Adisarwanto and Wudianto, 1999)

Pruning is one of the efforts to increase soybean productivity by cutting the upper section of the plant (Adisarwanto, 1999). Pruning improves crop yields by increasing root growth and availability of nitrogen nutrients (Saidi et al. 2007), increasing vegetative growth, triggering the growth of buds underneath which were initially dormant (Lakit 1995; Pinkard 2002) and increasing the leaf area index (Sadewa, 2000), have significant effect until very significant on the variable number of pods, number of seeds, weight of crop production, wet wet weight, weight of 100 dry beans of soybean plants (L 1995 and Bimasri 2013). Wachjar (1984) explains that basically pruning aims to regulate vegetative growth in the generative direction

Pruning stimulates the formation of productive branches in the hope that more flowers are formed so that the number of pods formed will increase more (Adisarwanto, 2002). the increase and growth of the number of branches is closely related to the increase in the number of fertile books (Sholeh et al. 2016). Several studies on other plants including cowpea mentioned that crop pruning is known to increase crop yields (Dugum et al. 1988; Ezedinma 1973), increase the number of pods in chickpea plants (Srirejeki et al. 2015), in tomato plants increase the percentage in fruit formation, total fruit weight (Febiola 2004; Esrita 2012) and addition of the number of side shoots due to cutting of shoots on Jatropha (Raden et al. 2009) and patchouli plants (Irawati and Setiari 2009)

There are several types of soybean species that develop in Indonesia, namely yellow soybeans, green soybeans and black soybeans. In addition to the yellow, black, and green soybeans above, there are also brown seeded soybean plants, a collection from Dr. Kisman which consists of several lines including KH7, KH8, KH9, KH14, KH50A, KH36D which are the results of crossing of soybeans and local soybeans in Lombok. The results of the initial description note that brown soybeans are able to grow and develop in the shade, so to improve the yield of brown soybeans it is necessary to conduct research on the application of shoot pruning technology in low light intensity conditions. Therefore research has been conducted on "the effect of topping time on topping on the growth and yield of cocoa beans under shading conditions".

II. MATERIALS AND METHODS

The method used in this study is an experimental method, by conducting a planting experiment using a poly bag. The experiment was carried out in a paranet house located on the South Ring Road, Sekarbela District, Mataram City. The experiment was arranged using a Completely Randomized Design (CRD) with two factorial experiments, as follows: Factor 1. Types of cocoa (G) strains consisted of: G1 = KH, G2 = KH14, G3 = KH1, G4 = Dena, G5 = Anjasmoro and Factor 2. Pruning time (T) consisting of: T1 = No Trimming, T2 = 3 MST, T3 = 4 MST, T4 = 5 MST, T5 = 6 MST. The combination of these two factors obtained 25 treatments. Each treatment was repeated 4 times to obtain 100 treatment units. All these polybag units are randomly placed under the paranet. The tools used in this experiment were scales, polybags, analytical scales, measuring devices (meters), scissors, labels and stationery - writing. The ingredients used in this experiment were the brown soybean strain of KH9, KH14, KH1 and Dena and Anjasmoro varieties of yellow soybean, inorganic fertilizer (NPK Ponska), manure and Marshall 25 ST pesticides, Dursban and Dethane M 45. The observed variables included: Plant height, number of books, number of productive branches, age of flowering, number of filled pods, number of planting seeds, weight of planting seeds, weight of 100 dry seeds and harvest age. The observational data were analyzed by Analysis of variance (Anova) at 5% significance level. Significantly different data (F arithmetic> F table), further tested using the DMRT (Duncan's Multiple Range Test) advanced test.

III. RESULTS AND DISCUSSION

Results of Analysis of Diversity (ANOVA) on Observed Variables

The results of the diversity analysis (ANOVA) of the observed variables showed that the treatment of prune time and strain type there was no interaction for all variables, while the treatment of prune time affected all observed variables. Likewise, the type of strain also affects all variables. (Table 1). Variables observed were plant height, number of productive branches, number of books, number of filled pods, number of seeds planted, weight of dry seeds of cropping, weight of 100 dried seeds, age of flowering and age of harvest.

Table.1 Results of analysis of variance (ANOVA) on some growth characters and soybean yields under pruning

Source	DF	Mean Square								
		(1*	(2*	(3*	(4*	(5*	(6*	(7*	(8*	(9*
Topping Periods	4	1894.14 s	85.84 s	565.46 s	8854.04 s	32399.10 s	324.14 s	1.41 s	1.37 ns	938089.10 ns
Lines	4	3713.31 s	72.69 s	356.16s	5257.01 s	20300.73 s	249.53 s	417.14 s	503.15 s	27.60 s
Topping periods * Strains	16	145.86 ns	2.83 ns	42.32 ns	955.93 ns	3382.95 ns	10.65 ns	0.76 ns	1.14 ns	2924.44 ns
Error	75	274,41	4,52	33,81	572,26	2161,21	15,03	0,84	0,48	36,08
Total	100									

Note : ns = non significant; s = significant;(1*hight plant;(2*Number of Branch;(3*Number of Node;(4*Number of fillet pod;(5*Number of seeds per plant;(6*Number of weight per plant;(7*Number of 100 seeds;(8*Days to Flower;(9* Harvesting date

Effect of soybean strain on the character of growth and yield of soybean plants under shade

In Table 2 it can be seen that the highest plants were obtained in the KH9 (G1) strain which was 119.65 cm while the lowest was obtained in Anjasmoro (G5) 85.86 cm. The highest number of productive branches was obtained in KH14 (G2) line which was 9.29 branches while the least number of branches in Anjasmoro (G5) was 4.76 branches which were not significantly different from Dena (G4) which were 5.45 branches. The highest number of books was seen in the KH14 (G2) line which was 19.20 books while the lowest was in Anjasmoro (G5) which was 9.35 books which were not significantly different from Dena (G4) 10.35 books and KH1 (G3) namely 10.9 books. The fastest flowering age was found in the Anjasmoro (G5) strain 33.71 days, while the longest flowering age was in the KH9 (G1) strain which was 45.24 days

Table 2 Effect of strain type on plant height, number of productive branches, number of books, age of flowering, number of filled pods, number of seeds planted, weight of dry seeds of planting, weight of 100 dried seeds, and age of harvest

Strain	Plant Height (cm)	Number of productive branches	The number of books	Flowering age (HST)	Number of filled pods	Number of planting seeds	Weight of planting seeds (g)	Weight 100 dry seeds (g)	Harvest Age (Days)
G1(KH9)	119.65d	7.33b	15.85 b	45.24d	79.80b	153.61b	8.68a	5.84a	114 d
G2 (KH14)	106.72c	9.29c	19.20c	39.45c	94.15c	180.44c	12.96b	7.29b	104.7c
G3 (KH1)	91.76 b	6.53b	10.90a	34.3b	59.35a	114.05a	18.08c	15.80d	91.43b
G4 (Dena)	95.29 b	5.45a	10.35a	33.96a	56.80a	110.84a	15.41b	14.95c	85.00a
G5 (Anjasmoro)	85.86 a	4.76a	9.35 a	33.71a	60.80a	109.81a	15.40b	14.52c	89.15b

Note: Figures followed by the same letters in the same column are not significantly different in the 5% DMRT follow-up test.

Table 2 shows the number of filled pods most obtained in the KH14 (G2) strain of 94.15 pods and the lowest in the Anjasmoro (G5) strain of 60.80 pods. The highest number of planting seeds obtained in the KH14 (G2) strain was 180.44 seeds while the least was Anjasmoro (G5) which was 109.81 seeds, which was not significantly different from KH1 (G3) which was 114.05. The highest seedling weight was obtained in the KH1 (G3) strain which was 18.80 g while the lowest in KH9 (G1) was 8.68 g. The highest weight of one hundred dry seeds was obtained in KH1 (G3) strain of 15.80 g while the lowest was obtained in KH9 (G1) strain of 5.84 g. The fastest harvest age is from the Dena (G4) variety that is 85 HST and is significantly different from other strains while the longest KH9 (G1) strain is 114 days.

Effect of soybean pruning time on the growth character and yield of soybean plants under shade

Table 3 shows that the highest plants were obtained at T1 (without pruning) ie 115 cm while the lowest was obtained at the pruning time of T5 (pruning 6 MST) ie 91.32 cm; the highest number of productive branches was obtained at the T2 prune (prune 3 MST) which was 9.85 branches, while the least was obtained at the pruning time T1 (without prune) at 4.80 branches; the most number of books obtained at the T4 pruning (pruning 5 MST) is 21.10 books while the least is obtained at T1 (without pruning) which is 7.20 books. The fastest flowering age was indicated at T1 (without pruning) at 36.96 while the longest at T2 (pruning 3 MST) was 37.65; The number of filled pods that were most obtained at the T2 pruning time (pruning 3 MST) and not significantly different from the T4 pruning time (pruning 5 MST) ie 93.15 and 90.45 respectively while the least obtained at the pruning time T1 (without pruning) ie 44,15 pods.

Table.3 Effect of pruning time on plant height, number of productive branches, number of books, age of flowering, number of filled pods, number of seeds planted, dry seed weight of planting, weight of 100 dried seeds, age of harvest

Prune Time	Plant height (cm)	Number of productive branches	The number of books	Flowering age (HST)	Number of filled pods	Number of planting seeds	Weight of planting seeds (g)	Weight 100 dry seeds (g)	Harvest Age (Days)
T1 (tanpapangkas)	115 d	4.80a	7.20a	36.96a	44.15a	59,75a	9.64 a	11.08a	94.70 a
T2 (pangkas 3MST)	102.8 c	9.85c	19.55c	37.65b	93.15c	141.8c	20.17 c	11.50 a	97.60 a
T3 (pangkas 4MST)	94.60 b	7.80b	14.35b	37.40ab	63.40b	103.45b	13.96 b	11.37a	97.30 a
T4 (pangkas 5MST)	93.48 b	6.80b	21.10c	37.45ab	90.45c	139.56c	20.20 c	11.72a	97.45 a
T5 (pangkas 6MST)	91.32 a	5.10a	13.45b	37.20ab	59.75b	93.10b	11.55b	11.71a	97.15 a

Note: Figures followed by the same letters in the same column are not significantly different in the 5% DMRT follow-up test.

Table 3 shows the number of planting seeds that were most obtained at the T2 pruning time (pruning 3 MST) and not significantly different from the pruning time of T4 (pruning 5 MST) ie 141.8 seeds and 134.56 seeds while at least at T1 pruning (without pruning) ie 59.75 seeds. The highest seed weight was obtained at the T2 pruning time (3MST pruning) and not significantly different from the T4 pruning time (5 MST pruning) ie 20.17 g and 20.20g respectively while the lowest was obtained at T1 (without pruning) ie 9.64 g. The weight of 100 dried seeds was not significantly different at all pruning times, with a weight ranging from 11.8 g - 11.70 g. The harvest age was not significantly different at all pruning times, which ranged from 97.15 - 97.70 days after planting.

IV. DISCUSSION

Effect of strain on the growth and yield of cocoa beans on shaded stress conditions

Light has a very important role in the process of growth and development of soybean plants. Low light intensity will affect the yield of these plants. It can be seen in table 2 that the KH9 (G1) strain had a plant height of 119.65 cm which was significantly higher than the other lines. Rahayu and Sumpena (2015) stated that differences in plant height can be caused by genetic factors and different adaptability of each soybean variety to the environment. The results of Mulyana's research (2006) show that the mechanism of morphological adaptation in shaded plants is to increase plant height. This result is clarified by Zhamal (2008); Harjadi and Yahya (2007) that the higher form of the plant (etiolation) is due to the activity of growth hormone, namely auxin. The results are in line with the report of Chozin et al. (1999) that the increase in height of rice plants varies between genotypes in a shaded environment

Based on Table 2, KH14 soybean strain (G2) has more productive branches than KH9, KH1, Dena and Anjasmoro soybean lines. The number of productive branches in each line ranged from 4.76 - 7.33 planted branches. Growth in the number of diverse branches is thought to be influenced by differences in genetic traits in each line, besides the difference in the number of branches can also be caused by environmental influences. The greater number of productive branches in KH14 is thought to be related to the greater number of books in KH14 lines. This condition is in line with what was reported by Irwan (2006); Adisarwanto (2007) that the number of branches in soybean depends on the environment and the variety. In line with the study of Muzaiyanah et al (2016) that the number of books correlates significantly positively with the number of branches in soybean plants

Based on Table 2, the fastest flowering age is Anjasmoro (G5) and Dena (G4) varieties at 33.71 days and 33.96 days, while the longest flowering age is obtained in KH9 (G1) soybean strain ie 45.24 day. The difference in flowering age in the various lines was suspected due to the genetic diversity factor of the strains tested. Similar results were reported by Suprapto (2001); Soverda et al. (2012); Kustera (2013) which states that the flowering age character in several soybean genotypes differ between genotypes

Based on Table 2, the highest number of pods produced by KH14 (G2) soybean lines was 94.15 pods, while the least was obtained at Anjasmoro (G5) which was not significantly different from KH1 (G3) and Dena (G4). The number of pods is determined at fertilization ie when pollen cells fertilize an egg in the ovary (Mimbar, 2004). The reduced number of crop pods is thought to be a result of reduced sunlight received by plants so that the process of photosynthesis is disrupted which results in reduced photosynthates which are allocated for pod formation. Chairudinet., Al (2015) reported that the decline in pods at various shading levels was caused by the inhibition of plant metabolic processes due to low light intensity. This implies a decrease in the amount of photosynthate to the seeds so that there is a decrease in the number of pods.

Based on Table 2, the number of seeds per plant line KH14 (G2) was higher than soybean lines KH9, KH14, Dena and Anjasmoro. This is thought to occur because KH14 (G2) soybean has more pods than other soybean strains. Saeed et al., (2007) reported that the number of seeds per plant had a significant positive correlation with the number of pods per plant. Results of the same study on green bean plants were reported by Makeen, et al., (2007); Rohman, et al. (2003); Judge (2008). Although KH14 (G2) soybean has the highest number of planting seeds, KH14 (G2) soybean has a lower seed weight than KH1, Dena and Anjasmoro because it has a relatively smaller weight of 100 seeds (7.29 gr / 100 seeds) and vice versa KH1 strain has a relatively small number of seeds per plant but has a higher seed weight per plant which is 18.08 g. . Weight increase of seeds per plant is not always followed by weight of 100 seeds. When associated with the number of filled pods, varieties that have many filled pods will have small seed sizes, due to competition between seeds to get photosynthate (Susanto and Adie, 2006)

Weight of 100 strain lines tested ranged from 5, 84 - 15.80 g / 100 seeds. According to Juwita (2012) the weight of 100 seeds is a quantitative character that is able to describe the size of the soybean seeds. Cahyono (2007) states that seed size is classified into three classes, namely small seeds (<10 g / 100 seeds), medium (11-14 g / 100 seeds) and large (> 14 g 100 seeds). In Table 4.2, there were three strains that had large seed sizes namely KH1, Floor Plan and Anjasmoro respectively 15.80 g / 100 seeds, 14.95 g / 100 seeds and 14.52 g / 100 seeds while KH9 and KH14 included soybean seeds each weighed 5.84 g / 100 seeds and 7.29 g / 100 seeds. This condition was explained by Wijayantiet., Al, (2014) not a positive positive correlation between the number of seeds per plant with a weight of 100 seeds. The difference in weight of 100 seeds of each strain is thought to be influenced by the genetic diversity of each strain, so strains with small seed weight will produce small seed weight sizes in other words, genetic factors are more dominant in determining the weight of 100 seeds of various lines in test.

The lines tested were significantly different in age at harvest. Dena (G4) tends to have a shorter harvest age while the longest harvest age is obtained in the KH9 (G1) strain. This is in line with the parameters of flowering age because the faster the age of flowering, the faster the age of harvest. According to Trihantoro (2010) flowering age is related to the physiological ripe age because the age of a plant is influenced by the flowering rate. Another factor that affects the age of soybean harvest is plant height. According to Rahajeng and Adie (2013) plant height affects the age of harvest and crop yields. The higher the plant, the longer the pod's ripe age will be, because the vegetative phase is longer

Effect of pruning time on the growth and yield of cocoa beans on shading stress conditions

Table 3 shows the best pruning ie at the T5 pruning time (prune 6 MST) which was significantly different from the other treatments. Pruning in this case aims to reduce the height of soybean plants under the shade. At the T5 pruning (pruning 6 MST) the height was 91.32 cm, shorter than the T4 treatment (93.48 cm), T3 (94.60 cm), T2 (102.80 cm), T1 (115 cm) . This condition is in line with research conducted by Srirejeki et al. (2015), namely bud pruning in bean plants can stop apical dominance so that stem length growth is slower. Other studies also conducted by Munawaroh and Aziz in Novianti (2016) report that pruning treatment on torbangun plants can inhibit plant height increase.

The most number of branches obtained at the T2 pruning (pruning 3 MST) is 9.85 branches, however the slow pruning time does not cause the addition of the number of branches at T3 (pruning 4 MST) and T4 (pruning 5 MST) as well as T5 (pruning 5) 6 MST) significantly. The growth of lateral branches due to pruning is influenced by the hormones auxin and cytokinins produced by plants (Takei et al., 2004). Significant increase in the number of branches was seen in pruning age of 3 MST, presumably because the plant was in the phase of maximum vegetative growth rate, consequently all buds with the potential to grow were maximally stimulated to produce more new branches, in contrast to pruning ages 5 and 6 of plant MST must divide the direction of vegetative growth into generative so that the formation of new shoots does not occur optimally. These results are in line with research reported by Rochayat et al., (2017) on frangipani plants, that pruning treatment shows an increase in the number of branches every week until the age of 6 MST and tends not to increase again at the age of 8 MST and so on. The same research results were also reported from Jatropha (Raden et al., 2009), patchouli plants (Irawati and Setiari 2009), Bitter (Januwati et al, 1996). According to Heddy (1986) this condition occurs because pruning of plant organs in the vegetative phase will cause the auxin growth hormone to accumulate at the point of growth which causes the emergence of new shoots and vice versa in the generative phase the hormone begins to decrease, the same thing is also reported (Lakit 1995; Pinkard, 2002)

Table 3 shows that the most number of books obtained at T4 pruning time (5 MST pruning) is 21.10 books and not significantly different from the T2 pruning time (3 MST pruning) which is 19.55 books, while the least at pruning time T1 (without prune) is 7.20 books. This is thought to occur because T4 (pruning 5 MST) has more branches than other pruning time treatments so that the chances of book formation are even greater. The increase and growth in the number of books is closely related to the number of branches where the more branches the more the number of books. The results of this study are in line with research reported by Anggraini et al 2017; Zamriyetty and Rambe (2006); Sholeh et al. 2016)

Table 3 shows that there were no statistically significant differences in the age of flowering in all treatments of soybean crop pruning time with control plants. Flowering age at all treatments when pruning ranged from 36-37 days after planting. This shows that the different pruning times did not affect the age of flowering days in all soybean lines which were pruned under the shade but were thought to be more influenced by genetic factors. This is in accordance with the opinion of Baharsja (1985) the main factor of flowering in soybean plants is more influenced by plant genetic traits

The number of crop pods is one of the components that affects the weight of soybean crop seeds. Zahara et al. (1994) states that in determining results, number of pods is the most important criterion. The highest number of pods was at the T2 pruning time (3 MST pruning) and not significantly different from the T4 pruning time (5 MST pruning) ie 93.15 and 90.45 pods, while the lowest at the T1 pruning time (without pruning) namely 44.15 pods. Overall treatment of pruning time increased in number of pods compared to plants without pruning. The results of this study are in line with those reported by Lakitan (1995); Bimasri (2013) that pruning has a significant effect on the number of pods, weight of crop production, wet weight

Table 3 obtained the highest number of seeds at T2 (prune 3 MST) and not significantly different from T4 (prune 4 MST), each of which was 141.8 seeds and 134.56 seeds while the lowest was at T1 (without prune) ie 59 .75 seeds. This happens because the treatment T2 (prune 3 MST) and T4 (prune 4 MST) produce more branches and pods compared to other treatments. Muzaiyanahet., All (2016) states the number of pods is positively correlated and is the factor that provides the greatest contribution to the number of seeds per plant

The highest seedling weight was at the T2 pruning time (pruning 3 MST) and not significantly different from the T4 pruning time (pruning 5 MST) ie 20.17 g and 20.20 g while the lowest at the pruning time T1 (without pruning)) which is 9.64 g. This is thought to occur because the number of seeds at the pruning time of T2 pruning (pruning 3 MST) and the pruning time of T4 (pruning 5 MST) is more than the other pruning time. Hapsari and Adie (2010) stated that the number of planting seeds was positively correlated with yields of crop seeds. Similar results were also reported by Wijayantiet., Al (2014); Muzaiyanah et all (2016)

The weight of 100 seeds reflects the size of the seeds. The dry weight of 100 seeds at all pruning times was not significantly different, with an average weight of 11.8 g - 11.70 g. These results indicate that the pruning time under shade does not spur increased seed weight. The difference in mean weight of 100 soybean seeds from each strain was assumed to be influenced by the genetic characteristics of each strain tested. Tulus (2011) in his research said that the maximum seed size is determined by genetic factors, whereas the actual size of the seeds produced is determined by the condition of the seeds during the filling period.

Table 3 shows that the age of harvest at all pruning times was not significantly different, with an average weight ranging from 94.60 days to 97.70 days. These results indicate that the pruning time under the shade does not affect the age of the harvest. According to Trihantoro (2010) flowering age is related to the physiological ripe age because the age of a plant is influenced by the flowering rate. The results of the same study were also reported by Pandiangan (2012) the age of harvest in soybean plants is very closely related to the age of flowering.

V. CONCLUSION

From the results of data analysis and discussion, it can be concluded that: 1) There is no interaction between pruning time and soybean strain on growth character and soybean yield 2) Under shading stress conditions, KH14 brown soybean strain shows better strain growth character than brown soybean plants KH9, KH1 and Dena and Anjasmoro varieties, while KH1 soybean strain showed 100 seeds weight and plant seed weight were higher than other strains. 3) Prune time 3 MST shows the highest character of growth (plant height, number of productive branches, number of books), as well as the highest yield and yield components not different from the age of 5 MST pankas

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Tropospheric Ozone Monitoring in Pune City

E. Ravendiran[1*], V. M. Motghare[2], Ajay R. Ojha[3], Prasad R. Pawar[3], Mohamad Nadeem Shaikh[3]

[1]Member Secretary, Maharashtra Pollution Control Board, Mumbai, India
[2]J.D. APC, Maharashtra Pollution Control Board, Mumbai, India
[3]Technogreen Environmental Solutions, Pune, India

***Abstract*— *Certain levels of stratospheric ozone are essential for existence of life. On the other hand, it has been proven that even very low levels of tropospheric or photochemical ozone damages plants and materials such as rubber and have harmful effects on the human respiratory system. With increasing industrialization and the tendency of majority of industries to congregate in areas which are already heavily industrialized, the problem of air pollution has begun to be felt in India. Short-term studies conducted by National Environmental Engineering Research Institute, Nagpur have confirmed that cities like Calcutta, Mumbai, Delhi and Pune are facing the impact of air pollution on a steadily increasing level. The present study deals with ground level ozone concentration at different sites in Pune. The sites have been categorized as residential cum commercial (Nalstop), commercial (Swargate), industrial (Bhosari), mixed land use (Karve Road), and area outside Pune (PimpriChinchwad Municipal Corporation). The average concentration of ozone in Pune as measured during the study period ranges from 0.39 ppmv to 3.36 ppmv. Bhosari has recorded maximum average concentration of ozone whereas Karve road shows the minimum average concentration of ozone.*

Keywords*— *Stratospheric ozone, photochemical, air pollution, urban air, major Indian cities.

I. INTRODUCTION

Ozone is one of the secondary air pollutants whose high concentration is harmful to humans and plants [3]. Ozone is known to be a toxic agent to humans and phytotoxic to vegetation [2]. Stratospheric input and photochemical ozone formation in the troposphere are the two main sources determining the ozone levels in the surface layer of the atmosphere. Because of the importance of ozone in controlling the atmospheric chemistry and its decisive role in the heat balance of atmosphere, leading to climate change, the examination of its formation and destruction are of great interest [4]. Several studies have been made concerning ozone behavior in the natural troposphere. However, the importance of studies on tropospheric ozone being realized, particularly in rapidly developing countries located in the tropics and subtropics, which are also regions of very limited measurements of ozone and its precursor gases(particularly Asia) [8]. Many studies measuring ozone concentrations have tended to focus on traffic intersections such as the ITO junction in Delhi [10[. Since ozone precursors and ozone travel large distances, it is important to measure ozone away from traffic corridors. Ozone concentrations may very well be much higher in non-traffic areas.

However, information on ozone levels in tropical areas, especially in Asian countries, which are at the threshold of rapid industrial development, is scanty. The Honorable Supreme Court of India has declared Pune as one amongst the 16 most polluted cities in India. With regards to these, various studies have been conducted in Pune since 2002 [7]. However, these studies have been restricted to aerodynamic diameter of 10 microns (PM_{10}). Like any other city, Pune is also witnessing a spurt in the number of vehicles. There are approximately 33,00,000 vehicles in Pune city. This year the growth rate of vehicles has doubled. The trips of the vehicles have also increased thereby making the growth rate of traffic 25% [7]. In the contextof the same, it was anticipated that surface ozone in Pune might be a critical parameter and indicator of air quality thereby scoping our studies to O_3 monitoring in Pune as an urban area.

II. MATERIALS AND METHODS

2.1. Study Area

Pune (18°32'N, 73°51'E) is situated on the Deccan Plateau, on the lee side of the Western Ghats (range of hills) and is about 100 km inland from the west coast of India. It is situated at a height of 560 meters above the mean sea level, near the confluence of Mula andMutha Rivers. Over the years the number of vehicles in Pune has increased. Around 10,000 vehicles are added to the Pune roads every month. This not only adds to congestion but also deteriorates the air quality of the city. It is estimated that about 60% of the Pune Municipal Corporation (PMC) roads in the heart of the city are congested whereas remaining 40% of the roads in the fringe area have relatively lower traffic volumes. Based on the criteria shown in TABLE 1,O_3 monitoring sites have been selected. The locations of the monitoring sites are represented in the map of the Pune city as shown in Fig. 1.

Fig.1. Map showing monitoring locations in Pune.

2.2. Meteorology

During monitoring period (January) prevailing wind direction was from the east with an average speed between 1.9 – 7.5 km/hr. The temperature averaged between 20-24˚C with a relative humidity ranging from 35 - 40%.

2.3. Frequency of Sampling

Monitoring was carried out at locations as described earlier for a period of one month. Samples were collected twice a week from Nalstop, Swargate and Bhosari as per the National Ambient Air Quality Monitoring Program (NAAQMP) protocol. Total samples collected from these three sites were twenty-four, eight samples from each site. At Karve road and Pimpri Chinchwad Municipal Corporation (P.C.M.C.) monitoring was carried out daily making in all 62 samples.

2.4. Analytical Protocol

High Volume Sampler (HVS) (Envirotech APM 460DX) was used for sampling. The O_3 concentrations were estimated by Neutral Buffered Potassium Iodide (NBKI) (pH=6.870.2) method, by bubbling a known volume of ambient air (1 l/min for 8 hrs). The absorbing reagent consists of 1 % potassium iodide in a neutral buffer composed of potassium dihydrogen phosphate annhydrous disodium hydrogen phosphate. The absorbing reagent was stored in the amber colored bottle and kept at room temperature for one day before using. The absorbing reagent was stored in the amber colored bottle in the refrigerator for several weeks [1]. The iodine liberated in the absorbing reagent is determined spectrophotometric ally by measuring the absorption of a tri-iodide ion at 352 nm. The range of this method extends from 0.01 ppm to 10 ppm. The reaction can be given as follows:

$O_3 + 3\ KI + H_2O \rightarrow KI_2 + 2\ KOH + O_2$

III. RESULTS AND DISCUSSIONS

Variations in the ground level ozone concentration observed at different sites, in Pune have been represented and discussed in the following sections.

3.1. Ozone Concentration at Karve Road

Results of ozone concentration at Karve Road are shown in Fig. 2; ozone shows marked temporal variation at Karve Road. The average concentration of ozone at this site was found to be 0.54 ppmv with a standard deviation of 0.06. At this site concentration of ozone varies from 0.398 ppmv – 0.65 ppmv. This sampling site is close to the center of the city, therefore, it primarily reflects the vehicular sources that are contributing to the ozone-forming precursors [6].

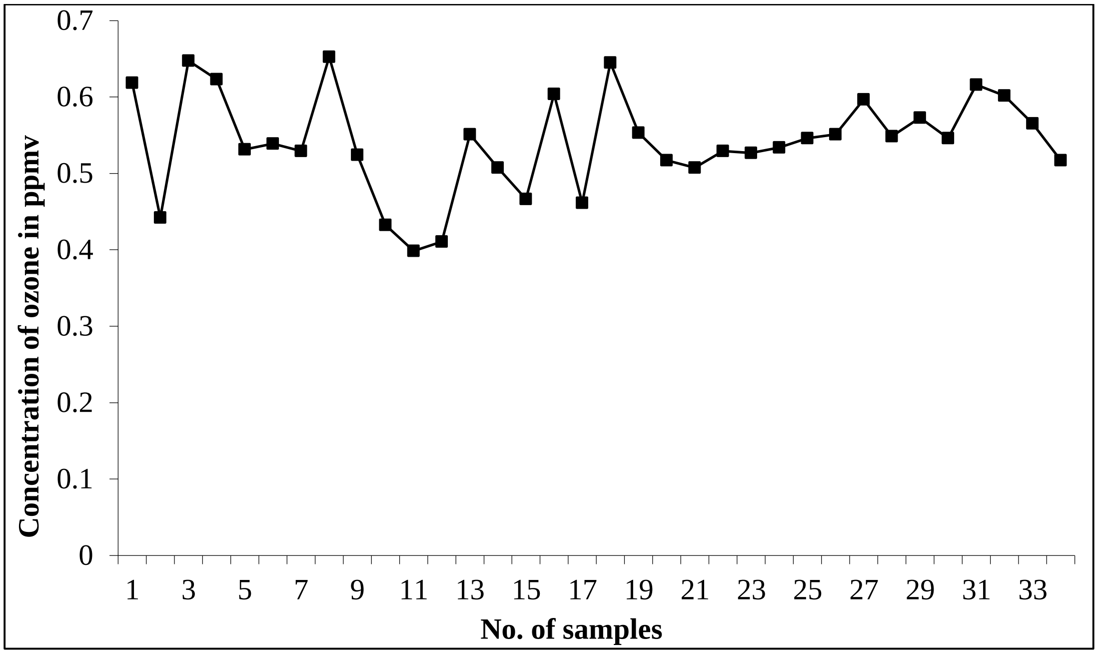

Fig.2. Concentration of ozone at Karve road.

3.2. Ozone Concentration at P.C.M.C.

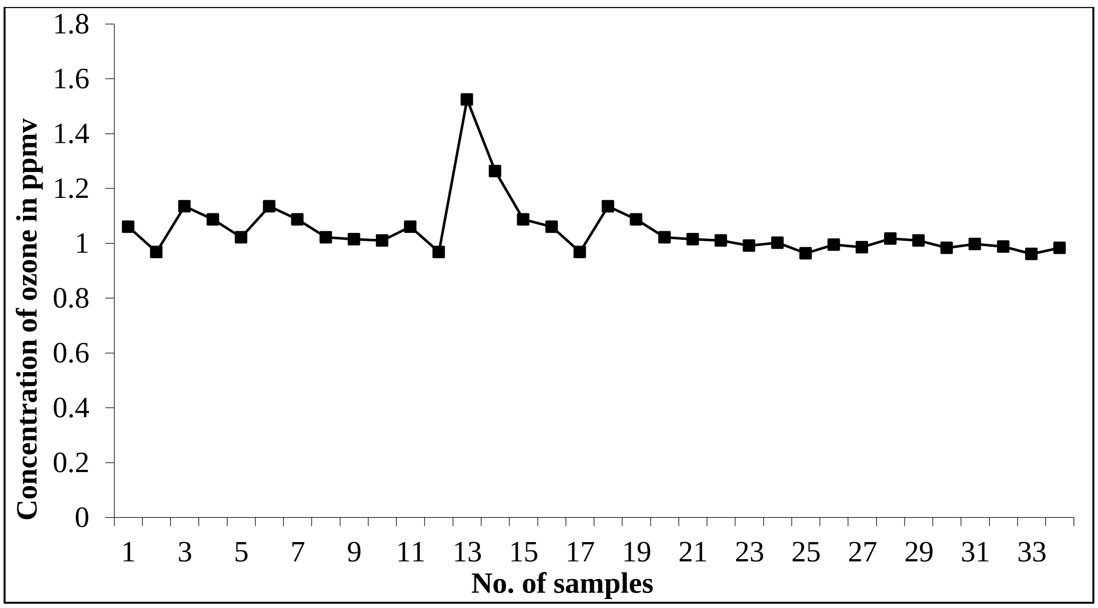

Fig.3. Concentration of ozone at Pimpri Chinchwad Municipal Corporation.

Fig. 3 shows comparatively higher temporal variation in ozone at P.C.M.C. than Karve Road. This site was selected to make a comparison between sites in Pune and site outside the Pune. The average concentration of ozone at this site was found to be 1.04 ppmv with standard deviation 0.1. At this site concentration of ozone varies from 0.96 ppmv to 1.52 ppmv. Ozone concentration observed at this site was higher than that of Karve Road. This area is of mixed land use; therefore there might be many different types of sources of ozone precursors.

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3. 3. Comparative Account of Ozone Concentration across Three Different Areas

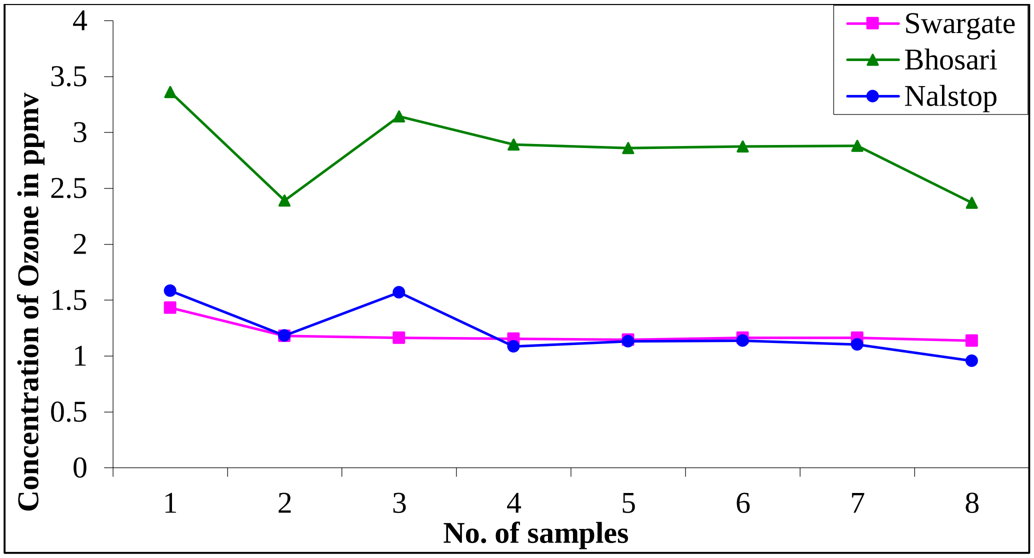

Fig.4. Concentration of ozone at three different areas.

Nalstop, Swargate and Bhosari sites are not regularly monitored. There is a marked similarity between the trends in ozone concentration at Swargate and Nalstop (Fig. 4).Swargate is a commercial area and it also represents the traffic intersection, with high vehicular density. Nalstop on the other hand is a residential cum commercial site. At these two sites, one of the major sources of ozone precursors seems to be traffic. Whereas the industrial site Bhosari shows a different pattern of ozone concentration revealing that there might be a number of different sources contributing to ozone precursors in this area.

3.4. Comparative Account of Ozone Concentration across Two Different Areas

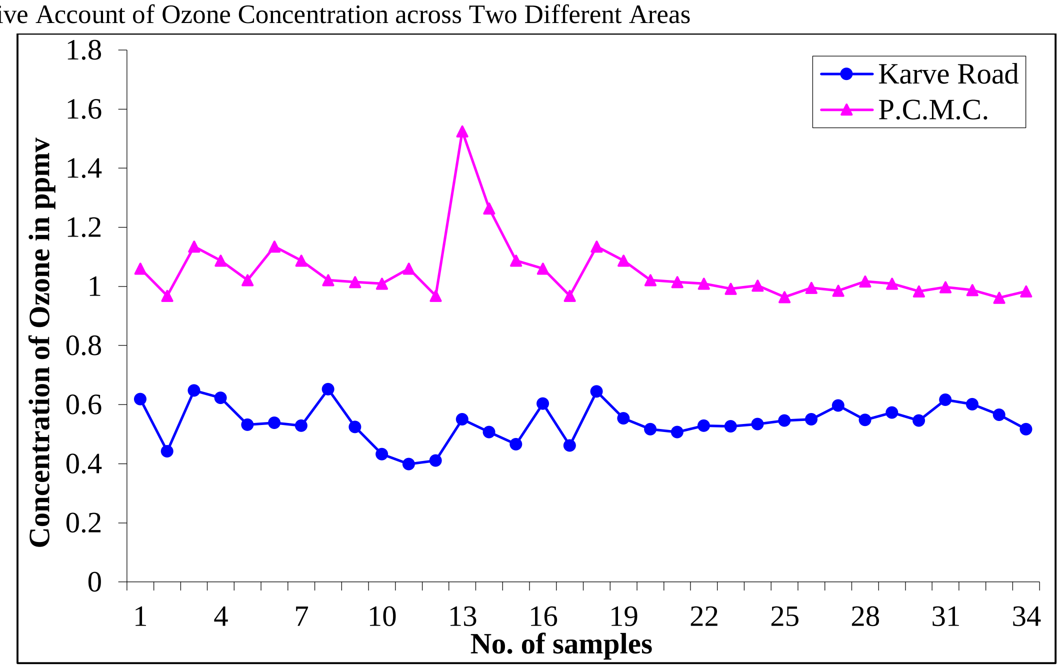

Fig.5. Concentration of ozone at two different areas.

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Karve road and P.C.M.C. sites were monitored daily for a period of one month. The concentration of ozone at P.C.M.C. was found to be much higher than the concentration at Karve road and is represented in Fig. 5.There might be various reasons of increased ozone concentration at P.C.M.C. such as it being near to the industrial zone, Bombay-Pune highway and in addition to that there was a road construction activity that resulted in the traffic congestion. On the other hand, at Karve Road only one source that is traffic, which might be contributing to a source of ozone precursor [5].

3.5. Spatial Variation of Ozone

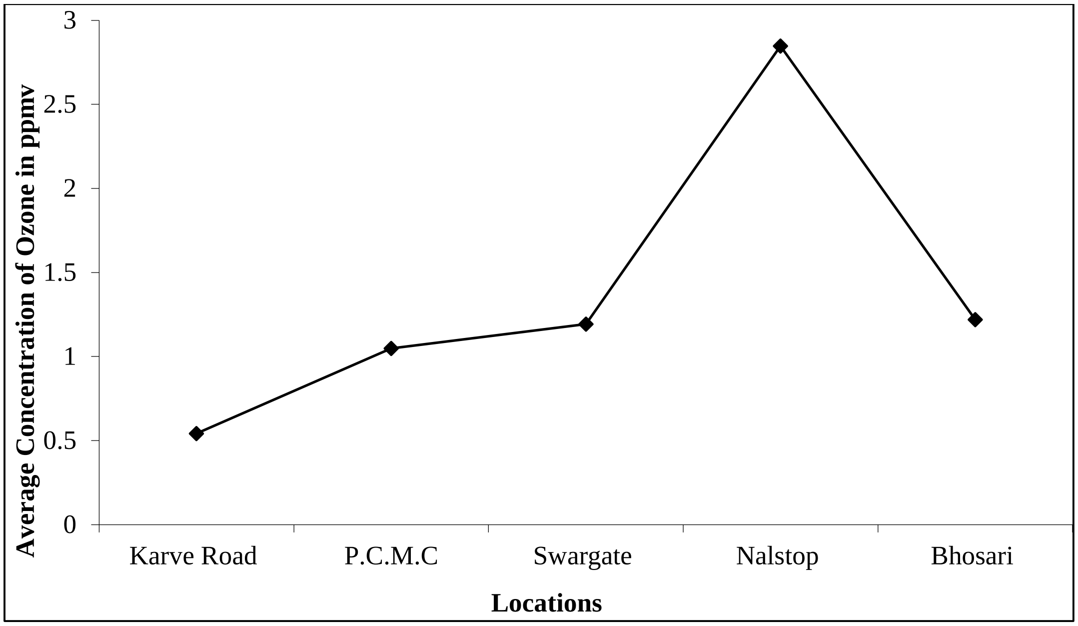

Fig.6. Spatial variation of ozone.

As shown in Fig. 6**,** Bhosari has recorded maximum average concentration of ozone. Bhosari being an industrial zone comprising mainly of petrochemical, chemicals, and other air polluting industries, it is quite expected to have high amounts NOx and VOC that indeed leads to increase in the concentration of ozone. Karve road shows the minimum average concentration of ozone. This is because there might be very low concentrations of the precursors required for ozone formation.

3.6. Comparison with the NOx data

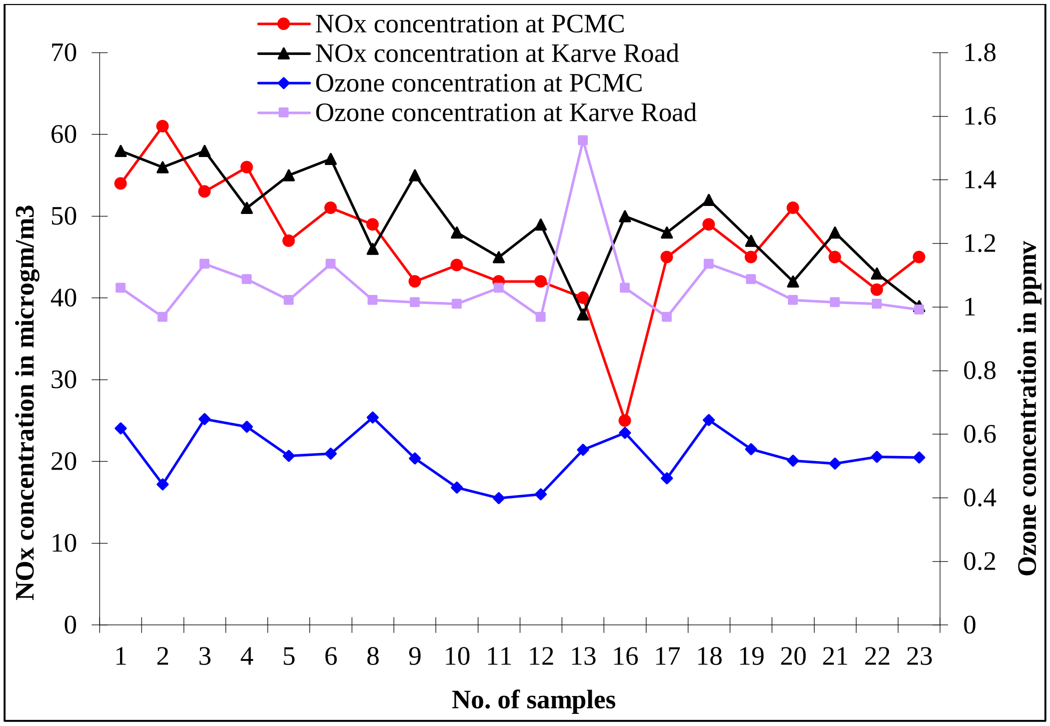

Fig.7. Comparison with the NOx data.

Maharashtra Pollution Control Board (MPCB) has carried out a sampling of NOx at different sites in Pune, under National Air Monitoring Program. High concentration of NOx encourages ozone production [5]. In order to sustain high levels of ozone, large amounts of precursors like NOx are needed. This leads to very low levels of these precursors, as they are used up for ozone production. As observed in Fig. 7, NOx and ozone concentrations show almost similar trend because the rate of emission of NOx is equivalent to the rate of ozone formation.

IV. CONCLUSION

Measurement of tropospheric ozone concentration in Pune shows the signature of the polluted site in tropics. The ambient air standard of 0.12 ppm adopted by the Environment Protection Agency (EPA) for ozone is violated on many occasions [9]. Differences in the ozone concentrations at various sites may be due to differences in the concentration of the precursors, secondary chemical reactions and more of the atmospheric chemistry related to ozone formation. Bhosari shows high variation in ozone concentration, which may be due to the significant increase of the precursor load from the industrial area. Most of the study sites are concentrated in the central part of the Pune city; hence ozone concentrations have been expected to primarily reflect the impact of vehicular sources. There is still a direct need for more thorough study investigation on the diurnal variations as well as measurement of precursors for a better understanding of the ozone behavior in fast developing cities of India.

V. ACKNOWLEDGEMENT

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Effect of Supplementation of Rumen-Protected Amino Acids to Barki Sheep on Some Blood Parameters

Younis, F. E. and Reda, A. Abd-Elazem

Animal and Poultry Physiology Department, Animal and Poultry Production Division, Desert Research Center, Egypt.

Abstract— *The objective of this study was to evaluate the effects of supplementing a traditional diet with rumen-protected (RP) either methionine (MET) or lysine (LYS) or their combination, on some physiological responses of ewes. Twenty-four adult Barki ewes were used were divided into four groups (6 ewes for each). The first group (Control) was fed only the control diet. The second group (LYS) fed the control diet and rumen-protected LYS (6g Lysi pearl /kg concentrate i.e. 3g /animal/day). The third group (MET) was fed the control diet and rumen-protected MET (14g Smartamin/kg concentrate i.e. 7g /animal/day). The fourth group (MIX) was fed the control diet and mixture of 3g LYS and 7g MET/animal/day. The experiment lasted for six months. Blood total proteins, urea, creatinine, total cholesterol, glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase were detected. Total lipids (TL), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and some plasma essential and non-essential amino acids were also analyzed.*

Supplemented ewes, especially with mixed LYS and MET, showed higher levels of TL, HDL, LDL, but lower creatinine. Treatment resulted in increased plasma Arginine, Lysine and Glutamine, while Leucine and Proline decreased. Other measured parameters were not affected.

Keywords— ***sheep, protected-Methionine and Lysine, blood biochemical parameters.***

I. INTRODUCTION

Protein is an important nutrient in ruminants fed low-quality forages. It becomes very necessary when animal attains its optimum growth or peak production. Moreover, nutrient requirements of ruminants vary according to the physiological state like growth, lactation and pregnancy. It is well accepted that amino acids, as nutrients, are building blocks of protein and play an essential role in the nutritional composition of a feedstuff.

The ruminants derive their amino acids from dietary protein which escapes rumen degradation and microbial protein synthesized in the rumen. The amount of protein and amino acids that escapes rumen degradation varies greatly among different feeds, depending on their solubility and the rate of passage to the small intestine (Kaufman and Lupping, 1982). In some situations, animal's requirements for amino acids not fully met from the normal sources of dietary protein.

Rapid and extensive degradation of valuable proteins in the rumen lead research to develop the concept of protein protection from ruminal degradation with the principal objective of enhancing the supply of essential amino acids to the productive animal and reduction of nitrogen losses as urea in the urine (Annison, 1981). Ruminant animals fed on poor quality forages with inadequate protein showed better performance with supplementation of quality protein or rumen-protected amino acid (RPAA) particularly methionine and lysine.

The main objective of this research was to investigate the effect of supplementation with rumen-protected amino acids, methionine or/and lysine on some plasma biochemical that indicate protein, energy and fat metabolism in Barki sheep in addition to plasma content of some essential and non-essential amino acids.

II. MATERIALS AND METHODS

The present study was conducted at Maryout Research Station, belonging to Desert Research Center, Ministry of Agriculture and Land Reclamation (Latitude 31.02°N, longitude 29.80°E) located 35-km south-west of Alexandria. The experiment was carried out from September 2016 to February 2017.

Animals and Management:

Twenty-four adult dry Barki ewes were used in this study. Age of ewes ranged between 3 and 4 years and average live body weight was 28.95±1.37 kg. The experimental animals were clinically examined to be healthy and free from diseases. They were fed on Berseem hay (*Trifolium alexandrinum*) that offered *ad-libitum* plus concentrate feed mixture (14% CP and 60% TDN,) at a rate of ½ kg/ewe/day. Concentrate feed mixture was composed of 50% cottonseed cake, 18% wheat bran, 15% yellow corn, 11% rice polish, 3% molasses, 2% limestone and 1% common salt. Proximate analyses were determined by the standard AOAC (2005), while nitrogen-free extract (NFE) was determined by the calculated difference (Table 1). Drinking water was provided twice daily.

Table.1: Proximate chemical analysis of different experimental roughages and concentrate feed mixture, (CFM) on Dry matter basis.

Items	Experimental rations	
	Hay	CFM
Dry Matter%	**88.80**	**89.66**
Crude Protein%	**17.50**	**14.20**
Crude Fiber%	**28.20**	**8.14**
Ether extract%	**1.38**	**3.36**
Nitrogen free extract%	**41.30**	**69.74**
Ash%	**11.61**	**4.56**

Experimental Design:

The experimental ewes were divided according to their live body weight into four groups (6 ewes each). The first group (Control): fed only the control diet. The second group (LYS) fed the control diet and rumen-protected lysine (3 g Lysipearl/animal/day). The third group (MET) fed the control diet and rumen-protected methionine (7 g Smartamin/animal/day) individually. The fourth group (MIX) fed the control diet and mixture of 3 g LYS and 7 g MET/animal/day). The rumen-protected amino acids in this experiment were obtained from United Biomed Company, Egypt.

Blood Biochemical Analyses:

Blood samples were collected from the jugular vein of each animal at the end of the experiment. The samples were collected in tubes containing anticoagulant (EDTA) and centrifuged at 3000 rpm for 30 min. Plasma was determined and stored at -18 ºC until laboratory assays. Biochemical analyses included some energy and protein metabolism indicators which were total protein (TP), glucose (Glu) and lactate dehydrogenase (LDH) activities. For lipid profile, cholesterol (Chol), total lipids (TL), high-density lipoproteins (HDL), and low-density lipoproteins (LDL) were assayed. For liver and kidney function plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine were examined. The analysis was conducted by standard methods using commercial kits supplied from Rosche Diagnostics (D-68298, Mannheim, Germany) in Roche Cobas C111 Clinical Chemistry Analyzer.

Amino acids Analysis:

Evaluation of plasma amino acids was executed using amino acid analyzer (Sykam Clarity Amino Acid Analyzer SW, Central Lab of Desert Research Center) according to (Pellet and Young, 1980).

Statistical Analysis:

Statistical analysis was carried out using A General Linear Model (GLM) procedures by SAS (2008) was used for statistical one-way analysis of variance. Duncan's New Multiple Range Test (Duncan, 1955) was used for separated differences among treatment means.

The linear model was: $\mathbf{Y_{ij}=\mu + G_i + e_{ij}}$

Where: Y_{ij}= Dependent variable.

μ= is the overall mean.

Gi= is the group effect i^{th} (i= 1, 2, 3, 4 where 1= control group, 2= lysine group, 3= methionine group and 4= lysine and methionine mixture group).

e_{ij}= The random errors assumed to be normally distributed with mean=0 and variance=^{2}.

III. RESULTS AND DISCUSSIONS

The effects on metabolic plasma indicators:

The effect of supplementation with protected amino acids on plasma biochemical parameters of Barki ewes that indicate metabolic statues are presented in Table (2). No significant differences were observed among treatments concerning the plasma contents of TP, Glu and Chol. Moreover, they were within the normal range as stated by Peter *et al..* (2002). On the other hand, TL increased (P<0.05) by treatments. Adding LYS or MIX could keep the level of HDL, but decreased LDL. However, adding MET decreased (P<0.05) HDL, the same trends reported by Hosseintabar et al., (2015) and Ma et al., (2014).

The glucose (Glu) and free fatty acids concentrations are good indicators of the energetic status. The dynamic interactions between these two major energy substrate pools, without hormonal mediation seems to indicate that the utilization of one nutrient (e.g. glucose) directly inhibits the use of the other (in this case fatty acids) in accordance with Hosseintabar et al., (2015) and Arslan et al., (2014).

Bouyeh and Gevorgyan (2011) observed a decrease followed by an increase in plasma cholesterol in birds feed Lys and Met above NRC recommendations.

These differences might be explained as the ability to completely oxidize non-esterified fatty acids (NEFA) and synthesize very-low-density lipoprotein (VLDL) is limited, thereby increasing the incidence rates of ketosis and fatty liver (Grummer et al.., 2004). Choline and methionine help in the transport of hepatic lipids by promoting the synthesis of phosphatidylcholine to package VLDL. Specifically, both of them play vital roles in 1-carbon unit transfer of dairy cows, modulate the synthesis of S-adenosylmethionine (SAM) in methionine cycle, and SAM functions as the most important methyl donor in the phosphatidylethanolamine to phosphatidylcholine formation (Osorio et al., 2014). Supply of methionine and choline enhance the capacity of liver to export triacylglycerol in the form of VLDL and help ameliorate the negative effects of fatty acid accumulation in the liver.

Table.2: Effect of adding lysine and methionine on some blood biochemical parameters of Barki ewes

Parameter	Treatment				Treatment
	CON	LYS	MET	MIX	SE
Total Protein, g/dl	6.08	6.74	6.70	6.77	0.25
Glucose, mg/dl	68.75	70.58	70.70	70.50	2.30
Cholesterol, mg/dl	84.10	87.33	85.64	91.33	3.41
Total Lipids, mg/dl	731.41^{b}	895.16^{a}	857.98^{a}	878.45^{a}	39.78*
HDL, mmol/l	38.37^{ab}	39.51^{a}	35.78^{b}	40.53^{a}	1.02*
LDL, mmol/l	32.58^{b}	27.73^{b}	36.82^{b}	50.40^{a}	4.56*

CON, Control group; LYS, Lysine group; MET, Methionine group and MIX, Lysine and Methionine mixture; HDL, high-density lipoproteins; LDL, low-density lipoproteins. $^{a,\,b}$: values in the same row for each trait with different superscripts significantly differ at P<0.05.

The effect on kidney and liver functions:

The effect of adding protected amino acids on the plasma compounds indicating kidney and liver functions of Barki ewes is presented in table (3). No significant differences were observed between treatments concerning the entire blood indicators of kidney and liver functions (urea, ALT, AST and ALP). Moreover, they were within the normal average as described by Peter *et al.* (2002). On the other hand, creatinine decreased (P<0.05) by adding rumen-protected LYS or MET and to a lesser extent by adding a mixture of both amino acids.

These results might point to that supplementation of both LYS and MET significantly decreased urea N concentration in plasma. Von Keyserlingk *et al.* (1999) observed that multiparous cows receiving a diet supplemented with bypass methionine and bypass protein had significantly lower blood urea nitrogen and creatinine levels when compared with the control ones. They added that cows receiving the bypass methionine and bypass protein with lower crude protein diets were able to maintain milk production. Moreover, Hellwing *et al.* (2007) explained that the plasma concentration of creatinine tended to decline with increasing protein meal levels.

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Table.3: Effect of adding lysine and methionine on some Kidney and Liver functions of Barki ewes

Items	Treatments				±SE
	CON	LYS	MET	MIX	
Urea, mg/dl	32.25	33.02	31.26	28.47	1.81
Creatinine, mg/dl	1.10[a]	0.76[b]	0.73[b]	0.95[ab]	0.07*
ALT, U/L	24.80	22.94	24.40	25.51	1.11
AST, U/L	33.51	34.84	36.06	33.40	2.21
Alkaline Phosphates, g/dl	35.85	41.99	41.51	36.01	3.96

CON, Control group; LYS, Lysine group; MET, Methionine group; and MIX, Lysine and Methionine mixture; NS, non-significant. * Significant at P<0.05, a, b: Values in the same row for each trait with different superscripts significantly differ at P<0.05.

Changes in plasma content of amino acids:

The effect of adding protected amino acids on plasma content of essential (EAA) and non-essential amino acid (NEAA) of Barki ewes are presented in Table (4). Adding protected LYS or/and MET caused a significant increase in plasma levels of arginine, lysine and glutamine, while it caused significant decrease in proline and to some extent leucine.

Table.4: Effect of addition protected amino acids on plasma content of essential amino acid and Non-essential amino acid (%) in Barki ewes

Amino acid	Treatments			
	CON	Lysine	Methionine	MIX
<u>**Essential amino acids**</u>				
Arginine	2.79±0.05[b]	4.00±0.32[a]	4.20±0.24[a]	4.10±0.24[a]
Histidine	3.61±0.07	3.30±0.21	3.92±0.23	3.60±0.21
Isoleucine	3.07±0.06	3.00±0.17	2.96±0.17	3.10±0.18
Leucine	9.43±0.16 [a]	8.20±0.47[b]	8.46±0.49[ab]	8.60±0.50[ab]
Lysine	8.83±0.16[b]	10.60±0.61[a]	10.20±0.59[a]	10.40±0.60[a]
Phenylalanine	5.30±0.09	5.10±0.29	5.50±0.32	5.40±0.31
Threonine	5.81±0.14	6.60±0.38	6.58±0.38	6.60±0.38
Valine	7.07±0.08	7.40±0.43	7.90±0.46	7.10±0.41
Methionine	0.26±0.16	0.4±0.02	0.32±0.02	0.33±0.02
<u>**Non-essential amino acids**</u>				
Tyrosine	4.43±0.11	4.80±0.28	4.60±0.27	4.70±0.27
Cysteine	2.30±0.10	2.20±0.13	2.40±0.14	2.30±0.13
Glutamine	12.49±0.12[b]	14.80±0.85[a]	14.50±0.84[a]	14.62±0.84[a]
Glycine	5.53±0.16	4.10±0.24	3.90±0.23	2.12±0.24
Proline	6.06±0.06[a]	3.70±0.21[b]	3.40±0.20[b]	3.84±0.22[b]
Serine	5.38±0.12	5.40±0.31	5.30±0.31	5.20±0.30
Alanine	4.94±0.05	5.20±0.30	4.90±0.28	5.10±0.29
Asparagine	8.23±0.35	9.50±0.55	9.61±0.55	9.60±0.55

Mean values with different superscripts within the same row are significantly different at (P<0.05)

Han *et al.* (1996) stated that supplementing rumen-protected lysine in the diets of sheep caused an increase in plasma concentrations of lysine, arginine, asparagine, threonine, serine, valine and leucine compared with control ones. Oke *et al.* (1986) found that feeding rumen-protected lysine or rumen-protected methionine and abomasal infusion of lysine caused similar increase in plasma concentration of these amino acids, but feeding unprotected lysine and methionine did not increase plasma levels of these amino acids. Other researchers have demonstrated that blood concentration of limiting amino acids remains relatively constant until tissue requirements are met (Nimrick *et al.*, 1970 and Bergen, 1979). Also, when sheep were infused of 1.06 g methionine, in 2 equal doses daily, there was an increase in molar proportions of lysine and methionine in plasma and there decreases in plasma threonine (Amos *et al.*, 1974). However, Reis *et al.* (1973) confirmed that supplementation of dietary methionine (ten grams per day) didn't cause any change in the total content of plasma amino acids nor in the concentration of any individual amino acid.

IV. CONCLUSION

Adding protected amino acids lysine or/and methionine did not affect plasma TP or glucose, while caused a significant increase in some blood AA without any adverse effect on kidney and liver functions. Rumen protected amino acids must be provided in a ruminant diet as they will be available in the small intestine for complete absorption. Among rumen-protected amino acids, Lys and Met are so important since their deficiency limited the milk production and growth of ruminant animals. Supplementation of these amino acids was especially useful in rations with, particularly low protein content.

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Characterization of some local varieties of fig tree (*Ficus carica* L.) in Oued Laou region of Morocco

El Oualkadi A*, Hajjaj B*

*INRA- Regional Agricultural Research Center of Tangier, Morocco
Corresponding author. E-mail: ai.oualkadi@gmail.com

Abstract— *A Prospection was conducted at the Oued laou region of Morocco in order to evaluate the existing genetic resources and collect the local fig cultivars of the zone. As a result, a total of 121 different accessions were collected and studied for preliminary variety identification, which was confirmed on the basis of biometric observations. Several synonymies and homonymies were detected. A total of 13 different cultivars were identified. Comparison of the ecotype shows the high significatif difference. Conservation of the local cultivars is highly recommended.*
Keywords— *fig tree, genetic resources, biometric analysis, Oued Laou, Morocco.*

I. INTRODUCTION

Common fig (*Ficus carica L.*) is an fruit crop cultivated in Mediterranean countries since antiquity. Common fig is considered, together with grape (*Vitis vinifera* L.) and olive (*Olea europaea* L.), classical fruit trees associated with the beginning of horticulture in the Mediterranean Basin [1], and consequently it is one of the earliest domesticated fruit tree species [2] ; [3]; [4]. In fact, recent studies [5] indicate that common fig is probably the first domesticated crop of the Neolithic revolution. This fruit crop is widespread in the Mediterranean basin countries since it is well adapted to either different soils or climates [6]. Despite its socioeconomic and historical importance, fig is considered a minor fruit species in Morocco. The regions in which the fig tree assumes economic importance are Taounate (22230 ha), Chefchaouen (7050 ha), Al Hoceima (5000 ha), Ouazzane (3150 ha), Tetouan (2000 ha) [7]. In Morocco, the production of fresh figs in 2018 growing season for fresh consumption was approximately 57000 tons with a total area of 46000 hectares (ha) of fig plantations in Morocco [7]. Particularly, in rural area, fig production assumes economic importance mainly in Ouad Laou area. Surveys done in different regions of Morocco contributed to identify and describe numerous cultivars [8]; [9]; [10]; [11]. In the present work, a prospection has been carried out during the period from 2015 to 2016 in the Northwestern (Oued Laou region) of the Morocco, in order to collect unknown or endangered varieties and avoid their extinction. The study focuses in old plantations by contributing to identify and collect minor or neglected varieties that in many cases had local names, and were unknown in others. Pomological parameters description has been carried out in order to identify the studied varieties.

II. MATERIAL AND METHODS

Prospection and sampling have been carried out at different localities in Oued Laou region in Northwestern of morocco (fig. 1). In total 121 accessions were gathered. In many cases, either isolated plants or plants located at old fig plantations areas were sampled (Table 1).

Fig. 1. Collection sites.

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Table 1: Key data for location of prospecting sites

Number of ecotype	Douar	Caîdat	Locatlity	Altitude	Geographic coordinate
25	Riffiyine	Oued laou	Sidi kassim	25m	35°N - 31,12' 0,5° W 13,949'
24	Tamernoute	Amsa	Sidi kassim	33m	35°N - 31,026' 0,05°W -10,832'
24	Awchtam	Amsa	Sidi kassim	35m	35°N - 30,293' 0,5°W - 10,093'
24	Tamernoute	Khandk lghrik	Kaâ asrass	71m	35°N - 24,300' 0,05°W - 03,200'
24	Tarzoute	Arbaa beni hassan	Talamboute	298m	35°N - 15,9871' 0,5°W - 13,822'

Plant material

Fig fruits from eight cultivars (Ferzaouia, Baghi, Gaouzi, Tabli, Baghi assal, Harchi, Roudane, Kharaza, Meltoufa, Harchi lkhal, harchi labyad, Hazouta and Tahadakte) were harvested from the five respective areas during cropping seasons 2015 and 2016. Cultivars were selected for their large distribution and their commercial value in the five regions. Samples of 121 homogenous fruits (three replicates of 10 fruits each) were chosen for each ecotype. Fruits were selected ripe and free from diseases.

Pomological characters

Biometric Approach

To examine the characteristics of the fruit for each ecotype, it was considered useful to approach a biometric study of the fruit based on the evaluation of the weight, caliber, dimensions and ostiole of the fruit. For each ecotype, a sample of twenty four fruits was randomly collected from different branches of the tree. Fruit weight was measured using a laboratory precision balance. Dimensions of the fruits such length, width, height and ostiole width were measured using a caliper (Figure 2). The descriptors used were adapted list drawn up by European program GEN LMBO 029 [12].

General appearance of the fruit:

The general appearance of the fruit corresponds to its external form. In this aspect, we were interested in the shape and size of the fruit.

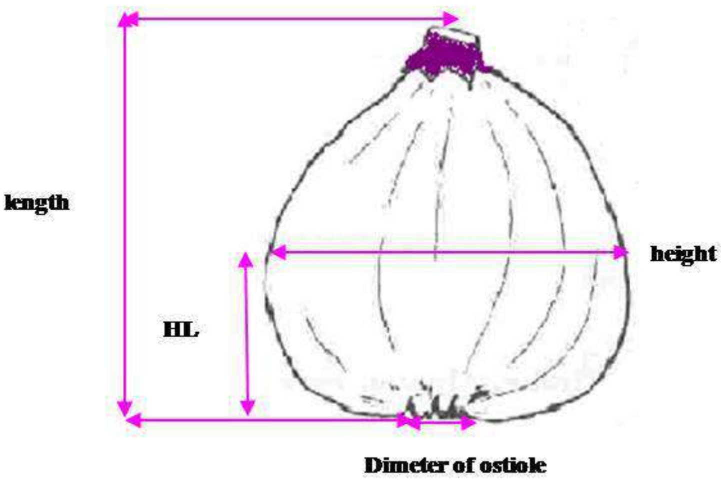

Fig.2: Representative diagram of measurements made on fig fruit

Fruit Shape

The fruits are of variable shape within the same tree and during the same season [13]. To avoid this hazard, we often rely on the presence or absence of neck. Other authors rely on three dimensions of the fruit to differentiate the varieties: the length C, the diameter D, and the distance A separating the base from the center of the circle of diameter D. The study of the shape of the fruit is important because it is related to the treatment that is applied to it. This is how the flattened shape with a short neck is ideal for canning. Other forms require certain precautions during transport; and others may facilitate the marketing of fresh fruits.

Statistical analysis

Comparison between the ecotype was made by statistical analysis of collected data. Statistical analyzes are performed

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with SPSS software version 21. Ghraphs was made by Excel version 2013.

III. RESULT AND DISCUSSION

Many of the sampled accessions were collected either as unidentified or with local names. As result of the biometric approach the prospected plant material was characterized. 121 accessions were identified in owed law region. The average weight of the varieties studied (Fig 3) varied from 23.1g to 57.92g. The Tahadakte variety shows the highest weight while the Hafzaouia variety shows the lowest weight. The analysis of variance showed 10 homogeneous groups from the weight fruit (Table 2). The variety Hazouta, Harchi lkhal, Rhoudane and Gaouzi are significantly not different for the parmeter weight of fruit. The varieties Ferzaouia and Tabli are significantly not different. The varieties Kharaza, Baghi, Tahadakte, Harchi lbayd, Meltoufa, Harchi and Baghi assal form each one a group significanlty diffrent from pthers for wight fruit (Table 2).

The average length of the fruit varied between 3.9 cm and 5.25 cm (Fig. 4), the variety Hazouta showed the greatest value of the length of the fruit whereas the variety Tabli showed the lowest value of the length of the fruit. Gaouizi, Rhoudane, kharaza, Meltoufa, Tahadakte and Hazouta , are not significantly different. Ferzaouia, Baghi assal are not significantly. The variety Tabli, Harchi, Harchi Labyad, Baghi, Harchi lkhal formed each one group significantly different from each other and from others groups (Table 2).

The width of the fruit varied between 3.43 cm and 5.11 cm (Fig 5), the Rhoudane variety shows the weaker the value of width fruit while the variety Ferzaouia shows the greatest value of the width of the fruit (Fgi.5). The variety Gaouizi, Harchi, Harchi labayd, Tahadakte and Hazouta groued in one group and they are significantly not different from the width of fruit. Also the variety Kharaza and Baghi are significantly not different (Table 2).

The value of HL varied between 2.53 cm and 1.83 cm (Fig.6), the variety Ferzaouia shows the greatest value (2.53 cm) while the variety Tahadakte shows the lowest value 1.83 cm. The variety Gaouizi, Baghi assal, Harchi and Rhoudane are not significantly different, also the variety Ferzaouia, Tabli and Meltoufa are not significantly different. The variety Harchi Labyad and Baghi are not significantly different from the parmeter HL fruit (Table 2).

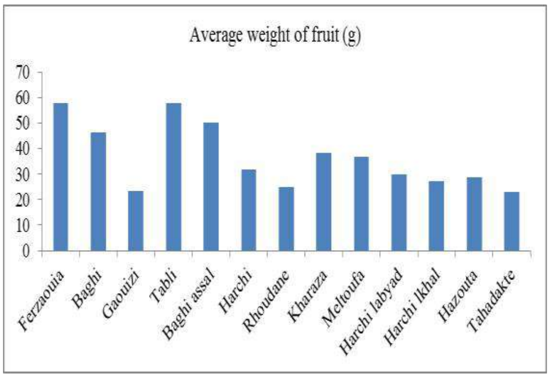

Fig 3 : Average weight of fruit

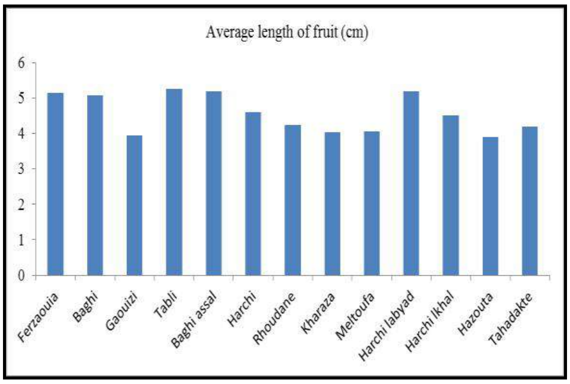

Fig 4: Average length of fruit (cm)

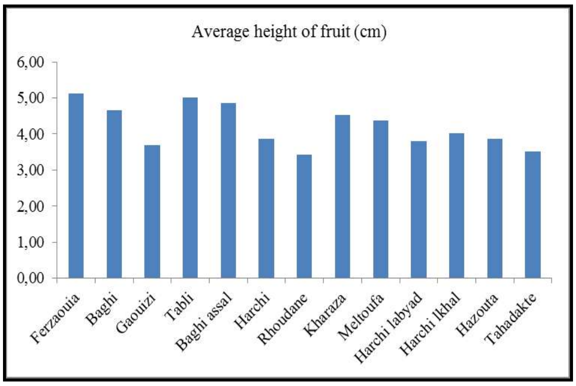

Fig 5 : Average height of fruit (cm)

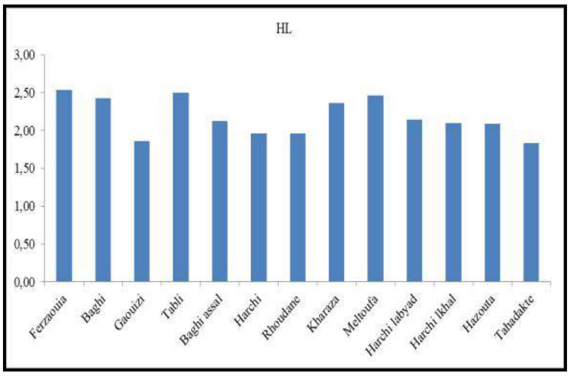

Fig 6: HL of fruit

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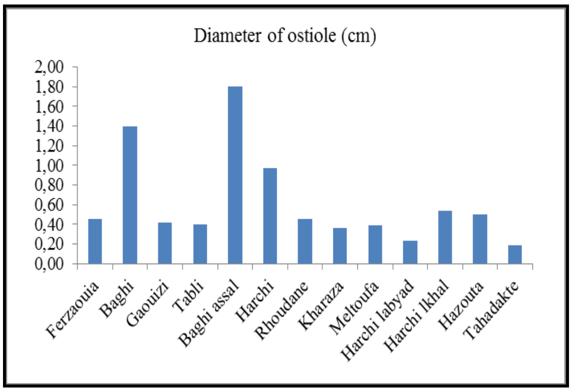

Fig 7: Dimeter of ostiole

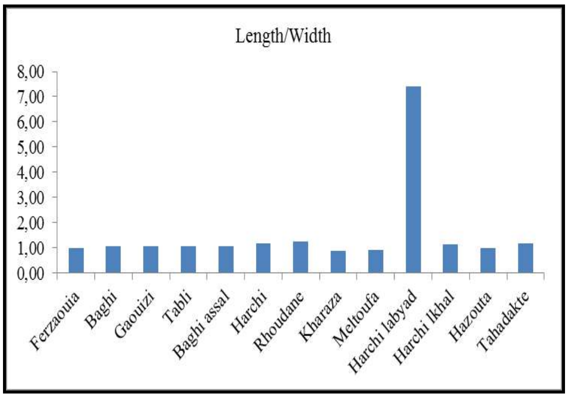

Fig 8: Length / width of fruit

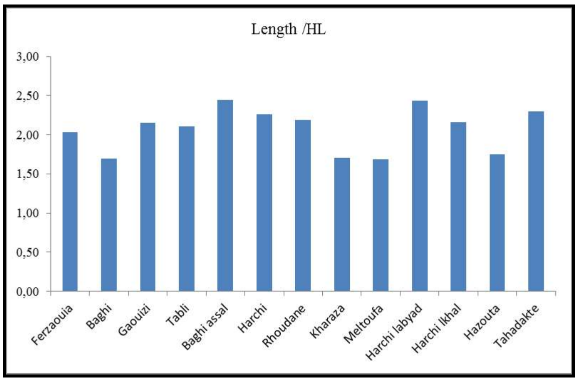

Fig 9: Length / HL of fruit

The diameter of the ostiole varied between 0.19 cm and 1.80 cm (Fig.7) the variety Tahadakte shows the smallest value of the diameter of the ostiole whereas the variety Baghi Assal shows the greatest value of the diameter of the ostiole. The analysis of variance show that the variety Ferzaouia, Baghi assal, Harchi, Rhoudane, Baghi, Harchi lkhal and Hazouta are not significantly different. The variety Gaouizi and Tabli are not significantly different. Also the variety Kharaza and Meltoufa are not significantly different from diameter of ostiole (Table 2)

The length/ Width ratio of the fruit varies between 0.89 and 7.42 (Fig. 8), the greatest value of the length / width ratio of the fruit was observed in the Harchi labyad variety, while the lowest value was observed in the variety Kharaza. The analysis of variance shows that the variety Gaouizi, Baghi assal and Harchi lkhal are not significantly different. Also the variety Ferzaouia and Hazouta are not significantly different (Table 2).

The length / HL of the fruit varied between 1.68 and 2.45 (Fig. 9), it is found that the variety Meltoufa showed the lowest value of the ratio length / HL while the variety Baghi Assal showed the most great value. Table 2 show that the variety Harchi, Rhoudane, Baghi and Tahadakte are not significantly different.

In these results, we find that the variety Ferzaouia showed the greatest value of the weight of the fruit, HL and the width of the fruit. And the Baghi assal variety showed the greatest value of the ostiole diameter and the length / HL when the Tahadakte variety showed the lowest value of fruit weight and ostiole diameter.

Table 2: Biometric characteristics of fig cultivars harvested from the region

Variety	Weight (g)	length (cm)	width (cm)	HI (cm)	Diameter of ostiole (cm)	Length/Width	Length / HL
Ferzaouia	57,92 *f*	5,13 *bcd*	5,11 *f*	2,53 *d*	0,46 *c*	1,02 *bcd*	2,03 *bc*
Gaouizi	23,47 *ab*	3,96 *a*	3,69 *ab*	1,86 *ab*	0,42 *bc*	1,08 *def*	2,15 *cde*
Tabli	57,78 *f*	5,26 *d*	5,00 *ef*	2,50 *d*	0,*40 bc*	1,06 *cde*	2,11 *bcde*
Baghi assal	50,28 *ef*	5,14 *bcd*	4,76 *def*	2,06 *ab*	0,56 *c*	1,08 *def*	2,51 *e*
Harchi	31,80 *bcd*	4,59 *abc*	3,87 *ab*	1,96 *ab*	0,52 *c*	1,18 *efg*	2,26 *de*
Rhoudane	25,08 ab	4,24 *a*	3,43 *a*	1,96 *ab*	0,46 *c*	1,24 *g*	2,19 *de*
Kharaza	38,10 *d*	4,03 *a*	4,51 *de*	2,36 *ce*	0,36 *abc*	0,89 *b*	1,71 *b*
Meltoufa	36,86 *cd*	4,05 *a*	4,36 *cd*	2,46 *d*	0,39 *abc*	0,93 *bc*	1,68 *b*
Harchi labyad	29,85 *abc*	5,19 *cd*	3,81 *ab*	2,14 *bd*	0,23 *ab*	0,00 *a*	0,00 *a*
Baghi	47,06 *e*	5,62 *d*	4,57 *de*	2,16 *bd*	0,54 *c*	1,*23 fg*	2,39 *de*
Harchi lkhal	27,20 ab	4,52*ab*	4,02 *bc*	2,10 abc	0,48 *c*	1,14 *def*	2,16 *cde*
Tahadakte	23,10 *a*	4,19 *a*	3,52 *ab*	1,83 *a*	0,19 *a*	1,19 *efg*	2,30 *de*
Hazouta	28,63 ab	3,90 *a*	3,87 *ab*	2,08 *abc*	0,50 *c*	1,00 *bcd*	1,75 *bc*

Pα = 0.05	*0*	*0*	*0*	*0*	*0*	*0*	*0*

Significant differences within the same column and means followed by the same letter do not differ at Pα ≤0.05 according to Duncan test.

IV. CONCLUSIONS

The present study highlights the necessity to identify the cultivars in the region of Oued Laou in Northwestern Morocco. The plant material used corresponds to very old varieties or local denominations. Although the Prospection had been made on a limited area, it had shown the existence of a great varietal diversity in this region. Indeed, 13 “local varieties” were listed in this study and showed high biometric characteristic. The minor varieties detected in the present study should be preserved in germplasm banks in order to prevent their extinction and maintain the biodiversity of the region.

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Impact of Fertilizer Usage on Crop Productivity at Siguidolo

Djeneba Dembele[1*], Mamadou Ballo[2], Abdou Ballo[3], Bocar dit Sire Ba[4]

[1]Laboratoire sol-Eau-Plante, Institut d'Economie Rurale (IER), Mali,
[2]Laboratoire d'Ecologie tropicale, Université des Sciences Techniqueset des Technologies de Bamako, Mali
[3]Département de Géographie, University des Sciences Sociales de Bamako, Mali
[4]Danish cooperation, Mali
*Corresponding Author: Dr. Djeneba Dembele, djenebademb@yahoo.fr

***Abstract*— *On a poor soil, fertilizers application is the best ways to meet crop need and restore the lost nutrients. However, its application is constrained in Mali due to the low access to fertilizers by smallholder farmers, resulting in low crop production. Today, make available to farmer's fertilizer and information needed for its proper use is an essential asset for sustainable agriculture and food security. Before, assessing the existing fertilizer usage and it impact on yield is a prerequisite. A study was done to characterise fertiliser management practices at Siguidolo and assess the impact on millet and sorghum yields. Thus, normalized difference vegetation Index (NDVI) and visual interpretation were used to estimate vegetation cover as sources of organic matter (OM).* Investigation was done*to get information about manure production and areas occupied by crop production. Geographic Information System and Survey was done to obtain information on fields, fertilizer used and yields. The results showed low vegetation cover (0.02 to 0.24) indicating low source of organic matter. The mean manure produced was 14373kg/ha/year while 69300kg was recommended. Organic fertilizers and integrated organic and mineral fertilizers occupied greater area. About 24.1% of the area did not received fertilizer. The extremely low fertility areas tended to receive more nutrient application. Indications are that, even in those areas where fertilizers were applied, the right quantities were seldom used. Low yield for millet and sorghum was recorded ranging from 414.66 kg ha^{-1} to 703.80 kg ha^{-1} for millet and 404.12 kg ha^{-1} to 448.04 kg ha^{-1} for sorghum.*

***Keyword*— Fertilizer, usage, crop, productivity, Siguidolo.**

I. INTRODUCTION

One of the main factors limiting crop efficiency and food security in Mali is the low natural fertility of the soils and its continued decline over years of cultivation. The decline in soil fertility and low nutrient application is the leading cause of the declining per capita food production in Sub-Sahara Africa (Chianu, 2012; Bremanet al., 1983;Kihara et al., 2016;IFDC, 2006;Shapouri et al., 2010;Muller et al., 2012). Food production index per capita showed a declining trend over the last decades and about 29% of the population is estimated to be undernourished (FAO stat, 2008). To reverse this trend, it is important to replenish the removed nutrients which results from crop uptake, harvest, erosion and leaching. Fertilizers application is one of the best ways to meet crop need and restore the lost nutrients(Gatti et al. 2019). Fertilizersare any material of natural or synthetic origin which, when applied to soils, provide one or more nutrients essential to plants growth and yield (Barker,2019). They occupy a prominent position in food security challenges on poor soils (Stewarta et al., 2012). However, their application is severely constrained in Mali due to the low access to fertilizer (IFDC, 2015) by the poor smallholder farmers, resulting in low agriculture production. Today in Mali, make available to farmer's fertilizer and information needed for its proper use is an essential asset for promoting sustainable agriculture production and food security while preserving environment. Before, assessing the existing fertilizers usage and it impact on crop production and food security is a prerequisite in the identification and development of appropriate improved practice. The objective of this study was to characterise the existing fertilisers management practices at Siguidolo and assess their impact one millet and sorghum yield in order to develop an improved method.

II. MATERIALS AND METHODS

2.1 Study site

Siguidolo is located in Segou region of Mali in a depression surrounded by the plateaux. It is between -6.80399° and -6.78583° N and 12.91624° and 12.91229° W. The mean annual rainfall is 700 mm. Soils are mostly sandy loam. During the rainy season, interrill (sheet) erosion is common with intermittent rills and gullies, some of which measure up to 1.0 m deep in the relatively hilly sites. In the depressions and valleys, particularly along the streams, waterlogged conditions often prevail during the rainy season presumably due to high water table.

2.2 Vegetation cover mapping

Normalized Difference Vegetation Index (NDVI) calculation and visual interpretation were used on a Quick bird image covering the study site at about 75%. NDVI is the dimensionless index that is indicative of vegetation density and is calculated by comparing the visible and near-infrared sunlight reflected by the plant surface (reflectance). The vegetation Index was used to quantify the density of green leaf vegetation by the formula:

$$\mathrm{NDVI} = \frac{(NIR - R)}{(NIR + R)}$$

Where

NI= Near Infra;

R= Red band

When NDVI calculation is applied on an image or a pixel, the output gives a value ranging between minus one (-1) to plus one (+1). A zero value means no green vegetation and close to +1 (0.8 - 0.9) indicates the highest potential density of green leaves. The spatial distribution and the density of vegetation have significant implications on fertilizer practices in an area.

The map derived from NDVI calculation was used for ground verification. Visits were done on various locations of the study area to identify and characterise known and unknown features marked on the map.

2.3 Assessment of organic manure production capacity

Investigation was donebeside the households to get information about manure production capacity and the area occupied by agriculture. Dembele (1994) showed that, the average organic manure production by livestock was 153.5 kg/head/4 months in rainy season and 182 kg/head/4 months in dry season. These data and the number of livestock were used to calculate the average organic manure production during both seasons at Siguidolo. Data were then used to estimate the availability of organic manure per year.

2.4 Fertilizer practices mapping

Global Positioning System (GPS) was used to delimit farmers' fields. Survey was done to obtain information on the fertilizer practices used in each field. Then, GPS and survey data were combined in Geographic Information System (GIS) environment to map fertilizer practices.

2.5 Assessment of the impact of fertilizer practices on yield

Survey was conducted beside the households to found out information about the ways of fertilizer usage (spread or micro-dosing, etc.), the types and amounts of fertilizers used, and yields obtained on the sorghum (*Sorghum bicolor L. Moench*) and millet (*Pennisetumglaucum*) fields. 36 out 38 households were interviewed.

III. RESULTS

3.1 Vegetation cover mapping

Vegetation assessment resulting from NDVI processing is presented in Fig.1.The image used for NDVI calculation covered 75.5% of the study area. The vegetation index values ranged from -0.01 to 0.24. This vegetation was classified into three savannah vegetation comprising bare soil (-0.01 - 0.02), grassland with scattered trees (0.02 - 0.05), and woodland with grass cover (0.05 - 0.24). The bare soil covered 278.26 ha (31.69%) of the area. The grassland with scattered trees occupied 451.01 ha (51.36%) and comprised grasses, such as *Andropogonguayanus*, crop residues and shrubs, such as *Piliostigmareticulatum*. The woodland had an aerial extent of 148.93 ha (16.62%) and consisted of green vegetation including *Vitellariaparadoxa*, *Parkiabiglobosa*, *Adansoniadigitata*, *TamarindusindicaL.*, *Lanneamicrocarpa* and *Lanneaacida Piliostigmareticulatum* on the bottom of the toposequence, then some *Lanneamicrocarpa* and *acida*, *Zizziphusmauritiana*, *Guierasenegalensis*, *Combretummicranthum*, *Combretonglutinosiun*, *Bauhinia rufescens*, *Bauhinia reticulate*, *Pennisetumpedicelatum*, on the top of the toposequence.

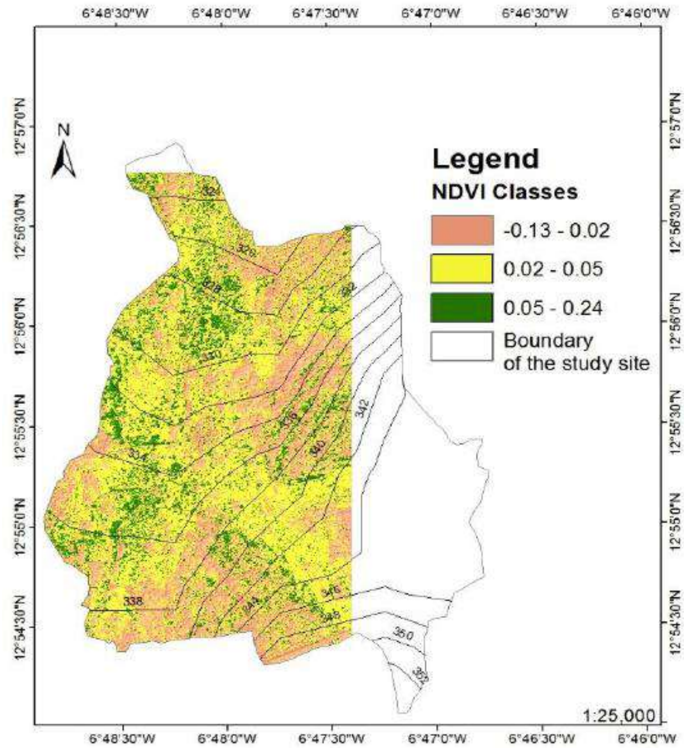

Fig.1: NDVI of the study area from Quick bird image in 2014

3.2 *Assessment of organic manure availability*

A total of 398 cattle, 274 sheep, 312 goats and 43 donkeys were identified in the village. Table1 shows the results of the basic statistical analysis about livestock. The mean values give the general magnitude of the data and the CV show the high variability between the household concerning livestock possession (minimum 0 and maximum 42). The mean bovine per household was higher than the others animals. Some households don't possess animal.

Table.1: Livestock available at Siguidolo in 2014

livestock	Bovine	Ovine	Goat	Donkey
Mean	11.06	7.61	8.7	1.19
Median	5.00	3.50	5	1
Minimum	0	0	0	0
Maximum	41	42	40	10
Coefficient of variation (CV)	102.56	131.32	116.73	158.62

Organic manure production capacity is presented in Table2. Organic manure production was high in dry season. The highest amount of organic manure was produced by bovine.

Table.2: Organic manure production capacity at Siguidolo in 2014

Average production of organic manure (kg)	Bovine	Ovine	Goat	Donkey	Total
Rainy Season (4 mouths)	1698	1168	1335	183	4384
Dry Season' (4months)	2013	1385	1583	217	5198

Year	**5566**	**3830**	**4378**	**599**	**14373**

The type of mineral fertilizer used in the study site in 2014 is presented in Table3. The Complexes Cereal is the most used with the mean of 102.1 kg/ha. NPK was not used at the site in 2014.

Table.3: Mineral Fertilizers used at Siguidolo in 2014.

Mineral fertilizer (kg/ha)	**Complexes Cereal**	**DAP**	**NPK**	**Urea**
Mean	102.1	4.29	0	38.21
Median	50	0	0	0
Minimum	0	0	0	0
Maximum	500	50	0	150
CV	118.5	331.37	0	123.66

3.3 Fertilizer practices mapping

Fertilizer's practices data collected on the site was overlaid on the soil fertility status map (Fig. 2) in order to understand the link between soil fertility status and farmer's fertilizers application decision. The fertility status varied from low, to extremely low. The very low status covered the greater area. Their respective area of coverage was 42.54 ha (4.81%), 134.9 ha (15.26%) and 706.61 ha (79.93%).

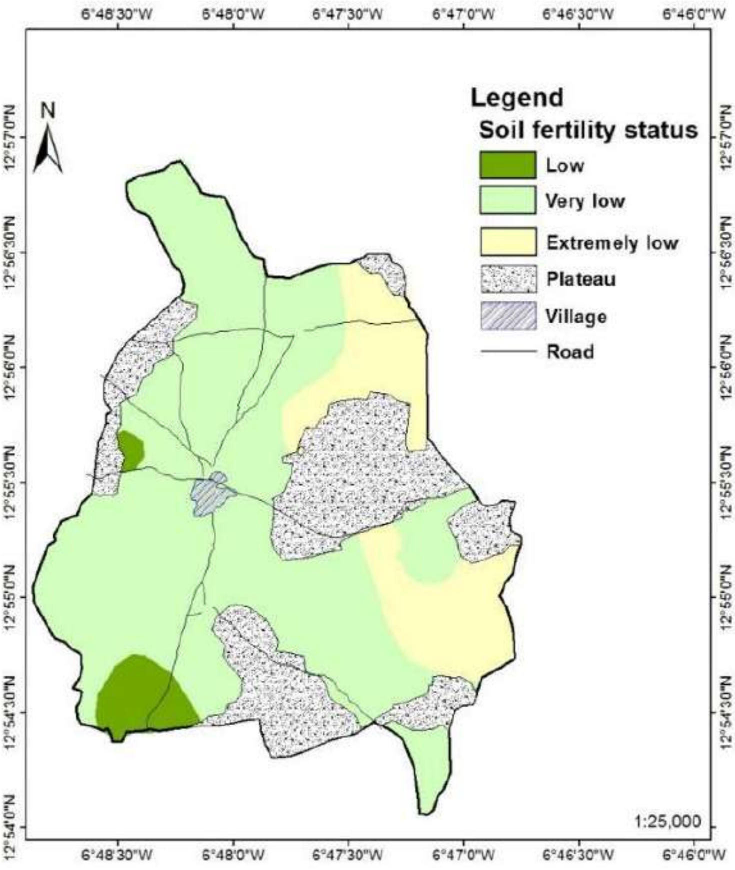

Fig.2: Spatial distribution of soil fertility status at Siguidolo in 2013.

The spatial distribution of the fertilizers used in 2013 is presented in Figure3. Fertilizer used were: complex cereal (CCl), complexecoton (CCt), urea (U), DAP, NPK, Organic fertilizer (OF). The extremely low fertility area tended to receive more nutrient application.

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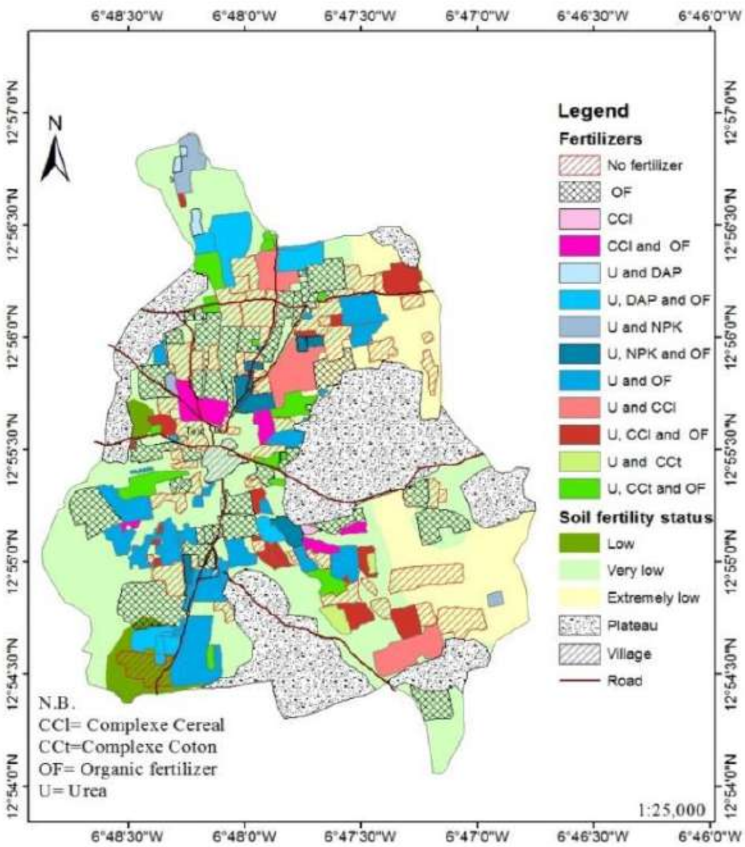


Fig.3: Spatial distribution of fertilizers usage at Siguidolo in 2013

Table4 shows the areal coverage of fertilizers. Organic fertilizers and integrated organic and mineral fertilizers occupied the greatest area of 333.51 ha over 506 ha. About 24.1% of the area did not received fertilizer.

Table.4: Areas covered by fertilizers in 2013.

Fertilizers	**Area (ha)**	**Area (%)**
No fertilizer	122	24.11
Organic fertilizer	132.01	26.08
Mineral fertilizer	50.2	9.90
Organic and mineral fertilizer	201.5	39.93
Total	506	100%

Table5 shows the soil amendments applied in 2013 and percentage area of coverage. The very low fertility areas tended to receive more nutrient application.

Table.5: Soil amendments applied in 2013 and percentage area of coverage.

Soil amendments	**Soil fertility status**		
	Low	**Very low**	**Extremely low**
	---------------% area covered---------------		
No fertilizer	55.57	19.67	28.96
Organic fertilizer	15.82	23.69	40.76
Mineral fertilizer	-	12.87	1.99
Organic and mineral fertilizer	28.61	43.77	28.29
Total	100	100	100

3.4 Assessment of the impact of fertilizer practices on yield

Information on soil fertility status, spatial distribution of millet and sorghum grain yields is presented in Figures 4 and 5, respectively. The means millet grain yield was 414.66 kg ha^{-1} on soil with low nutrient status, 703.80 kg ha^{-1} on soil with very low nutrient status and 558.2 kg ha^{-1} on soil with extremely low nutrient status. The corresponding yields for sorghum were 445 kg ha^{-1}, 448.04 kg ha^{-1} and 404.12 kg ha^{-1} respectively. The very low and extremely low nutrient status soils were compensated by greater amounts of fertilizer application with a consequent higher grain yield than the relatively better low fertility soils. The percentage area of the low, very low and extremely low fertility status soils that received fertilizers were 44.4, 80.33 and 71.01 respectively.

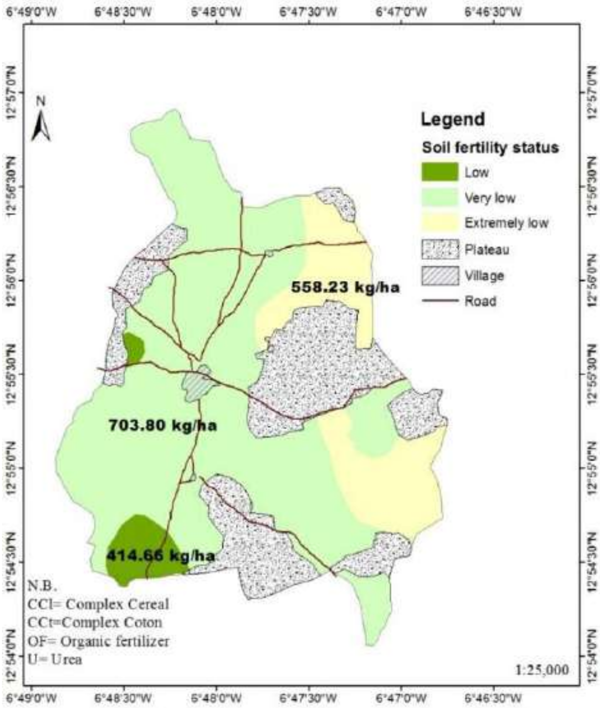
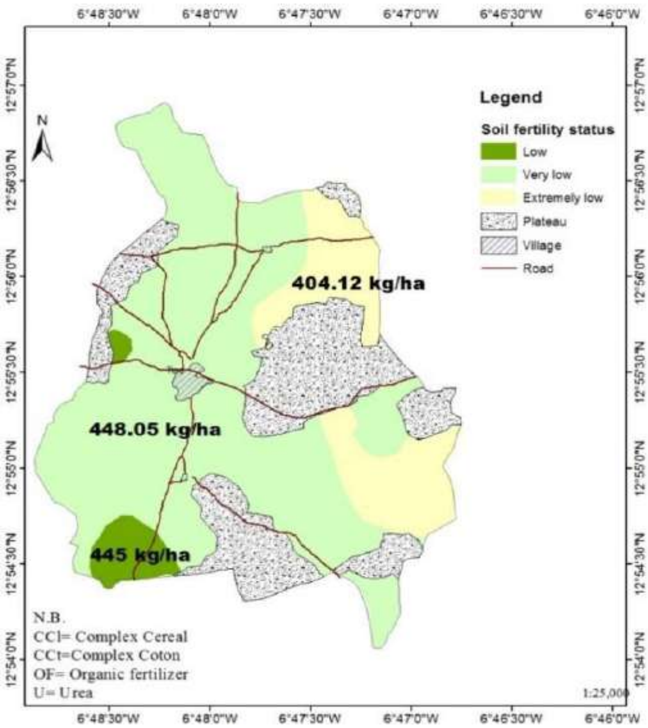


Fig.4: Spatial distribution of soil fertility status and millet grain yield in 2014.



Fig,5: Spatial distribution of soil fertility status and sorghum grain yield in 2014.

IV. DISCUSSION

Vegetation is source of organic matter (OM). OM has many beneficial effects soil (Erich et *al*., 2002; Sanyal et al., 1991). It improves the physical, chemical and biological properties of the soil.Thus, Maurice et al.(1998) used organic matter as an indicator of soil fertility, overall soil stability and a factor influencing the reduction of soil erosion, soil compaction and bulk density (Vorhees, W.B. 1992). Fen-Li (2006) found that the vegetation effect on soil erosion was predominant.As Soil Organic Matter increases, the quantities of N, P, K available, carbon and certain micronutrients increase (Oates, 1998, Dembele, 2017).Acquaye (1990) reported that organic matter was the main source of N, P and S for plant growth in small farms without fertilizer. Nitrogen (N) use in crop production is inevitable target (Rossner et al. 2014).In addition, soil water infiltration is more influenced by vegetal cover and soil organic matter(Sampaiode Almeida, et al., 2018)as well as nutrient and water holding capacities (Lal et al., 1998, Traore, 2003; Morgan, 2005; Barmani et *al*., 2013). They improvesoil microorganism activities (Havlin et *al*., 2005),soil aeration,plant root growthand elongation and pH stability.Furthermore, vegetation covers cushions the soil against the erosive forces of water and wind (Vásquez-Méndez et *al*., 2009,) and thereby reduce their detachment and transport capacities with a resultant reduction in erosion.Assessing the vegetation cover of the study site therefore became necessary. The effectiveness of vegetative cover in reducing erosion however depends, in part, on its density and spatial coverage, the continuity of the canopy and height (Nanko et *al*., 2008). NDVI at Siguidolo varied from -0.01 to 0.24 indicating low and sparse vegetation covert and very low source of organic matter. This condition has negative effects on soil fertility and influences fertilizer management strategies.

Manure production capacity impacts soil fertility in Mali because small scale farmers don't own enough money to afford mineral fertilizer at the recommended level (Jens and al., 2007; PRSP, 2002). A mean of 11.06 cattle's, 7.61 sheep, 8.7 goats and1.19 donkeys per household was investigated in the site which produce 14373 kg/year of organic manure. This amount is not sufficient to meet a mean of 13.86ha/household measured in the area where 5t/ha is recommended.ExceptComplexes Cereal, the mean of the other mineral fertilizers used in the site was significantly below the recommended rate of 50 kg/ha of DAP and 50 kg/ha of Uréanoted byOuendeba (2010). Analysis has shown that the very low fertility area tended to receive more nutrient application. Indications are that, even in those areas where fertilizers were applied, the right quantities were seldom used. Sorghum and millet yields recorded in the study site were significantly low compared to 1424 $kgha^{-1}$ and 1301 kg ha^{-1} for millet in 2013 and 2014, respectively and 1378 kg ha^{-1} for sorghum in 2014 harvested in research station by Traore et al. (2017).

Increasing Crop production and productivity atSuigidolo area, dominated by smallholder farmers require a set of accompanying soil conservation and efficient water utilization technologies.Sound soil fertility management, as recommended by Quansah (2000), should therefore use available livestock and poultry manure and crop residues wherever practical, taking appropriate nutrient credit for these materials and using mineral fertilizers to balance the crops nutritional requirements for realistic yield goals. These include ridge furrow system, tie-ridging, circular contour bunds, zai, cereal legume rotations and residue management. In contributing to this effort, the cropping systems in the area need to be study to show trends as a basis for recommending asustainable cropping systemable to improve the biophysical and socio-economic conditions of the farmers.

V. CONCLUSION

Agriculture development at Siguidolo involves the improvement of organic matter production, andthe adapted agriculture technologies.

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Feasibility of *Pole-and-Line* Fishery: Comparison of Milkfish (*Chanoschanos, Forskal*) and Anchovy (*Stolephorussp*) as Live Bait

Achmad Cahyo Rinaldi[1], Sri Suro Adhawati[2], Achmar Mallawa[2]

[1]Student of Master Degree Fisheries Science, Faculty of Marine and Fisheries Science, Hasanuddin University, Perintis Kemerdekaan St.Km 10, Makassar 90245, Indonesia

[2]Department of Fisheries Science, Faculty of Marine and Fisheries Science, Hasanuddin University, PerintisKemerdekaan St Km 10, Makassar 90245, Indonesia

*Email: mailto:ahmadcahyorinaldi@gmail.com

Abstract—*This study was conducted from May to July 2019 in Bitung City, North Sulawesi Province using pole-and-line vessels in Bitung Fishing Port (PPS Bitung).The method used in this study is a survey method with case studies. Some of the analyzes used: (1) comparative descriptive analysis; (2) business analysis; (3) criteria analysis for investment.The objectives of this study are to compare the level of financial feasibility anddetermine the level of difference between pole-and-line fishing businesses that use milkfish seed and those that use anchovy. The calculation results of business analysis of pole-and-line businesses that use milkfish seed and anchovy are based on the criteria of the businesses in gaining profits and a fast payback period, with an ROI value greater than the capital expense. Investment feasibility is based on the criterion value of an investment, that is NPV>0, Net B/C > 1, and IRR value > 10% interest rate so that pole-and-line business units that use either one of those live baitsmeet the requirements, especially business units that use anchovy. However, milkfish seed still deserves a development as an alternative bait, especially in the condition in which anchovy is difficult to find.*

***Keywords—Pole-and-Line*, Milkfish, Anchovy, Business Analysis, and Investment Criteria.**

I. INTRODUCTION

Bitung is one of the centers of skipjack fishing in Indonesia. *Pole-and-line* or *huhate*isan environmentally friendly fishing gear usually used to catch tuna, mackerel tuna, and skipjack tuna in Bitung. Based on the International Seafood Sustainability Foundation data (ISSF, 2010), *pole-and-line* catch is dominated by skipjack by 57%, yellowfin tuna 27%, bigeye tuna 11%, and albacore 5%. *Huhate*greatly relies on the availability of live bait in nature (wild bait). Wild baits often used are anchovy (*Encrasicholinaheteroloba* and *Encrasicholinadevisi*) and fingerscalesardine (*Sardinella fimbriata*). Those wild baits can be obtained from floating *bagan*fishermen and small boats (*pajeko*). In 2014, there were about 104 *pole-and-line* vessels in Larantuka, North Flores, but only about 50 vessels were able to operate because of various problems, one of which was the declining availability of live bait obtained from floating *bagan* fishermen. This condition is caused by the limitation of *bagan*, which can only operate during a dark moon. Moreover, the population of baitfish tends to decrease because of overfishing since the fish is used not only as live bait but also as the cheapest consumption fish in local markets (Tokan, 2017).

The competition to get the same resources between fishing gear *Lampara* and purse seine to meet the needs of home industry, as well as *bagan* which is considered as an environmentally friendly fishing gear also contributes to the decrease of live bait availability in the sea. To overcome this problem, some *pole-and-line* vessels in Larantuka and Bitung tried to use cultivated milkfish seed as live bait. Therefore, it is important to analyze the comparison of *pole-and-line*fishing businesses to determine the differences in financial feasibility between those businesses that use milkfish seed and those that use anchovy as live bait.

II. RESEARCH METHODS

This study was conducted from May to July 2019 in Bitung City, North Sulawesi Province using *pole-and-line*

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vessels in Bitung Fishing Port (PPS Bitung). The method used in this study is a survey method with case studies (Arikunto, 2000). Study objects consist of four vessels, namely KM River, KM. Primadona, KM. CahayaBintang, KM. CahayaDaulinsa with 25-27 GT which were examined using milkfish and anchovy. The time of fishing and the fishing ground were assumed to be the same for each vessel. The selection of respondents was adjusted to the needs of the study using a purposive sampling method (Sugiyono, 2006).

The respondents in this study were the owner and crew of *pole-and-line* vessels, five people in total. In addition, other stakeholders namely two representatives from the Fisheries Service and four company employees were also interviewed to enrich the data. Thus, there were 11 respondents in total. Data collected consists of primary and secondary data. The primary data were obtained through direct observation while the secondary data were obtained using literature search method.

A. Data Analysis

1. Comparative Descriptive Analysis

Comparative descriptive analysis provides thematic comparative frequency distributions in the form of tables and figures. This analysis aims to observe the tendency and comparison of business feasibility between *pole-and-line* businesses that use milkfish seed cand those that use anchovy as live bait.

2. Business Analysis

Components used in business analysis include production costs, gross and net income gained from the *pole-and*-line fishing business. Various analyses are conducted in business analysis, namely analyses of business income, revenue and cost balance (R/C), payback period (PP), and Return of Investment (ROI) (Hernanto, 1989).

Business income can be calculated using the following formula:

$$\mu = TR - TE$$

where:

μ = Profit

TR = Total revenues

TE = Total expenses

with the following criteria:

If TR > TE, the business activity gets profits.

If TR<TE, the business activity does not get profits.

If TR = TE, the business activity is at the break-even point, i.e business activity does not get profits or losses.

Revenue and cost balance (revenue-cost ration) can be calculated using the following formula:

$$\mathbf{R/C} = \frac{TR}{TC}$$

with criteria:

If R/C > 1, the business activity gets profits.

If R/C < 1, the business activity gets loss.

If R/C = 1, the business activity does not get profits or loss.

Payback Period (PP) is calculated using the following formula:

$$\boldsymbol{PP} = \frac{Total\ Investment}{Profit\ in\ a\ year}$$

Return of Investment (ROI) is calculated using this formula:

$$\mathbf{ROI} = \frac{Profit}{Investment}$$

3. Comparative Descriptive Analysis

Investment feasibility analysis for the development of *pole-and-line*fishing business uses analysis instruments namely Net Present Value (NPV), Internal Rate of Return (IRR), dan Net Benefit Cost Ratio (Net B/C). The purpose of financial evaluation in this study is to observe the cost benefits of *pole-and-line*fishing business in production(Charles B. Purba et al. 2008).

Net Present Value (NPV) is calculated using this formula:

$$\boldsymbol{NPV} = \sum_{i=1}^{n} \frac{(B_i - C_i)}{(l+i)^t}$$

where:

B_t = Benefitsof a project in the yeart

C_t = Project cost in year t

n = Technical lifespan of a project

i = Applicable interest rate

The feasibility criteria are:

If NPV ≥ 0,means the investment is feasible

If NPV < 0 means the investment is a loss or not feasible to be implemented.

Internal Rate of Return (IRR) can be calculated using the following formula:

$$\boldsymbol{IRR} = D_f P + \left\{\frac{PPV}{NPV} - (D_f N - D_f P)\right\}$$

where:

$D_f P$ = Discount factor that produces a positive present value.

$D_f N$ = Discount factor that produces a negative presentvalue.

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PPV = Positive present value.
NPV = Negative present value.

The feasibility criteria are:

If IRR >i, then the investment is feasible to be implemented, and

If IRR <i, then the investment is not feasible to be implemented.

Net Benefit Cost Ratio (Net B/C) is calculated using this formula (Kadariah,2001).:

$$\boldsymbol{Net - B/C\ - ratio} = \frac{\sum_{t=1}^{n} \frac{B_t - C_t}{(1+i)^t} [(B_t - C_t) > 0]}{\sum_{t=1}^{n} \frac{C_t - B_t}{(1+i)^t} [(B_t - C_t) < 0]}$$

The feasibility criteria are:

B/C > 1 means that the investment is feasible to be implemented

B/C < 1 means that the investment is not feasible to be implemented,and

B/C = 1 means that implementation decisions depend on the investor.

III. RESULTS & DISCUSSION

A.*Pole-and-Line* Catching Unit

Bitung is the main base of *pole-and-line* vessels in North Sulawesi. There were 37 *pole-and-line* vessels in 2017. These vessels are generally made of wood sized 20-100 GT, most of which are vessels sized 50-100 GT.*Pole-and-line* vessels in Bitung Fisheries Port are made of wood with a fishing spot at the bow of the vessel. The Bow of the vessels is specially designed with a flying deck to ease the anglers. The bow is also designed rather high so that the fish caught can slide down to the center of the vessel near the catch tank. This design is intended to ease the handling of the catch. *Pole-and-line* has *huhate* fishing gear made of a fishing rod, fishing line, and fishing hook. Each vessel has 15-20 crews/workers.

Machines used consist of three engine units, namely the main engine, water circulation engine, and lighting engine. Navigation equipment consists of GPS Furuno GP32, compass, and SOG-VMS. The vessel is also equipped with water spray pipes with flattened ends to maximize the spray reach. These pipes are placed in the bow of the vessels under the flying deck or angler seats. There are eight in total, two each in the left and right sides of the vessel, and 4 in the bow.Live bait tanks are placed in the hull of a vessel. There are two tanks of the same size, that is 1.5 m long, 1 m wide, 1.5 m high and have a maximal capacity of 20 buckets or ± 400 L. The bait tank is the characteristic of a*pole-and-line* vessel. It is equipped with water intake and discharge holes that function as a water circulating system. In addition, there is also a bait pot to ease the throwing of live bait.

Pole-and-line vessel also has two units of hatches served as catch storage sized 1 long, 1 m wide and 1.2 m high, with capacity of 2 tons and two units of hatch for ice storage sized 1 m long, 1m wide, and 1.2 m high with capacity of 2 tons (60-70 ice blocks) each which are placed on the deck in front of the pilothouse. The ice hatches can also be used as storage if the storage hatches are unable to contain all the catches. Other parts of the vessels are the pilothouse, engine room, 1,000-L fuel tank, break room for crews, clean water tank, kitchen, and toilet.

Table 1. Specifications of pole-and-line vessels used in the study

Specifications	**Details**
Dimension	
a) Length (LOA)	14,60 – 21,50 m
b) Width (B)	2,50 – 4,60 m
c) Height (D)	2,25 – 2,50 m
d) Draft (d)	1,35 – 1,50 m
Tonnage	20 – 27 GT
Engine	120 – 160 PK

Source: Primary Data, 2019

B. Live Bait

The operation of *hutate* depends on the availability of baitfish. About 20-40% of the number of sea days cannot be carried out because there is no bait available, especially in skipjack season (Naamin, 2000). The main type of live bait that fishermen often use is anchovy (*Stolephorussp*), which, in this study, is compared to milkfish seed (*Chanoschanos, Forskal*) as a substitute when the main bait is scarce. Anchovy (*Stolephorussp*) is obtained from *bagan* fishermen around the path to the fishing ground. The price is IDR 20,000 per 20-L bucket. A vessel usually loads 15 – 20 buckets on each operation, depending on the capacity of available tank and fund.

On the other hand, milkfish seed are obtained from milkfish farmers in North MinahasaRegency since there are no milkfish farmers who sell milkfish seed (*Chanoschanos, Forskal*) in Bitung City. The price is IDR 300 per fish. A vessel usually uses 12 bags in which a bag contains 1000 milkfish seeds.

C. Catch Composition

On average, a *pole-and-line* catching unit can catch 7,666 kg/trip using anchovy as live bait and 7,168 kg/trip using milkfish. This indicates that the catch of *pole-and-line* using anchovy is 4% more than *pole-and-line* using milkfish. Fish caught are large pelagic fish, namely

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skipjack (*Katsuwonuspelamis*), tuna (*Thunnus sp.*), *tongkol*(*Auxis sp.*), mahi-mahi (*Coryphaenahippurus*), rainbow runner (*Elagatisbipinnulata*).

The production of *pole-and-line* catches in 2017 landed at Bitung Fishing Port (PPS Bitung) reached 112,398.7 tons of skipjack, tuna, and *tongkol* as well as 30,971.4 tons of rainbow runner and mahi-mahi. The catches are dominated by skipjack by 33%. From January to July 2019, the catches of four *pole-and-line* vessels used in this study consistedof skipjack, tuna, *tongkol*, rainbow runner, and mahi-mahi, which were dominated by skipjack with 152 tons. The types of tuna caught were yellowfin tuna (*Thunnusalbacares*) and albacore (*Thunnusalalonga*). There were morealbacore caught thanyellowfin tuna.

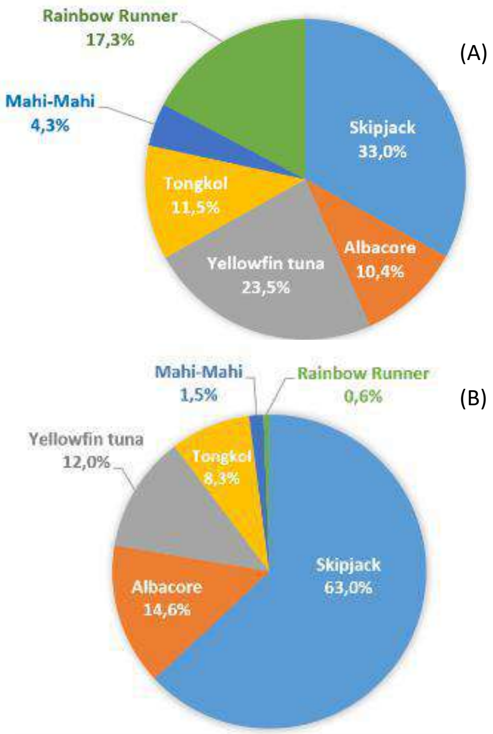

Fig 1. The composition of fish type caught using huhate in 2017 (A) and January-July 2019 (B)

There is a significant difference between the priceset by company and the local price used by fishermen to sell fish to retailers in fish season. These prices are set based on an agreement on the cutting operational costs.

Table 2. Fish prices set by company and local market

Fish Type	Price (IDR)	
	Partner Company	Retailer
Skipjack	9.000	13.000
Yellowfin tuna	13.000	25.000
Albacore	20.000	30.000
Tongkol	7.000	10.000

Source: Primary Data, 2019

On average, the income of *pole-and-line* using anchovy is IDR 877,731,765 per year while the income using milkfish seed is IDR 702,499,480 per year. This indicates that the income of *pole-and-line* using anchovy is greater by 11.1% than that of *pole-and-line* using milkfish seed. *Pole-and-line* vessels, either using anchovy or milkfish seed, have three fishing days (trip) per month in total, and 36 trips per year on average. The optimal fishing operation is nine months.

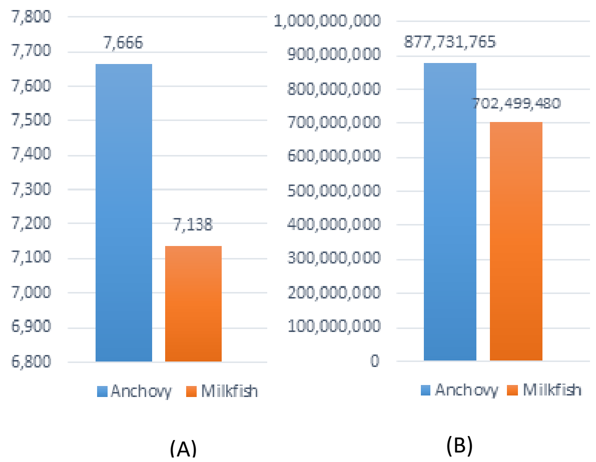

Fig 2. Production and revenue of pole-and-line catching unit: (A) catches per trip (B) Revenue per year

D. Business Analysis

1. Investment Costs for *Pole-and-Line* Fishing Business

Analysis of business feasibility is carried out to examine the financial and economic benefits of activities of each business unit so that the investment used will provide maximal benefits to fishermen's income. Investment costs are the initial costs incurred by the owner (fishermen) to start the business.

Table 3. Average prices of invested components of pole-and-line fishing business in Bitung

Type of Pole-and-line Investment	Price (IDR)	
	Anchovy Bait	Milkfish Bait
Vessel	680.000.000	680.000.000
Main Engine	287.500.000	287.500.000
Auxiliary Engine	32.000.000	32.000.000
Navigation Equipment	23.000.000	23.000.000
Catching Tool/Huhate	1.295.000	1.295.000
Sibu-sibu/Palo/Drain	140.000	140.000
Bait tank	1.500.000	1.500.000
Water Sprayer	3.500.000	3.500.000
Pickup Car	-	120.000.000
Total	**1.028.935.000**	**1.148.935.000**

Source: Primary Data, 2019

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2. Fixed Costs for *Pole-and-Line* Fishing Business

Fixed costs are costs that must be incurred even if there is no fishing operation. The components of fixed costs for *pole-and-line* business consist of maintenance costs and depreciation of vessels, machinery, navigation equipment (7 years), fishing gear (2 years), *sibu-sibu* (3 years), bait tank, water sprayer (5 years), and pickup car (10 years).

Table 4. Average prices of fixed-cost components of pole-and-line fishing business in Bitung

Type ofFixed Cost	Price (IDR)	
	Anchovy Bait	Milkfish Bait
Vessel Depreciation	97.142.858	97.142.858
Vessel Maintenance	15.000.000	15.000.000
Main Engine Depreciation	41.071.428	41.071.428
Auxiliary Engine Depreciation	4.571.429	4.571.429
Engine Maintenance	5.000.000	5.000.000
Navigation Equipment Depreciation	3.285.714	3.285.714
Catching Tool/Huhate Depreciation	650.000	650.000
Sibu-sibu/Palo/Drain Depreciation	11.667	11.667
Bait Tank Depreciation	300.000	300.000
Water Sprayer Depretiation	700.000	700.000
Pickup Car Depreciation	-	12.000.000
Total	**167.733.096**	**179.733.096**

Source: Primary Data, 2019

3. Variable Costs of *Pole-and-Line* Fishing Business

Variable costs are costs that are incurred only during fishing activities.

Table 5. Average prices of variable-cost components of pole-and-line fishing business in Bitung

Type of Variable Cost	Price (IDR)	
	Anchovy Bait	Milkfish Bait
Diesel Fuel	148.500.000	148.500.000
Oil	50.400.000	50.400.000
Ice	25.200.000	25.200.000
Consumption	108.000.000	108.000.000
Freshwater	1.620.000	1.620.000
Crew's Wage	1.464.783.339	1.353.750.625
Anchovy	100.800.000	-
Milkfish	-	129.600.000
Bensin	-	7.200.000
Total	**1.899.303.339**	**1.824.270.625**

Source: Primary Data, 2019

3. Calculation Results of Business Analysis

Analysis of *pole-and-line*fishing business is conducted to determine the level of business success that will be achieved financially and the feasibility of business development.

Table 6. The calculation results of business analysis on pole-and-line fishing business in Bitung

Business Analysis	***Pole-and-line Fishing Business***	
	Anchovy	Milkfish
Annual Profits (IDR)	877,731,765	702,499,480
Revenue and Cost Balance Ratio (R/C Ratio)	1.4	1.33
Payback Period (PP)	15.6 month	21.84 month
Return of Investment (ROI)	83%	60%

Source: Primary Data, 2019

4. Calculation Results of Analysison Investment Criteria

There are a few assumptions used in the analysis of investment criteria in *pole-and-line* business. First, *pole-and-line*fishing business in Bitung City is new. Second, project lifespan is determined based on investment with the longest technical lifespan, i.e vessel with a technical lifespan of 10 years, and the first year of the project started in 2019. Third, prices used are fixed throughout the project lifespan and during the study. In addition, the catches are assumed to be fixed throughout the lifespan of the project so that the revenue is also fixed at IDR 2,929,566,250 for *pole-and-line* vessels with anchovy as bait and IDR 2,707,501,250 for *pole-and-line* vessels with milkfish as bait. Furthermore, operational costs used throughout the project lifespan are considered fixed. The value of the discount factor is 10% per year (bank interest).

Table 7. Feasibility criteria of pole-and-line fishing business using anchovy and milkfish

Business Analysis	***Pole-and-line Fishing Business***	
	Anchovy	Milkfish
Net Present Value (NPV) at DF 10%	4,364,346,731	3,287,620,197
Net B/C at DF 10%	5.3	3.8
Internal Rate Of Return	85.81%	60.9%

Source: Primary Data, 2019

E. Marketing

The marketing of the catches in Bitung City is done in two ways:

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Marketing Channel 1

Marketing Channel2

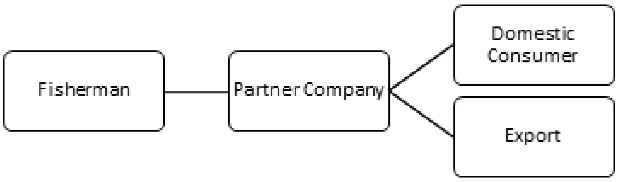

Fig 3. Marketing channels of fishermen's catches in Bitung City

F. Profit-Sharing System

After profit sharing (50% : 50%) between the vessel owner and the crew as a whole, based on the calculation results of crew's income in skipjack catching business in the waters of Bitung City, with the price of fish set by the company, then the average net income gained by the owner of the vessels is IDR 24,381,438 per *pole-and-line* vessels per trip for vessels that use anchovy as live bait and IDR 19,513,874 per trip for vessels using milkfish seed. The average income of the crew for vessels with anchovy is IDR 2,159,795 per person per trip and IDR 1,996,923 per person per trip. Assume that there are three trips in a month, then the average income of the crew of *pole-and-line* vessels using anchovy is IDR 6,479,384 and IDR 5,990,768 for the crew of *pole-and-line* vessels that use milkfish as live bait.

Based on the calculation, it can be concluded that the income of these fishermen is feasible since it has exceeded the provincial minimum wage set by the Provincial Government of North Sulawesi, which is a minimum of IDR 3,051,076 per month.

IV. CONCLUSION

Based on financial feasibility, a significant difference in income can be observed between *pole-and-line*fishing businesses that use milkfish seed and those that useanchovy as live bait. The results of the business and investment feasibility indicate that *pole-and-line*fishing businesses that use anchovy as bait are still prospectively more profitable. However, the results also indicate that *pole-and-line*fishing businesses in Bitung City that use milkfish seed as bait still deserves a development.

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Insects from light trap: Do they represent total diversity?

Joshua Matata Kimondiu[1]*, A R V Kumar[2], and K.N. Ganeshaiah[1]

[1] Department of Forestry and Environmental Sciences, GKVK 560065, Bangalore India
[2] Department of Agricultural Entomology, GKVK 560065, Bangalore India
*kjoshuamatata@yahoo.com

Abstract— *Insects collected in the light traps over a period of two years in the agroecosystems of GKVK campus represented 13 of the 21 orders of India. Among these orders collected, five orders viz., Coleoptera, Hemiptera, Hymenoptera, Lepidoptera and Diptera were the most predominant. We compared the relative proportions of these five speciose orders with those expected at national and at global levels to test if the light traps collections can be used as a surrogates of national and global diversity of these groups. We found that while orders Coleopteran and Hemipteran insects are over represented, those of Lepidoptera and Hymenoptera are under-represented. We discuss the possible reasons for these differentia representation.*
Keywords— ***Insects, light trap, GKVK campus.***

I. INTRODUCTION

Assessment of diversity is central to ecology and conservation (Whittaker, *et al*., 2005). Terrestrial arthropods are extremely important ecosystem components since they exert control over the stability and functioning of ecosystems; they are key players in nutrient cycling and also create substantial economic value via ecosystem services such as pollination, parasitization and predation of crop pests, *etc*. (Pyle *et al.,* 1981). Moreover, terrestrial arthropods are by far the most diverse group of organisms on our planet, as insects alone account for an estimated 57% of all species living on our planet (Millennium Ecosystem Assessment, 2005). The best approach to collect a wide range of terrestrial arthropods has been a topic of long-lasting debates (Brehm and Axmacher, 2006). When selecting an appropriate sampling method, the most important parameters to be considered are the design of the sampling tools and their costs, as well as the ecological traits and habitat conditions of the target taxa (Gullan and Cranston, 2005). Specific methods are indeed needed to sample different arthropod taxa. For example, pitfall traps are highly useful for ground-dwelling beetles and ants, malaise traps for flies or parasitic wasps, light traps for moths and many other nocturnal insects (Kitching *et al.,* 2001). For this reason, the capture effectiveness of diverse sampling methods and their improvements are continually studied (Sabu and Shiju, 2010).

Light traps capture highly diverse orders of insects like Coleoptera, Hemiptera, Lepidoptera, Diptera, Hymenoptera, *etc* (Ramamurthy *et al.*, 2010). Earlier studies on insect species diversity and long term monitoring programmes were based on light trap catches (Holloway, 1983, 1987; Taylor, 1978; Taylor *et al.*, 1976; Wolda, 1981 a, b; Wolda and Roubik, 1986). Though several limitations such as un-interrupted power supply or even the very availability of power in remote forest areas, restrict the use of light traps (see Wolda 1981a), they are used widely for studying the abundance of agricultural pests, community structure, population variability, and incidence of density dependence (Gaston and Macardle, 1994).

Though it is known that the light traps sample only the nocturnal insects, in the present study we attempted to assess the efficacy of the light traps used over a two year period in trapping the total insect diversity of an agro ecosystem. While a comprehensive insect profile of the studied ecosystem is not available we tested the extent to which the insects attracted to light traps reflect the overall diversity at the national and global level based on the available information.

II. MATERIALS AND METHODS

The study was taken up at Gandhi Krishi Vignana Kendra (GKVK) campus of the University of Agricultural Sciences, Bengaluru, Karnataka State, India. The campus, spread over an area of about 1500 acres has several cropping systems such as plantations (mango, guava, citrus, sapota, *etc.*), dry land farming systems, irrigated crops and even agro-silviculture systems. The rain-fed crops such as cereals, pulses and oilseed crops, horticultural crops such as flowering plants, fruits and vegetable crops such as tomato, bhendi, chillies, *etc.*, are grown during different seasons of the year. Besides, the campus also has several experimental plots covered by soybean, sunflower, minor millets, arid legumes and agro-forestry crops. Surrounding the campus there is very little vegetation as the area is dominated by residential areas.

Funnel and vane type of light trap fitted with a container to collect the insects was set up almost in the middle of the campus. The light source for the trap was a mercury vapour lamp of 165 Watts (make: Philips). Dichlorvos mixed with water in a ratio of 1: 1 served as the killing agent for insects trapped. The traps were run between 8th May, 2015 and 6th December, 2016 at 21 day intervals from 6.00 p.m. to 6.00 a.m. Thus there were totally 26 sampling dates.

The trap catches were initially air dried and sorted. It was not possible to identify all the collected insects' up to the species level. Therefore, based on external morphology, all potential taxa were assigned to different Operational Taxonomic Units (OTUs). These OTUs were assigned to different families and orders.

Comparing diversity of insects at GKVK with global and national level.

Among the insects collected, five orders *viz.*, Coleoptera, Hemiptera, Lepidoptera, Diptera and Hymenoptera were the most abundant in terms of the OTUs represented; the numbers of OTUs in other orders were very less (Matata, 2017). Therefore, only these five orders were considered for comparison with the global and national level. We hypothesized that if the proportions of these five orders in our collections were similar to those at the national and global level then light traps could be used to assess the overall diversity of insects.

Of the 31 orders of Insects, Zoraptera, Grylloblattodea and Mantophasmatodea are not found in India. Further, Collembola, Protura, Diplura, Archaeognatha and Thysanura are primarily wingless, while all species of Siphonaptera and Phthiraptera are secondarily wingless. Leaving out these orders, it can be anticipated that members of as many as 21 orders are to be found at the light trap. For this, we first computed the proportion of each of these orders as the ratio of their numbers of species of these orders to that of the sum of their numbers. Such proportions were computed for the global level (Ghosh, 1996) and also for the national level (Ghosh, 1996). Using these proportions at the national and global level, we derived the expected numbers of each order and compared them with the observed numbers of OTUs using chi square test.

III. RESULTS

From 26 sampling days during the study period of 546 days, 209,098 insects were collected and were classified in to 764 OTUs. These OTUs represented 13 orders of insects, *viz.*, Isoptera, Psocoptera, Neuroptera, Odonata, Mantodea, Blattodea, Trichoptera, Orthoptera, Hymenoptera, Lepidoptera, Diptera, Hemiptera and Coleoptera. Therefore, fifteen of the 28 orders that are expected to be found at the lights had no representation in the light trap catches (Table 1).

As many as 740 of the OTUs collected were from the five orders (Table 1) with 351 from Coleoptera, 133 from Hemiptera, 110 from Diptera, 108 from Lepidoptera and 38 were Hymenoptera. Thus Coleoptera represented 45.76 per cent, Hemiptera were 17.34 per cent, Diptera 14.34 per cent, Lepidoptera 14.08 per cent and 4.95 per cent were from Hymenoptera (Figure 1).

These proportions were different from those known at global and national level (Figure 1). Therefore in order to understand the extent of deviation from the general representation we computed the expected number of OTUs for these five orders based on the proportionate representation at global and national level.

Chi-square computation showed that the relative diversity of the five orders collected at GKVK differed significantly from those expected at the global (Chi-square = 90.16; p<0.001) and at the national level (Chi-square = 139.03; p<0.001; Table2). Order Coleoptera, Diptera and Hemiptera were found to be over represented while the other two were under represented (Table 2) at GKVK light traps considering both the global and the national levels. Clearly the insect diversity collected at light traps at GKVK did not match the representation of the five most speciose orders at both the national and the global level insect diversity.

The results of the present study suggest that light traps from a single locations may not provide a relative representation of insect diversity across a larger geographical area. This is anticipated because a single locality cannot capture the diversity of the diverse habitats over a large geographic scale. For example as many as 65 species of tiger

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beetles are known from a single stretch of Siliguri to Darjeeling (Pearson and Ghorpade, 1987) owing to continually changing habitats in this stretch. While the entire stretch of Western Ghats is home for less than 300 species, the state of Sikkim alone harbors 689 species of butterflies (Haribal, 2003) again due the habitat diversity created by the altitude variations. Thus the insects from a single locality can be expected to differ from that of a wider geographical area.

Many factors may influence nocturnality in insects. Potentially the night hours being cooler, the cost of flight may be of great importance in being nocturnal in many insects (Price, 1997). Nevertheless, advantages such as avoidance of abiotic stresses (Casy, 1981; Heinrich 1977, Janzen 1973), restricted availability of resources as a mechanism to avoid competition (Sheehan 1994) and possibly predator avoidance (Wcislo et al., 2004; Basset & Springate 1992, Heinrich 1977) could drive some insects to become nocturnal. Given these potential advantages, do nocturnal insects outnumber diurnal insects? The available records do not always favour this possibility and suggest all the three possibilities of either the diurnal or the nocturnal insects to be relatively more abundant than the other or almost a similar representation of both the groups (Springate and Basset, 1996; Basset and Springate, 1992). However, the relative abundances can vary across taxa and the general idea of herbivores to be more abundant during nocturnal hours seems to be better supported with predatory and parasitic species being more active during day time (Rosenthal, 2004).

Few studies have examined the entire fauna of trapped insects and most studies restrict themselves to single taxonomic groups. As a result, although similar trapping methods are likely to provide more constant relative representation (Kitching et al., 2001), it is unlikely that the results would hold when diverse kinds of habitats are explored. Light trap catches have been rarely examined from this point of view. The results thus suggest that the light trap collections may not be expected to represent total diversity on a gross scale for the above mentioned reasons. Nevertheless, it may be of interest to examine the relative differences between the GKVK collections and that of global and national level diversity.

Order Coleoptera, Diptera and Hemiptera were found to be over represented at GKVK while Lepidoptera and Hymenoptera were under represented. Although, Coleoptera were over represented in the GKVK collections, it is of interest to note that among the five orders considered, it had best match with the global diversity (Table 2), while the difference was maximum when compared to the national level diversity. Further considering the 21 orders expected at lights, the representation of Coleoptera in India seems to be far poorer among all insects, compared to the relative representation of the order at the global level (Table 2). As a result, it is tempting to suggest that the Coleopteran fauna of India seems to be far poorly known, and hardly matches with the global proportions. Further, the relatively large diversity of the phytophages among the Coleoptera may have provided a better representation of the over all diversity of the group at the lights.

Similarly, a substantial proportion of Hymenoptera being parasitic (high abundance of Ichneumonoidea (Shapiro and Pickering, 2000), underrepresentation of the group could be because of the overwhelming diversity of predatory and parasitic species in the order which by and large are expected to be diurnal (Rosenthal, 2004; Springate and Basset, 1996). However, the under representation of Lepidoptera despite having a large proportion of moths (90 % or more) which are all, by and large, herbivores and nocturnal and the over representation of Diptera is enigmatic. General representation of the Lepidoptera in the plains of southern India that are relatively dry could be poorer relative to the more wet habitats such as the Western Ghats and the North-Eastern parts of the country, as exemplified by the diversity of butterflies in the state of Sikkim. Many anthropophilic Diptera are expected to be nocturnal as also the scavengers. Other flower visiting Diptera and midges could potentially be crepuscular or nocturnal (Inouye et al., 2015) and are likely to be trapped at lights. A detailed analysis of the dipterans caught at lights may throw some light on the general ecological features that might be contributing for better representation at lights. But these results are invariant with studies using Malaise traps that expect Diptera to be more abundant (Kitching et al., 2001) and consequently more active during day time. Yet, GKVK campus being quite an island in the midst of a growing city, it is possible that a considerable diversity is trapped in the island situation, which gets reflected in the trap catches. A similar possibility may explain the over representation of Hemiptera. An analysis of the subcategories and the juveniles (Basset and Springate, 1992) may provide a meaningful explanation for the observed results.

These results apparently are a product of both the geographic variations in representation and the variability in the relative diel activity of different taxa. Better appreciation of the results are possible through comparative studies with other ecologically matching environments and a detailed analysis of the insects caught in the study at taxonomic subcategories. Such detailed studies will provide better opportunities to evaluate the constancy of the patterns of collections across different taxa and help strengthen the observed results vis-à-vis general patterns of diversity.

Different collecting methods are expected to provide different kinds of samples that can be delineated by taxa. But light traps catch a greater diversity of insects when compared to many other method of sampling; yet few studies have made efforts to assess the overall diversity of all taxa attracted to light and make comparisons with the general diversity patterns. Most studies are target taxa specific and broad comparisons are rare to come by. In the present study, in an insular environment, it was possible to collect representatives of as many as 13 different orders among the possible 21 orders of insects. Potentially a greater diversity of insects by major groups can be found at lights in a more open environment. Thus despite the limitations, the light traps continue to be the choice method of sampling for many groups of insects and in studies to address broad ecological questions. Present investigation further points to many surprising results that call for greater in depth studies to understand and explain the variations found.

Table 1. Number of species in different orders of insects

Sl. No.	Order	Species / order * (India)	Species / order * (World)	Species / order Study site (GKVK)
1	Archaeognatha	2	250	0
2	Strepsiptera	8	300	0
3	Mecoptera	15	350	0
4	Diplura	16	355	0
5	Protura	20	260	0
6	Thysanura	23	1250	0
7	Embioptera	33	200	0
8	Siphonaptera	52	2000	0
9	Phasmida	60	2500	0
10	Psocoptera	85	2500	2
11	Ephemeroptera	94	2146	0
12	Plecoptera	113	2100	0
13	Blattodea	156	4200	4
14	Mantodea	161	2000	3
15	Collembola	200	5000	0
16	Isoptera	300	2000	1
17	Neuroptera	315	5000	2
18	Dermaptera	320	1800	0
19	Phthiraptera	400	3000	0
20	Odonata	491	5500	2
21	Thysanoptera	691	6000	0
22	Orthoptera	759	14491	7
23	Trichoptera	812	7000	6
24	Hymenoptera	5000	100000	38
25	Diptera	6093	96600	110
26	Hemiptera	6500	80000	133
27	Lepidoptera	13000	142500	108
28	Coleoptera	15000	350000	351
	Total	50719	839302	767

***From Ghosh, 1996.**

Table 2.Comparison of the proportions of five speciose orders at GKVK with those of global and national level diversity.

Sl. No.	Order	No. of species observed at GKVK	No. of species expected from national proportions	No. of species expected from Global proportions	Per cent deviation from national level	Per cent deviation from global level
1	Hymenoptera	38	77	93	-50.4504	-59.0181
2	Lepidoptera	108	199	132	-45.8364	-18.2633
3	Diptera	110	93	90	17.7032	22.80722
4	Hemiptera	133	100	74	33.40283	79.29575
5	Coleoptera	351	230	325	52.56068	8.155201
	χ^2		**139.03****	**90.16****		

** Chi square values are significant at 1 %

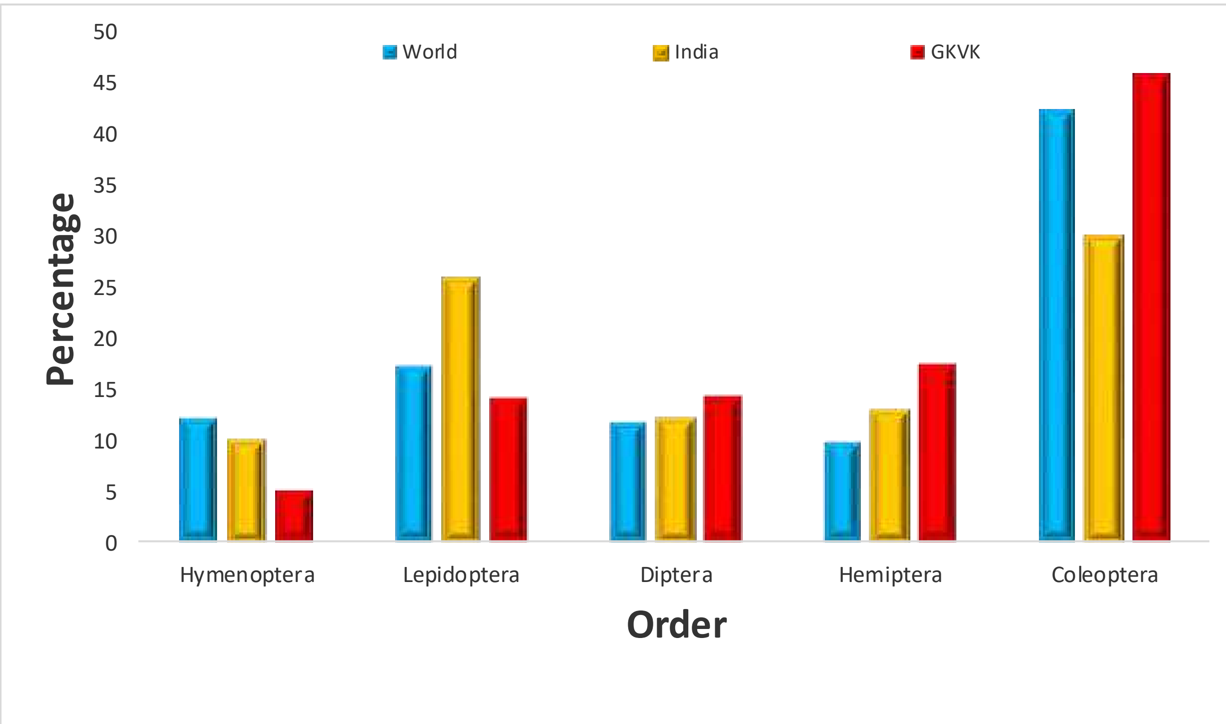

Fig.1: Percent species of the five orders of insects at global, national and at GKVK campus. These values are computed as a percent of the total number of species in 21 of the 31 potential orders that are expected to be attracted to light.

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Effect Inhibitor Packlobutrazol of Against Type and Summed Dominance Ratio (SDR) Weeds in Potato (*Solanum tuberosum* L.) Plant

Rohmi Suwinda[1], Warnita Warnita[2], Irawati Chaniago[2] and Zul Irfan[3]

[1]Department of Agronomy, Faculty of Agriculture, Andalas University, Padang, Indonesia
E-mail: rahmisuwinda@gmail.com
[2]Department of Agrotechnology, Faculty of Agriculture, Andalas University, Padang, Indonesia
E-mail: warnita@agr.unand.ac.id
[3]Researcher, Balai Pengkajian Tekmologi Pertanian of West Sumatera, Solok, West Sumatera, Indonesia

***Abstract*—*The research aimed tostudy determine the effect of plant growth regulator and dominance Paclobutrazol against this type of weeds in potato fields. Weed identification is done by using squares method using a rectangular plot with a size of 1 x 1 m and then the data were analyzed using the formula SDR. The results obtained are 21 species of weeds that grow at the age of 0 WAP (before if the land), 4 WAP (before application Paclobutrazol) and 8 WAP (after application Paclobutrazol). Dominace most in before if the land is Pennisetumpurpupoides with a value of SDR 41.10%, in the period of 4 WAP Chips are Ageratum conyzoides L dominace's with a value of SDR 24.58% and weeds Setariabarbata land dominace's research in the period 8 WAP Chips with value SDR 26.78%.*

Keywords*— *types, SDR, dominance, weeds, potatoes.

I. INTRODUCTION

Potatoes are the main food crop 4th in the world after wheat, rice, and corn [1] is also one of the commodities horticultural crops the main vegetables world that have the potential to be developed as a source of carbohydrate in order to support the diversification program food [2]. Nutrient content per 100 grams of potato tuber ie 2 g protein, 0.1 g fat, 19.1 g carbohydrates, 11 mg calcium, phosphorus 50 mg, iron 0.7 mg, fiber 0.3 g, 0.09 mg vitamin B1 , vitamin C 16 mg and calories 83 cal[3].

Indonesia potato production can only meet 10% of the national potato consumption, ie 8.9 million tonnes per year [4], whereas the intensive cultivation of potato productivity can reach more than 35 tons / ha. According to Central Bureau of Statistics Indonesia [5] the productivity and the production of potato Indonesia in 2014 and 2015 respectively was 17.67 tons / ha (1,347,815 tons) with total area of 76 291 ha and 18.20 t / ha (1,219,269 tons) with total area of 66 983 hectares.

In the potato plant maintenance can not be separated from weed control, because of the presence of weeds in potato crop is often regarded as one of the causes of the decline in the results of the potatoes. The yield reduction is highly dependent on the type of weed, density, time of competition, as well as compounds that issuedalelopati by the weeds. As a result of the decline occurring is yield loss can exceed the yield losses caused by pests and diseases in plants.

According Kastanja[6], many factors can affect the type and diversity of weeds in an area, such as soil type, technical culture, and altitude. Sembodo[7] stated that the high technical culture will affect the low competitiveness of weeds on crops. Further, he said the density of weeds on agricultural land varies according to the season. During the rainy season, water supplies enough that the weed population increases and vice versa.

Land of potatoes grown conventionally, weeds growing on the land can be controlled through tillage and weeding, but these control activities require time, effort and cost is enormous. Inhibiting Substance Usage Growing considered a solution to weed control in addition to saving time and labor, it also saves the cost incurred for such activities. The purpose of this study is to determine the effect of plant growth regulatorPaclobutrazol and dominance against this type of weeds in potato fields.

II. MATERIALS AND METHODS

2.1 Implementation Research

This research was conducted in June-September 2018, in field trials Institute for Agricultural Technology (BPTP) West Sumatra Sukarami located in Solok, West Sumatra with altitude of ± 928 m above sea level. The method used to identify the types of weeds are squares method to create a sampling frame using a rope with a size of 1 x 1 m at 0 WAP (at the time if the land), 4 WAP (before giving Paclobutrazol) and 8 WAP (after giving Paclobutrazol) Research this was conducted in June-September 2018, in field trials Institute for Agricultural Technology (BPTP) West Sumatra Sukarami located in Solok, West Sumatra with altitude of ± 928 m above sea level.

2.2 Data analysis

To determine the dry weight of weeds, weeds example from the field is cleaned, then dried by the oven for 2 x 24 hours with a temperature of 60ºC. Weeds that have been dried tersebutnya weighed to determine dry weight of each species. After the calculated frequency of each species squared plot. The next of these indicators are calculated relative frequency, relative density, relative dry weight and value summed Dominance Ratio (SDR)

a. The density of a kind (KM)
$$KM = \frac{Jumlah\ individu\ suatu\ jenis}{Luas\ petak\ contoh}$$
b. Relative density (KR)
$$KR = x\ 100\% \frac{Kerapatan\ suatu\ jenis}{Kerapatan\ semua\ jenis}$$
c. The frequency of a particular type (FM)
$$FM = \frac{Jumlah\ petak\ ditemukan\ suatu\ jenis}{Jumlah\ seluruh\ petak\ contoh}$$
d. The relative frequency (FR)
$$FR = x\ 100\% \frac{Frekuensi\ suatu\ jenis}{frekuensi\ seluruh\ jenis}$$
e. Biomass is a type (BM)
$$BM = \frac{Biomasa}{Luas\ petak\ contoh}$$
f. Relative biomass (BR)
$$BR = \frac{biomasa\ suatu\ jenis}{biomasa\ serelatif\ semua\ jenis}$$
g. Dominance summed Ratio (SDR)
$$SDR = \frac{KR+FR+BR}{3}$$

III. RESULTS AND DISCUSSIOND

3.1 Type Weeds

The results of the analysis of vegetation of weeds in before if the land, period 4 WAPand 8 WAP found as many as 21 species of weeds such as; 10 species of weeds in before if the land (Table 1), 13 species of weeds in 4 WAP period (before application Paclobutrazol) (Table 2) and 12 species of weeds in a period of 8 weeks after planting (after application Paclobutrazol) (Table 3).

Table 1. Identification of Weeds in Potato 0 WAP

No.	Family	Species	Indonesian name
1	Asteraceae	*Bindens leucorrhiza*	Ajeran
2	Poaceae	*Pennisetum purpupoides*	Rumput gajah
3	Asteraceae	*Ageratum conyzoides* L.	Babadotan
4	Fabaceae	*Mucuna bracteata*	Sengon
5	Rubiaceae	*Oldenlandia corymbosa*	Rumput mutiara
6	Fabaceae	*Crotalaria striata*	Orok-orok
7	Fabaceae	*Mimosa pudica*	Putri malu
8	Euphorbiaceae	*Euporbia heterophylla* L	Katemas
9	Verbenaceae	*Stachytharpheta jamaicensis*	Pecut kuda
10	Asteraceae	*Crassocephalum crepidioides* (Benth)	Sintrong

Table 2. Identification of Weeds in Potato 4 WAP

No.	Family	Species	Indonesian name
1	Asteraceae	*Bindens leucorrhiza*	Ajeran
2	Asteraceae	*Ageratum conyzoides* L.	Babadotan
3	Fabaceae	*Mimosa pudica*	Putri malu
4	Euphorbiaceae	*Euporbia heterophylla* L	Katemas
5	Poaceae	*Setaria barbata*	Bulu rubah
6	Poaceae	*Enchinochloa colona*	Rumput bebek
7	Poaceae	*Cynodon dactilon*	Rumput griting
8	commelinaceae	*Ottochloa nodosa*	Gedong puser
9	Graminae.	*Brachiria humidicola*	Rumput bede
10	Araceae	*Colocasia esculenta L.*	Talas
11	Rubiaceae	*Borreria latifolia*	Rumput setawar
12	Compositae	*Galinsoga quadriradiata*	Bribil
13	Cyperaceae	*Cyperus cyperoides*	Pako

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Table 3. Identification of Weeds in Potato 8 WAP

No.	Family	Species	Indonesian name
1	Asteraceae	*Bindens leucorrhiza*	Ajeran
2	Asteraceae	*ageratum conyzoides* L.	Babadotan
3	Rubiaceae	*Oldenlandia corymbosa*	Rumput mutiara
4	Fabaceae	*Crotalaria striata*	Orok-orok
5	Fabaceae	*Mimosa pudica*	Putri malu
6	Euphorbiaceae	*Euporbia heterophylla* L	Katemas
7	Poaceae	*Setaria barbata*	Bulu rubah
8	Fabaceae	*Brachiria humidicola*	Kaliandran
9	Rubiaceae	*Borreria latifolia*	Rumput setawar
10	Cyperaceae	*Cyperus cyperoides*	Pako
11	Poaceae	*Elusin indica*	Rumput belulang

3.2 SDR

The results of the analysis of vegetation of weeds in before if the land, period 4 WAP and 8 WAPfound as many as 21 species of weeds such as; 10 species of weeds on land though Prior, 12 weed species in the period 4 WAP potato plants and 12 species of weeds in potato crop period 8 WAP (Table 4).

Table 4. The value of SDR various weed species identification results before if the land, 4 WAP and 8 WAP

Weeds name's	Classification of Weeds	SDR (%)		
		0 WAP	4 WAP	8 WAP
Bindensleucorrhiza	Broadleaf	15.01	17.05	14.56
Pennisetumpurpupoides	Grass	41.10		
*ageratum conyzoides*L.	Broadleaf	9.53	24.58	19.21
Mucuna bracteate	Broadleaf	6,90		
Oldenlandiacorymbosa	Grass	4.12		1.46
Crotalaria striata	Broadleaf	5.67		3.67
Mimosa pudica	Broadleaf	2.03	1.20	1.28
*Euporbiaheterophylla*L.	Broadleaf	2.00	6.82	3.17
Stachytharphetajamaicensis	Broadleaf	10.42		
Crassocephalumcrepidioides (Benth)	Broadleaf	3.23		
Setariabarbata	Grass		14.62	26.78
Enchinochloacolona	Grass		11.80	
Cynodondactilon	Grass		1.20	
Ottochloa nodosa	Broadleaf		1.34	
Brachiriahumidicola	Grass		5.29	5.43
Colocasia esculenta (L.)	Broadleaf		2.52	
Borrerialatifolia	Broadleaf		10.37	16.86
Galinsogaquadriradiata	Broadleaf		1.26	
Cyperuscyperoides	Credits		1.96	1.58
Elusinindica	Grass			2.02
Cyperusrotundus	Credits			3.99
Total		100	100	100

Pennisetum purpupoides otherwise known as the king of grass weeds dominate in the research area before the land though, it is suspected the farmers around the area of research with king deliberately planting grass for animal feed. During the rainy season, fodder crops grow, so available in abundance so the king of grass is almost up about half of the land if the study at the time before the land. According Suyitman [8] king grass is a very potential fodder given to include livestock ruminants. This grass is a hybrid between an elephant grass (*Pennisetum purpureum*) with Barja grass (*Pennisetum thypoides*). King grass is a perennial plant (perennial), grow upright form clumps. Roots in, looks similar to sugarcane, height 2-4 m, and if allowed to grow upright can reach 7 m,

Besides Pennisetum purpupoides more dominant weeds found in before if the land is Bindens leucorrhiza and Stachytharpheta jamaicensi. Bindens leucorrhiza has a value of SDRs amounted to 15.01% and Stachytharpheta jamaicensi has a value of SDRs amounted to 10.42%. Both of these weeds manifold broadleaf weeds.According Sumekar et al. [9] generally makes a broadleaf weed species are able to produce abundant seed number so it is difficult to control. This weed grows large habitus, so the competition is going on with plants, especially in terms of getting light [10].

Weeds that have the smallest dominance value based on the value of SDR 2% before if the land is *Euporbia heterophylla.* Is classified as a weed broadleaf weeds suspected Euporbia heterophylla it less competitive with king high grass and overgrown weeds cover Euporbia heterophylla so less gets light so underdeveloped. According Hayata et al. [11]*Euphorbia*able to bloom throughout the year so that reproduction and development was rapid, like the soil slightly moist, somewhat tolerant with ternaung atmosphere. In addition, because the study area includes plains (57 m asl) so Euphorbia hirta is able to grow well because these weeds live and spread the altitude 0-1400 m asl.

Other broadleaf weeds *ageratum conyzoides* L found to be most dominant in the period of 4 WAPKentangdengan SDR value of 24.58%. Weed *Ageratum conyzoides* is one kind of weed of the family Asteracea. *Conizoides ageratum* L. is an annual weed that is commonly found on farms and plantations in the lowlands to an altitude of 3,000 m above sea level. This type of flowering weeds throughout the year and is able to produce up to 40,000 seeds / plants that are easily dispersed by wind and water flow [12]. Knuuttila [13] states that the seeds - seeds of weeds and organs - vegetative organs such as risoma dormant in the soil will do regrowth under appropriate conditions. It is usually characterized by an increase in soil temperature and quality of the sunlight. According to Caton et al. [14], *Ageratumconizoides*L. a shade-tolerant weeds, can occur throughout the entire season, responsive to fertilizer,

Further more mendominance weeds in 4 WAP period after *ageratum conyzoides* is *Bindens leucorrhiza* the SDR value of 17.05 and Setaria barbata with SDR value of 14.62%. Setaria barbata SDR value increased 8 WAP period amounted to 26.78% and most mendominance among other gulama. Setaria barbata is a livestock animal feed ingredients that grow abundantly in the rainy season. Anticipated increase in SDRs on 8 WAP period is due to the high rainfall increased so Setaria barbata SDRnya value. After *Setaria barbata* found weeds *Ageratum conyzoides*L still mendominance on research fields at 8 WAP period with a value of SDR 19.21%. This period of dominance of weeds *Ageratum conyzoides* L decreased compared to the previous period. Unlike the weeds also mendominance at 8 WAP Borreria latifolia period increased from the previous period (4 WAP) which has a value of SDR 16.86% with an increase of 6.49%.

Weeds that have the lowest SDR value of 1.2% is *Cynodon dactilon* and *Mimosa pudica* in the period of 4 weeks after planting. Whereas in a period of 8 weeks after planting potatoes, weeds Oldenlandia corymbosa has the lowest SDR is 1.28%. According toMercado [15], changes in weed species because of a difference in crop management, including water management and fertilization as well as differences in morphological characters of plant constituent components that can alter microclimates planting environment giving rise to the different responses of each weed species.

The most dominant weed species found on land if the land prior research, 4WAP and the period 8 WAP Potato Chipsis a kind of broadleaf weeds and grasses. Dominace most in before if the land is Pennisetum purpupoides with a value of SDR 41.10%, in the period of 4 WAPpotatoes are *ageratum conyzoides* L mendominance with a value of SDR 24.58% and weeds Setaria barbata land mendominance research in the period 8 WAPPotatoes with a value of SDR 26.78%(Picture 1).

A B C

Fig.1: Weed dominant in before if the land, 4WAP and 8 WAP: (A) Pennisetum purpupoides (B) weeds Ageratum conyzoidesL and (C) weeds Setaria barbata

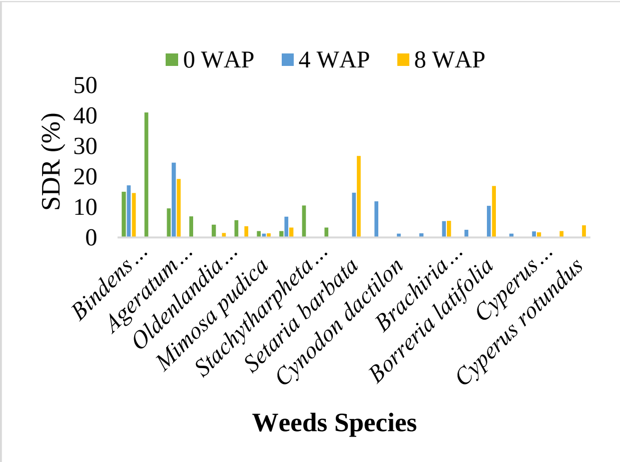

Fig.2: Bar chart value of SDR

Judging from the value of the SDR in the period 4 WAP and 8 WAP weeds*Setaria barbata, Brachiria humidicola, Borreria latifolia* and Cyperus cyperoisdes firstly not found in prior if the land grows well in periods 4 WAP and 8 WAPChipsit is suspected at the time of land preparation are initially dormant weed seeds, germinated due to the reversal of land that grows and develops.In accordance with the statement Paiman et al. [16] on agricultural lands many types of weeds whose seeds immersed enough in the result of the processing of the soil. The lifting of weed seeds to the top layer of surface soil and the availability of appropriate moisture for germination will encourage weed seeds to grow and thrive.

Judging from the development phase of cultivated plants, weeds do not have tocontrolled throughout the growth period of crops. Nietto et al. [17] stated that the presence of weeds throughout the life cycle of plants is not always negatively affect crop production. There is a phase in which the crop is sensitive to the presence of weeds and weed presence in this phase can significantly degrade results, referred to as the critical period. At this critical period of weeds should be controlled in order to avoid competition which can lead to a decrease in plant productivity.

Many factors affect the diversity of weed communities among which are cropping patterns, and the different methods of weed control. Certain weed species are ecologically grow well in areas with a particular crop cultivation and dominate the cropping area of cultivation. Ecologically crop rotation can prevent the dominance of weed species or groups of certain weeds in crop cultivation area [9].

IV. CONCLUSION

The most dominant weed species found on land if the land prior research, 4WAP period and the period 8 WAP Potato Chipsis a kind of broadleaf weeds and grasses. Dominace most in before if the land is *Pennisetum purpupoides* with a value of SDR 41.10%, in the period of 4 WAPpotatoes are *ageratum conyzoides* L mendominance with a value of SDR 24.58% and weeds *Setaria barbata* land dominate research in the period 8 WAPpotato with value of SDR 26.78%

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Constraints of paddy production in Western Terai of Nepal

Chetana Basyal*, Sarthak Ghimire, Bipin Panthi and Sudarshan Basyal

Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P., India
*corresponding author: 12chetana28@gmail.com

***Abstract*— *Paddy is important crop of Nepal, which have significant role in food security and supporting livelihood of the Nepalese population. Although Nepal has harvested the record production in recent year, but it is still far behind to substitute the import. Various strategies are required to be developed to make Nepal self-sufficient in rice. The objective of this paper is to identify the various constraints of paddy production in Nepal. This study utilizes the primary data collected through semi-structured questionnaires. A total 120 paddy growing household were surveyed in Kapilvastu and Rupandehi district (60 in each district) for the study. Study identifies, that among the various constraints faced by farmers major constraints are lack of improved varieties, inadequate supply of chemical fertilizer, lack of proper farm equipment and dependence on monsoon rain for successful crop.*
***Keyword*—Paddy, Agricultural Constraints, Food security, Western Terai.**

I. INTRODUCTION

Nepalese economy heavily depends upon agriculture, which contributes 27.1 per cent of the total GDPand provides livelihood to 65.6per cent of Nepalese population (MoALD, 2019). Among the various crops grown, Paddy is the most important staple crop of Nepal. It holds first rank in term of livelihood generation, area and production (Gauchan et al., 2012; MOAD, 2017). It is grown in about half of net cultivated area of Nepal and contributes about 20 % of the total Agricultural GDP (Gauchan et al., 2014). It supplies about 40% of total calories intake of Nepalese people. Hence, it can be said thatpaddy has significant role in food security and supporting livelihood of Nepalese people.

In Nepal, paddy is grown in varying agro-ecological conditions ranging from 50 to 3000 meter above sea level (Gauchan et al., 2014). Among the total production, about two-thirds of them are produced in southern plain. Due to the lack of all-year-round irrigation facilities, majority of the paddyproduced in the country is rain-fed (Tripathi et al., 2012). Success of paddy production depends upon monsoon rains in Nepal. Although Nepal has harvested the highest paddy production in the recent year, but still it was not able to substitute the import from other countries (Prasain, 2018). There exists lots of challenges in improving production of paddy in Nepal. Therefore, this study was carried to identify the constraints of paddy production in Nepal.

II. RESEARCH METHODOLOGY

This study was conducted in western Terai of Nepal. Kapilvastu and Rupandehi districts was purposively selected for the study because of their high potential for paddy production. This study utilizes primary data to fulfil its research objective. Primary data was collected by using semi-structured questionnaire. A total of 120 paddy growing families were selected for the study (60 from each district) in the month of September and October 2018, through simple random sampling method. Data was collected about their socioeconomic characteristics and the constraints they are facing in paddy production. Socioeconomic characteristics included gender of head of farming household, age, family size, education and landholding. To work out the constraints faced by the farmers in paddy production, constraints were characterized into four groups viz. agro-ecological, economic, technical and institutional constraints, which were listed out by key informant interview and group discussion. The raw data collected were coded and entered into computer for analysis. They were analyzed using M.S Excel and SPSS v25.0. Socioeconomic characteristics of farmers were presented using descriptive statistics like mean, frequency and percentage. And, constraints were ranked based on the percentage of farmers reporting it.

III. RESULT AND DISCUSSION

a. Socioeconomic characteristics

We need to scrutinize the socioeconomic characteristics of the studied population in order to have clear understanding of constraints perceived by them. The socioeconomic characteristics of the respondents are presented in Table 1.

It was found that males holding the position of head in farming household is much more than the female holding the similar position. Male holds position of head in 80% of the studied area, compared to female in only 20%.This situation can be taken as a remnant of patriarchal nature of ancient agriculture (FAO, 2019). According to the age, active population (15-64 years old) represented 89.17% of the total studied population. Only 10.83% of the population were inactive population (64 + years old).

For this study, farming households were classified into three categories based on the family size viz. small (0-50), medium (5-10) and large (>10). It was found that majority of the farmers belongs to medium category (65.83%), followed by small (27.50) and large (6.67%), respectively.

In term of education, it was found that majority of the farming household are medium-sized (65.83 %), followed by small (27.50 %) and large size (6.67%). Majority of farmers are educated up to secondary level (35.83 %), followed by primary level (30.83%) and university level (20.00 %). 13.33% of the total studied population never attended any institution for education.

Farming household were categorized based on their size of total land holding: below 0.5 Ha as a marginal farmer, 0.5 to 2 Ha as a small farmer and above 2 Ha as a large farmers. Table shows that majority of total studied farming household are small constituting 54.16% of total studied household. 35.00% of farmers are marginal, and 10.83% are large farming household, average farm size being 0.98 Ha, which is higher than average farm size of 0.8 Ha (CBS, 2011). Study shows that 48.33% farming household have operational land under agriculture in between 0.5 – 2 Ha, followed by 45.83% and 5.83 % farming household with operational land below 0.5 Ha and above 2 Ha, respectively. The average operational area was 0.92 Ha, and average paddy cultivated area was 0.72 ha.

Table 1: Socioeconomic characteristics of the respondents.

Characteristics	Variables	Frequency	Percentage
Sex of Head of Farming household	Female	24	20.00
	Male	96	80.00
Age	Active population (15 - 64)	107	89.17
	Inactive population (0-14 & 65+)	13	10.83
Household size	Small (0-5)	33	27.50
	Medium (5-10)	79	65.83
	Large (>10)	8	6.67
Education	Illiterate	16	13.33
	Primary level	37	30.83
	Secondary level	43	35.83
	University level	24	20.00
Total Land holding	Marginal (<0.5 Ha)	42	35.00
	Small (0.5 - 2 Ha)	65	54.16
	Large (>2 Ha)	13	10.83
	Average farm size	0.98 Ha	
Operational land holding	< O.5 Ha	55	45.83
	0.5 – 2 Ha	58	48.33
	>2 Ha	7	5.83
	Average operational land	0.92 Ha	
	Average paddy cultivated area	0.72 Ha	

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b. Constraints of paddy production as perceived by farmer

The constraints of paddy production perceived by the farmers are categorized into 4 different categories, i.e. agro ecological, economical, technical and institutional. Farmers were asked what are the constraints in paddy production and these constraints were ranked based on the frequency they are reported. Constraints perceived by the farmers are presented in Table 2.

Table 2: Constraints of paddy production as perceived by farmers

S.N	Constraints	Frequency	Percentage	Rank
A	**Agro-Ecological**			
	1. Late Commencement of rainfall	98	81.67	I
	2. Soil problems	85	75.83	II
	3. Rainfall Pattern	69	57.50	III
	4. Early cessation of rainfall	42	35.00	IV
	5. Topography	8	6.67	V
B	**Economic**			
	1. Chemical Fertilizer	102	85.00	I
	2. Farm Equipment	97	80.83	II
	3. Improved Seed	95	79.17	III
	4. Labour wage	63	52.50	IV
	5. Electricity Charge	24	20.00	IX
	6. FYM	57	47.50	V
	7. Pesticide	46	38.33	VI
	8. Land Distribution	38	31.67	VII
	9. Water cost	27	22.50	VIII
	10. Transportation fee	5	4.17	X
C	**Technical**			
	1. Lack of Improved varieties	106	88.33	I
	2. Unavailability of quality insecticide/pesticide	65	54.16	II
	3. Lack of proper irrigation system	64	53.33	III
	4. Lack of modern farm machineries like sprayer, harvester, thrasher, etc.	61	50.83	IV
	5. Post-harvest technology	28	23.33	IX
	6. Lack of agricultural labourer	54	45.00	V
	7. Heavy pest and weed Infestation	51	42.50	VI
	8. Occurrence of new paddy disease	51	42.50	VI
	9. FYM	43	35.83	VIII
	10. Intensive Field preparation	19	15.83	X
D	**Institutional**			
	1. Training	72	60.00	I
	2. Proper market	72	60.00	I
	3. Extension Services	66	55.00	III

	4. Weather Information	61	50.83	IV
	5. Poor Infrastructure	25	20.83	IX
	6. Crop Insurance	54	45.00	V
	7. Market Information	46	38.33	VI
	8. Electricity and fuel	46	38.33	VI
	9. Proper Credit	36	30.00	VIII
	10. Access to market	21	17.50	X

Agro ecological constraints

It is found that late commencement of rainfall in paddy growing season was perceived by majority of farmer as constraint related to agro ecological condition in the studied area. Farmers in the studied area grow paddy crop in the monsoon season, and commencement of monsoon is marks starting of sowing/transplanting crop. Late starting of monsoon has delayed their cropping time. Similarly, soil problem is perceived as the second constraint in the total studied population. Farmers reveled that decrease in the soil fertility is prevalent in the paddy growing fields in both the districts, which has forced them to have reduced productivity. Rainfall pattern and early cessation of rainfall were perceived as third and fourth constraint respectively. These two constraint along with late commencement of rains, can be related to the climate change (Kang et al., 2009). As the studied area is located in the plain topography which is most suitable for lowland paddycultivation, topography is the least perceived constraint, perceived by only 6.67 % of respondent.

Economic Constraints

Most important constraint perceived by the farmers in the area is high cost of chemical fertilizer, which is perceived by the 85.00% of the total respondent. Farmers revealed that high cost of primary fertilizer like Urea, DAP, MOP, etc. and micronutrient has left them incapable of using recommended dose of chemical fertilizer. It is also reported that in recent years Nepalese farmers are facing lack of chemical fertilizer in market, and often they pay high prices while buying from black market (Acharya, 2019). Similarly, high cost of the farm equipment is perceived as the second most important constraint by the farmers, which is perceived by 80.83% of total respondent. Paddycultivation requires intensive use of farm machinery from preliminary land preparation to thrashing. This could be the reason for lack of proper mechanization in Nepal (Shrestha, 2012). High cost of improved seed is third most important constraint in paddy production in the total studied population, which is perceived by 79.17% of respondent. Labour wage is another important constraint of the studied population, which is perceived by 52.50 % of respondent. Availability of the fully decomposed FYM is another important constraint of the paddyproduction, perceived by 47.50% of the farmers. High cost of pesticide is the sixth important constraint, perceived by 38.33% of the respondents. Other constraints perceived by the farmers are land distribution, high water cost, high electricity charges and high transportation charges, which is perceived by 31.67%, 22.50%, 20.00% and 4.17% of the total respondents, respectively.

Technical Constraints

Lack of improved varieties is the most important constraints as perceived by the farmers, which is perceived by 88.33% of respondents. Although various initiatives are being done in Nepal for improvement of rice varieties, still very less success is achieved (Ghimire et al., 2015). Similarly, Unavailability of the quality insecticide/ pesticide is second most important technological constraint, which is perceived by 54.16% of the respondents. Similarly, lack of proper irrigation system is identified as the third most important technological constraint which is perceived by 53.33% of the total respondent. Lack of modern farm machineries is the fourth most important technological constraints, which is perceived by 50.83% of the total respondent. Lack of agricultural labourer is the fifth important constraint, which is perceived by 45.00% of the respondents. Heavy pest and weed infestation and occurrence of disease is perceived another important technological constraint, which is perceived by 42.50 % respondents. Other technological constraints are unavailability of the farmyard manure, post-harvest technology and intensive land preparation, which is perceived by 35.83%, 23.33% and 15.83% of the total respondents, respectively.

Institutional Constraints

Poor market infrastructure and lack of training is the most important institutional constraints which is perceived by 60.00% of the total respondents. Another important institutional constraint is lack of extension services in the studied area, which is perceived by 55.00% of the total respondents. Similarly, 50.83 % of farmers reported that they do not have proper information related to weather. This supports the above-mentioned rainfall related constraints in agro ecological category. Other constraints perceived by farmers include lack of proper crop insurance policies (45%), lack of access to market information (38.33%), unavailability of sufficient electricity and fuel (38.33%) for irrigation and mechanization, lack of access to credit (30.00%), poor infrastructure (20.83%) and access to market (17.50%).

IV. CONCLUSION

This study explores the various constraints faced by the paddy farmers in the western Terai of Nepal, which have great potential of paddyproduction because of climate and geographical suitability. Study identifies, that among the various constraints faced by farmers major constraints are lack of improved varieties, inadequate supply of chemical fertilizer, lack of proper farm equipment and dependence on monsoon rain for successful crop. These findings calls for the governmental bodies, research institutions, local agencies and other stakeholders to design plan and implement a proper strategy to increase the paddy productivity in the area, and support the livelihood and food security in the region.

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Elaboration of a key to the determination of indigenous figs in North-West Morocco

El Oualkadi A*, Hajjaj B*

*INRA- Regional Agricultural Research Center of Tangier, Morocco
Corresponding author. E-mail: ai.oualkadi@gmail.com

***Abstract*— *Morocco, with its pedoclimatic potential, is a natural habitat for the fig tree, especially the autochthonous fig tree are the genetic diversity remains to be highlighted. In this work a study has been made of the characters that best differentiate autochthonous figs from northwestern Morocco. To establish our determination key, we proceeded to a hierarchy of characters to use. For this, we have based essentially on the practical aspect of the characters, that is to say the characters easy and obvious observation and whose different states are also easy to observe and appreciate.*

***Keywords*— Ficus carica L., Northwest Morocco, indigenous, key determination.**

I. INTRODUCTION

The fig (*Ficus carica L.)* may be the first domesticated plant of the Neolithic Revolution [1]. It is considered to have been cultivated for the first time in southern Arabia [2]. Wild or "almost wild" figs have been reported throughout much of the Middle East and Mediterranean region [3]. Today, it is cultivated worldwide, with a production rate of one million tones [4].

At the national level, the fig tree is a tree of great importance for the Moroccan population and fulfills several functions: social, economic and environmental [5]. Northern Morocco is characterized by a heterogeneous physical environment (climate, soil, relief, land use ...) [6]. It is a hot spot area for biodiversity in the Mediterranean region [7]. Demographically, it is the most populous region of the country [8].

Cultivation of the fig tree, of which 85% of the national orchard is located in the Rif, is the best illustration of such a situation. However, despite the maintenance of traditional survival agriculture, fruit trees and in particular fig trees represent a secondary agricultural activity threatened with marginalization. Local varieties of the fig tree maintained under ecological conditions and in areas with contrasting farming practices are probably characterized by high genetic diversity and considerable hardiness. Morphology, pomology and molecular markers are effective tools for assessing genetic diversity and classifying fig accessibility surveys for plant diversity, but these traits are strongly influenced by environmental conditions. To remedy this, a wide range of molecular markers is increasingly used to evaluate genetic polymorphism. Unfortunately, little research has been done on genetic diversity in fig genetic material [9]; [10]; [11]; [12]; [13]; [14]. To better conserve and use genetic resources, patterns of characterization of morphological variability within collections and selection of the most significant variables must be carefully performed [11]. In Morocco, apart from the work done on the Aïn Taoujdate domain collection at INRA Meknes [15], [16]. The Aïn Taoujdate varietal collection is considered as a reference collection for fig tree genetic resources in Morocco and is also the only collection analyzed and characterized at the pomological and molecular level [15]; [16]. Unlike other fruit trees, on the fig tree no work on the establishment of a determination key has been made. The development of determination keys using statistical programs [17] and automatic measurement methods by computer to obtain data in a fast and accurate manner [18]; [19] have thus been made possible. For example, for the vine species close to the fig tree, the use of computers and the generalization of computers have greatly favored the production of ampelographic files for the recognition of grape varieties [20]; [21]; [22], [23]; [24]; [25]; [26] [27]; [28]. In this work, we have mainly sought to differentiate autochthonous figs from northern Morocco and to develop a key to determine these figs based on pomological criteria.

II. MATERIAL AND METHODS

Plant material

The study looked at 96 ecotypes of fig trees prospected in northern Morocco. It is about 49 indigenous figs, well spread in the orchards of northern Morocco (Table.1). The work was based mainly on surveys carried out in 14 stations in four large areas in the north - west of the country. These stations were chosen according to the importance of fig orchards in agrosystems (Fig. 1).

- Béni Ahmed area: characterized by its richness in fig and caprifigue as well as a good knowledge of cultivation techniques in particular caprification.
- Areas of Moukrisset, Zoumi and Oued Laou: areas rich in figs with very diversified varieties, but with a lack of knowledge of caprification techniques.
- Khmiss anjra area: is a new and much diversified variety of resources especially in Douar Tafza, this area is also characterized by a neglect of caprification.

Table.1: List of varieties studied

The main varieties		
Rhoudane	Saadi	Jouhri
Gaouizi	Lemti	Ournakssi
Ferzaoui	Sinani	Kharar
Baghi assal	Achir	Hafer elbrel
Harchi el khal	Hafri	Sbaa ou rhgoud
Meltoufa	Chitoui	Kohli
Kharaza	Bakour	Zerki
Tahadakte	Larchan	Tbantou
Tabli	Mouslikh	Sibti
Hazouta	aroui	Kourti
Lmdar	L'khoumbiz	Silfaf
L'hmar	Lndbar	Smouni
Fassi	L'beidi	Khoumsi
El messari	harchi lbeid	Ounk Hmam
Makoutia	L'mdar eL khal	Ozilane
Qouti	Maalmouss	
Lassoune	Zenfoukh	

Statement of determination key:

To establish our determination key test, we proceeded to a hierarchy of characters to use. For this, we have based essentially on the practical aspect of the characters, that is to say the characters easy and obvious observation and whose different states are also easy to observe and appreciate.

So we adopted the following hierarchy to build our key:

A: Color of the epidermis.
B: Form of the fruit.
C: Type of collar.
D: Peduncle.
E: Cracks.
F: Form of the top of the fruit.
G: Ostiole.
H: Placenta.

The combination of these different characters allows us to make a satisfactory distinction of the varieties prospected during our study.

III. RESULT AND DISCUSSION

For the establishment of this key of determination, several problems arise which one can quote:

1 / Varieties such as: Kohli, Fassi, Ounq Hmam, Gaouzi, Koti, meet several definitions and are repeated in the descriptive sheets with different characters,

2 / Varieties that are considered both unified and biferous as Assal, Kohli, Koti, and Ounq hmam.

These various problems are due to several causes, on the one hand the insufficiency of the repetitions within each variety, to have an idea on the variability intra varietal, and on the other hand to the limited and probably very local value of the the same variety may have different names in two different localities, such as the case of the variety El messari which bears this name in the region of Beni Ahmed while the same variety is named Johri in the region of Moukrisset. There is also the problem of varietal knowledge among respondents, who may confuse varieties or who disagree about variety traits. Indeed, the same variety can be considered fig with tow production by a person while for another person it will be of fig of one production.

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Key for the determination of autumn figs

1. <u>Fig with one production</u>

A. Purple epidermis black to black
B. Flattened pyriform fruit to oblic turbiform
C. Col absent
D. Long and thin peduncle, little pubescent skin
F.F. Rounded Summit…………………………...1- Gaouizi
D.D. Short and thick peduncle
G. Ostiole small half open with a split 2.Tahadakte
G.G Ostiole large, open without splitting .... 3.El Smouni
C.C. Prominent collar …………………………………………4- Gaouizi
B.B. Short pyriform fruit

C. Col absent
D. Thick and long peduncle, little pubescent skin .... 5- Harchi lkhal
B.B.B. Flattened spherical fruit, very delicate and non-pubescent skin .... 6- Tabli
B.B.B.B Small fruit, elongated pyriform 7.Assal
A.A. Light green epidermis, yellow green, sometimes dark green
B. Elongated pyriform fruit, sometimes oblique
D. Short and thick peduncle
E. Small longitudinal cracks and tight furrows
G. rounded apex .... 8- Chitoui
G.G. flat top .... 9- Sbaâ or R'koud
E.E Fissures absent, and longitudinal furrows of minor importance ....
.... 10- Harchi lbyad
B.B. Flattened spherical fruit
C. Col absent
D. Short and thick peduncle
E. Cracks present
G. Fine paths .... 11. Herich
G.G Furrows big .... 12. Zenfough
E.E. Missing cracks
G. Strong furrows well marked .... 13. Koti
D.D. Long and thin peduncle, dark green ....14. Khoumsi
B.B.B. Small, flattened or turbidiform pyriform fruit
C. Col absent
E. Missing cracks .... .15. Bakour
E.E. Small cracks .... 16. Kohli
C.C. Thick collar
F. Few furrows and whitish spots ....17 Larchan
F.F Fine creases, yellowish spots .... 18 Koti
B.B.B.B. Fruit of small size elongated pyriform .... 19 Ounq hmam
A.A.A. Epidermis red brown to black
B. Flattened spherical fruit
C. Col absent
H. Fruit de très grande taille sans tâches,
G. Placenta blanc jaunâtre ………………….………...20. Mouslikh
H.H. Fruit de petite taille, avec des taches importantes
G.G. Placenta blanc verdâtre……………………..……21.Kharaz
B.B. Fruit de petite taille, pyriforme aplati
C. Col absent…………………………………………………...22 Ounq hmam

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C.C. Col épais…………………………………………………....23.Kourti

A.A.A.A. Epiderme jaune doré, parfois de couleur rose jaune très caractéristique du fruit……………………………………………………………...……24. Silfafe

C. Col absent

H. Very large fruit without stains,

G. Yellowish white placenta .... 20. Mouslikh

H.H. Small fruit, with important spots

G.G. Greenish white placenta 21.Kharaz

B.B. Small fruit, flattened pyriform

C. Col absent .... 22 Ounq hmam

C.C. Thick col 23.Kourti

A.A.A.A. Golden yellow epidermis, sometimes of a yellow pink color very characteristic of the fruit .... 24. Silfafe

2. Fig with two production

A. Reddish brown epidermis

B. Flattened pyriform fruit

C. Very long neck more or less thick

D. Short and thick peduncle, not very delicate skin

G. Flat Summit .... 25- Zerqui

C.C. Col. more or less long

D.D. Short and thick peduncle, very delicate skin

G.G Rounded Summit .... 26- Baghi

C.C.C. Col absent

Short and thick peduncle

E. Not very delicate skin

G. Half open ostiole ....27. Assal

G.G. Ostiole closed

H. Yellowish white placenta .... 28. Harchi

H.H. Greenish white placenta ....29. Kohli

C.C.C.C. Thick neck flattened

D. Long and thin peduncle .... 30- The mti

B.B. Flattened spherical fruit

C. Col absent

D. Short and thick peduncle .... 31. Hafer el bghal

D.D. Long and thin peduncle

F. Missing cracks 32.Hamra

F. F. Small longitudinal cracks .... 33. Fassi

C.C. Long, thick neck, thin on the side of the peduncle like the pigeon's neck ....34. Ounq hmam

C.C.C. Very short neck almost absent

Epidermis with yellowish, elongated, brownish patches 35.Hmir

C.C.C.C. Thick and short collar

Epidermis with few brown spots ....36. Lmdar lkhal

C.C.C.C.C. Thick neck long and flattened

Small flattened spherical fruit is consumed when it is still green …....... 37.Makoutia

B.B.B. Globular spherical fruit and absent collar ....38. Ounq hmam

B.B.B.B. Short pyriform fruit of average size .... 39. Tbantou

B.B.B.B.B.Fruit short or globose pyriform ....40. Fassi

B.B.B.B.B.B. Extended pyriform fruit ....41. Hafri

A.A. Epiderme vert clair à vert jaune

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B. Fruit sphérique aplati
C. Col absent
D. Pédoncule court et épais
F. Fissures de faible importance……………………………….…42.Lmdar lbyad
F.F. Fissures absentes
H.Ostiole demi ouvert…………………………….............43.Maalmnouss
H.H. Ostiole fermé………………………………………...44.Lassoun
D.D. Pédoncule long et mince……………………………………...45.Koti
C.C. Col épais et petit
B.B. Fruit turbiniforme oblique…………..………………………………...46.Lndbar
A.A. Light green to yellow green epidermis
B. Flattened spherical fruit
C. Col absent
D. Short and thick peduncle
F. Small cracks ....42.Lmdar lbyad
F.F. Missing cracks
H.Ostiole half open 43.Maalmnouss
H.H. Ostiole closed 44.Lassoun
D.D. Long and thin peduncle ....45. Koti
C.C. Thick and small collar
B.B. oblique turbiniform fruit 46.Lndba
B.B.B. Pyriform fruit, flattened
C. Short and thick neck
F. Fissures absent, well marked furrows
G. Delicate and pubescent skin ....47.El messari
G.G. Skin not very delicate and pubescent ....48 Gaouizi
F. Fissures absent, furrows in the form of abundant plumes, especially when the fruit is touched
....49.Ournakssi
C.C. Thick neck long and flattened 50.Saadi
C.C.C.Col absent
D. Short and thick peduncle
H. Large fruit with long brown spots ………………………. 51.Sebti
D.D. Long and thick peduncle
H. Small fruit with yellowish spots 52.Sinani
B.B.B.B. Small fruit, oblique pyriform ....53.Harchi lbyad
B.B.B.B.B. Very small fruit, very pigmented with yellowish spots
.... 54.Achir
A.A.A. Black epidermis
B. Small fruit, flattened pyriform or oblique turbiniform
C. Short and thick neck
F. Fissures absent, well marked furrows
G. Yellowish white placenta 55.Assal
F.F. Small cracks
H. Not very delicate skin 56.Kohli
F.F.F. Longitudinal cracks
H.H. Very delicate skin ....57. Rzilane
C.C. Thick and long neck
H. Flat top ....58. Harchi lkahl
H.H. Rounded summit ....59. Baghi
C.C.C. Thick and thin collar ....60. Ferzaoui
C.C.C.C. Col missing ....61. tibal
B.B. Small flat spherical fruit

D. Short and thick peduncle
H. Rounded Summit .... 62. Gaouzi
H.H. Flat top ....63. Meltoufa
B.B.B. Elongate pyriform fruit of medium size, sometimes oblique turbiniform
C. Col absent
D. Short and thick peduncle
F. Small cracks
G. Flat Summit .... 64. Aroui
D. Short and thick peduncle
H. Skin not very delicate, pubescent 65.Humoumbiz
H.H. Skin not very delicate and not pubescent, smooth .... 66. Hazouta
F.F Longitudinal cracks well marked ....67. Ghouddane

IV. CONCLUSION

The method used proved to be very useful in differentiating autochthonous fig tree varieties from northwestern Morocco. Classical morphological description is still the method most used in a practical way by a large number of people. The most discriminating characters are, ultimately, those that express the color of the epidermis, the shape of the fruit, the type of cervix, the peduncle, the cracks, the shape of the top of the fruit, the ostiol and the placenta because it allows to qualify the variables and gives us an exact idea of the different degrees taken by the parameters. The use of fig determination keys, from these parameters seems valid for a zone or a region delimited with a number. To improve the quality of this key, it would be necessary to increase the number of observations and to widen the surveys in order to have a clear vision on the different varieties. This pomological approach will be complemented by the use of molecular markers.

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Differentiation of autochthonous figs from northwestern Morocco with morphological criteria

El Oualkadi A*, Hajjaj B*

*INRA- Regional Agricultural Research Center of Tangier, Morocco
Corresponding author. E-mail: ai.oualkadi@gmail.com

***Abstract*— *The importance varietal diversity of fig tree in the traditional agro ecosystems of the Riffian Mountains remains ignored. To evaluate it, a prospection and characterization were carried out in North West of Morocco. Seventy-five morphological parameter traits were used to characterize the fig accessions. In the first way of hierarchical clustering, the fig accessions were distributed into three main groups. The groups were very heterogeneous and include many clusters separate under tow or more clusters. Thus, it could be concluded that there are a wide range of variability within the cultivated fig accessions under current study. This diversification could enrich the genetic base of this genus and required more studies to achieve the maximum usefulness from this diversification.*

***Keywords*— Fig, morphology, diversity, cluster analysis, Morocco.**

I. INTRODUCTION

Ficus carica L. is a diploid species (2n = 26) [1] that belongs to the Moraceae family. It is characterized by the presence of latex in all parts of the plant. The fig is probably the oldest cultivated crop [2], being widely used as a fruit tree in the eastern Mediterranean regions of Europe and Africa and in southwestern Asia [3]. It is the only species of this family cultivated for its edible fruits [1], [4]. In Morocco, the cultivation of the fig tree is ancestral, the villagers of some production areas (Taounate, Chaouen, Ouezzane) claim that its culture is very old and that the dried fruit traded with cereals, from the Gharb. Its cultivation, which once covered a number of flat lands, is currently limited to hills, sloping land and housing. Indeed, this fruit tree is considered secondary and sometimes grown on the edges of orchards to protect them. The fruits are mainly destined for the local market and eaten fresh or dried. This Mediterranean fruit has been linked and has been classified as a minor species despite the role it can play in the development of many areas, especially with the drought and the reduction in the cold availability necessary for fruiting. Intensive fruit species such as apple, peach, pear, etc. The marginalization of the *F. carica* culture exposes the species to severe genetic erosion. It is therefore imperative to put in place a safeguarding strategy aimed at preserving and protecting this heritage.

The varietal heritage of the fig tree (*Ficus carica L*) on the Mediterranean scale consists of a few hundred varieties whose genetic diversity has been characterized essentially morphologically [4], [5]. Several studies have reported the use of morphometric and pomological parameters as well as isozyme markers to discriminate fig cultivars [6], [7], [8]. In Morocco, the first work concerning the pomological description of fig tree varieties was made by [9] but from surveys limited to the Chefchaouen region. In order to analyze the constraints affecting the production and marketing of figs, important factors for the development of this sector, particularly the knowledge and characterization of local varieties, must be taken into account. In this context, a study identifying the differentiation of local varieties using morphological parameters was carried out in the northwestern region of Morocco.

II. MATERIAL AND METHODS

Plant material

The study looked at 96 ecotypes of fig trees prospected in northern Morocco. It is about 49 indigenous figs, well spread in the orchards of northern Morocco (Table.1). The work was based mainly on surveys carried out in 14 stations in four large areas in the north - west of the country. These stations were chosen according to the importance of fig orchards in agrosystems (Fig. 1).

- Beni Ahmed area: characterized by its richness in fig and caprifiguier as well as a good knowledge of cultivation techniques in particular caprification.

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- Areas of Moukrisset, Zoumi and Oued Laou: areas rich in figs with very diversified varieties, but with a lack of knowledge of caprification techniques.

- Khmiss anjra area: is a new and much diversified variety of resources especially in Douar Tafza, this area is also characterized by a neglect of caprification.

Table.1: List of varieties studied

The main varieties		
Rhoudane	Saadi	Jouhri
Gaouizi	Lemti	Ournakssi
Ferzaoui	Sinani	Kharar
Baghi assal	Achir	Hafer elbrel
Harchi el khal	Hafri	Sbaa ou rhgoud
Meltoufa	Chitoui	Kohli
Kharaza	Bakour	Zerki
Tahadakte	Larchan	Tbantou
Tabli	Mouslikh	Sibti
Hazouta	aroui	Kourti
Lmdar	L'khoumbiz	Silfaf
L'hmar	Lndbar	Smouni
Fassi	L'beidi	Khoumsi
El messari	harchi lbeid	Ounk Hmam
Makoutia	L'mdar eL khal	Ozilane
Qouti	Maalmouss	
Lassoune	Zenfoukh	

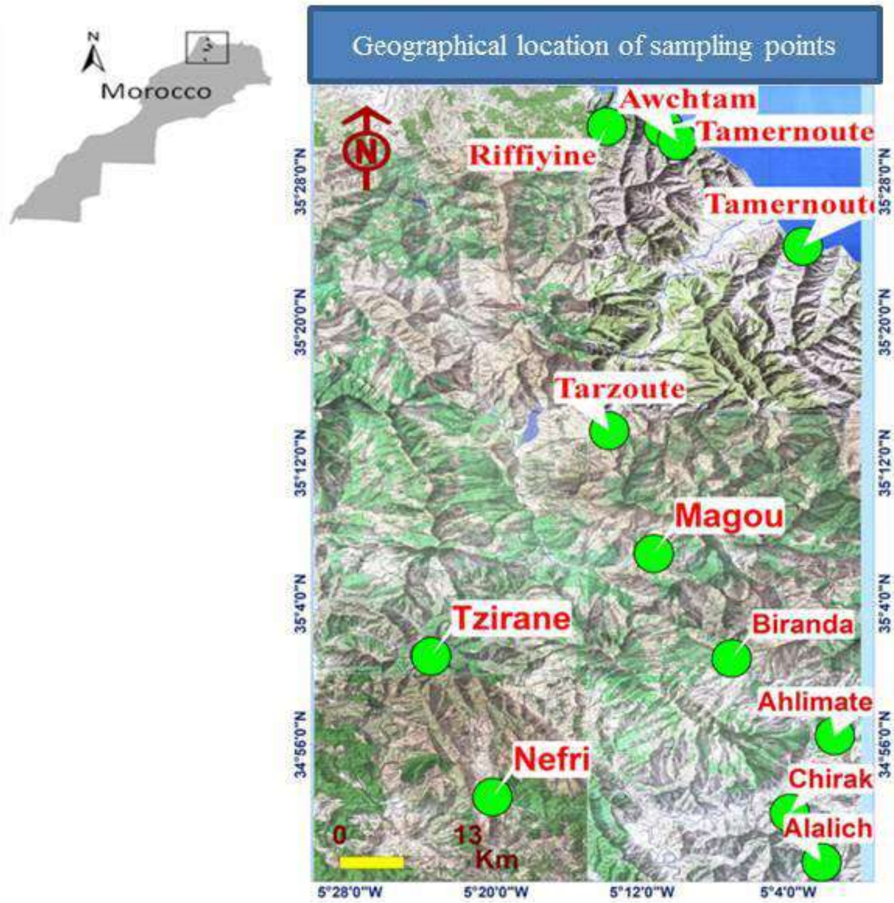

Fig. 1: Localization of the sites prospected

Morphological character

The characteristics studied concerned the vegetative development of the tree and, in particular, the measurement of certain parameters characterizing the growth of leaves and shoots developed during the years 2015 and 2016. For each ecotype, the measurements were carried out on two trees and, for each tree, six shoots selected on the south, north-east and north-west sides, at the rate of two shoots per exposure.

We took into account 3 characters: tree habit, leaf type and lobes. For each character, the different modalities are considered (Table 2).

Harbor of the tree:

It corresponds to the general appearance of the tree, there are 5 cases:

Erected: The outer shape of the set of leaves on the tree forms an angle of 60 °,

Semi-erect: The outer shape of all leaves on the tree forms an angle of 80 °,

Compact: The outer shape of all leaves on the tree forms an angle of 90 °,

Spread: The outer shape of the set of leaves on the tree forms an angle of 100 °,

Falling: The outer shape of all leaves on the tree forms an angle of 120 °.

Type of leaves:

This character corresponds to the number of leaves divided by 5 cases: leaves of a single division, leaves little divided, leaves moderately divided, leaves divided, leaves very divided:

Lobe :

The lobes of fig leaves differ in their depth, so we can distinguish leaves with lobes: very shallow, shallow, medium, deep, very deep.

Table. 2: Morphological characters studied

Character	Modality
Tree port	Erected
	Semi erected
	Compact
	Spread
	drooping
Sheet type	1 piece
	Little divided
	Middle divided
	divided
	Very divided
Lobe	Very little deep
	Shallow
	Middle Deep
	Very deep

Fruit general appearance

For the general appearance of fruits,

a set of qualitative and quantitative characteristics has been studied, several modalities have been taken according to each parameter (Table 3).

Table. 3: Fruits General Appearance

Character	Modality
	Very small
	small
Average height	Average
	Big
	Very big
	Very light
	Lightweight
Average weight	Way
	Heavy
	Very heavy
	Very delicate
Skin	Little Delicate
	hairy
	No Pubescente
Mountain peak	Round
	Dish
	flattened
	Spherical
Form	egg-shaped
	pyriform
	Turbiniforme
	Squash

External and internal characteristics of the fruit

For the general appearance of the fruit, 10 quantitative and 12 qualitative characters were studied. For the external characters of the fruit (Table.4): 35 qualitative and 3 quantitative characters, while for the internal characters of the fruit, 32 qualitative characters (Table.5).

Table. 4: The external characters of the fruit

Character	Modality
Epidermis color	Light green
	Yellow
	Green yellow
	Grey
	Brown
	Purple
	Dark purple almost black
	Violet tied dark wine
	Greenish
	Golden yellow
	Red-brown

	Black purple
	Violet - red
	Brown gray
	Purple
	Uniformly dark
Eye	Closed
	Open
	Half open
	small
	Big
	Providing duplication
Peduncle shape	Variable
	Long and thin
	Short and thick
Col	strong
	Long
	Well distinct
	Contrasted to the body
	Very affected
	+/- marked
	Thick
cracks	Low Importance
	absent
	Longitudinal
Average size of the ostiole	small
	Average
	big

Character	Modality
Placenta	Yellowish-white Greenish white
Pulpe	Salmon pink Black violet Red purplish Pink yellow Amber Amber rose Pale yellow Red brunette Hot pink Dark purplish red
Abundance seeds	Low Average important
Seed size	Low Average important
Perfume of the fig	Low perfume fragrant Very fragrant
vesicles	fines Pretty fine Grosses
Quality	Poor Fair Pretty good good excellent
State of the fig	Fresh fig Dried fig

Statistical analyzes

In order to facilitate the acquisition and processing of data, each modality has been assigned a code. The data processing is carried out using the NTSYSpc software for the UPGMA analyzes of similarities and algorithm for the construction of the clusters.

III. RESULT AND DISCUSSION

Port of tree :

According to surveys conducted in the region, most of the trees have a semi-erect habit (48.98%, effective 24), and 34.70% have a spreading habit (number 17), the others have a falling port 10 , 20% (effective 5) and 6.12% of the trees have a compact habit, while erect varieties have not been observed. [10] have shown that the variability of the vigor and the habit of the fig tree is due to a strong reiteration of the growth which is accompanied by a significant collapse of the branches. The carrying of the tree is also heritable and this heritability can change according to genotypic diversity and ecological conditions [11].

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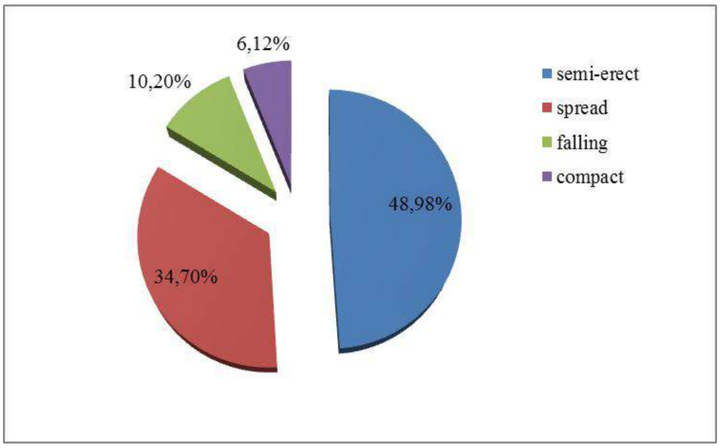

Fig .2 : port of tree

Type of sheet:
The importance of the morphological characteristics of the leaves (Fig. 4) for the varietal differentiation of the fig tree has been the subject of much research [12] and [13]. These traits are very important for the selection of genotypes by arborists and breeders [14].
For this trait, it was noted that the varieties' maturity is divided (63.27%, effective 31), 22.45% have medium division leaves, 12.24% have very divided leaves and 2.04 % have poorly divided leaves, whereas no variety has leaves with a single division. These values are different (14 to 21) from those recorded by [15] but close (6 to 13) to those of [16]. Nevertheless, the differentiation of fig varieties with their leaves, especially in the number of lobes [17] and the thickness of the petiole is difficult because they are polymorphic within the same individual.

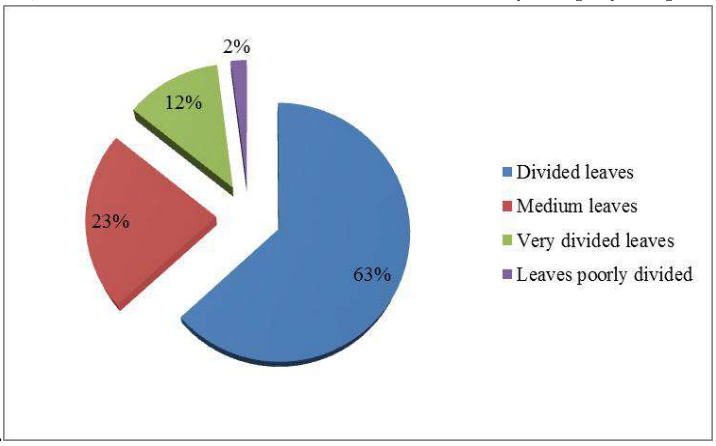

Fig.3: types of leaves

Lobe:
The study of the morphology of the leaves of the varieties showed that 53.06% of the varieties have a deep lobe (effective size 26), 28.58% of the varieties have an average lobe (effective size 14), while 10.20% have a shallow lobe and 8.16% have a very deep lobe (effective 4). [18] consider that the length of the petiole, the number of lobes per leaf, the length, the width and the leaf area are important parameters for the phenotypic analysis of the fig tree, whereas for [19] they are the form of the base of the leaf, the position of the small side lobes, the degree of approval, and the number and shape of the lobes that are important. In Turkey, [16] found that the color of the petiole is a character that can also change according to the type of fig tree, the cultivar and the conditions of the environment.

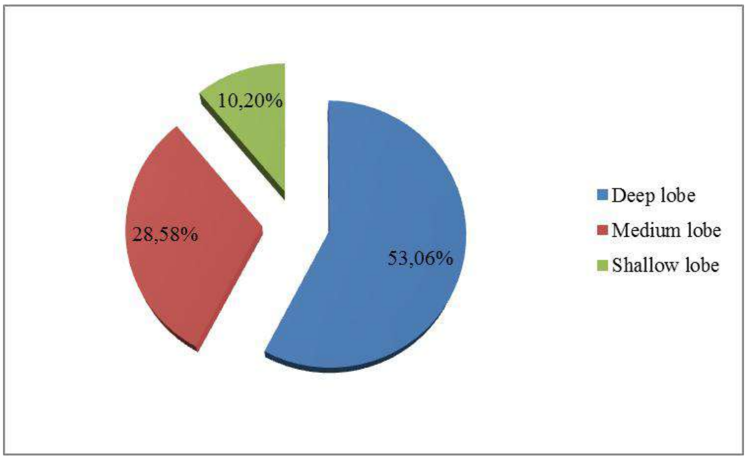

Fig.4: The different types of lobes

Hierarchical classification

In the first way of hierarchical clustering, the fig accesses were distributed into three main groups. The first group includes four clusters separate under two clusters. The first cluster included Rhouddane accessions, Baghi assal and Harchi lkhal, the second cluster included accessions, Lfassi, Lhmar. The third group was heterogenous (Fig.5).The analysis of the data shows that these types have a great variability as well as the variety. The varieties which bear the same name, with the same morpholigical characters, but it is a general case. We think that they may be in certain cases different varieties but bearing the same name (homonymy) or on the contrary (synonymy). As they can be in other cases polymorphic varieties, polyclonal nature of the varieties is shown in the evaluation of the national collection [20]. It is thus necessary to record the importance of the problems of denomination of the species listed in the prospected area. These problems are due to local names which vary from one locality to another and the absence of the syntheses and varietal characterization.

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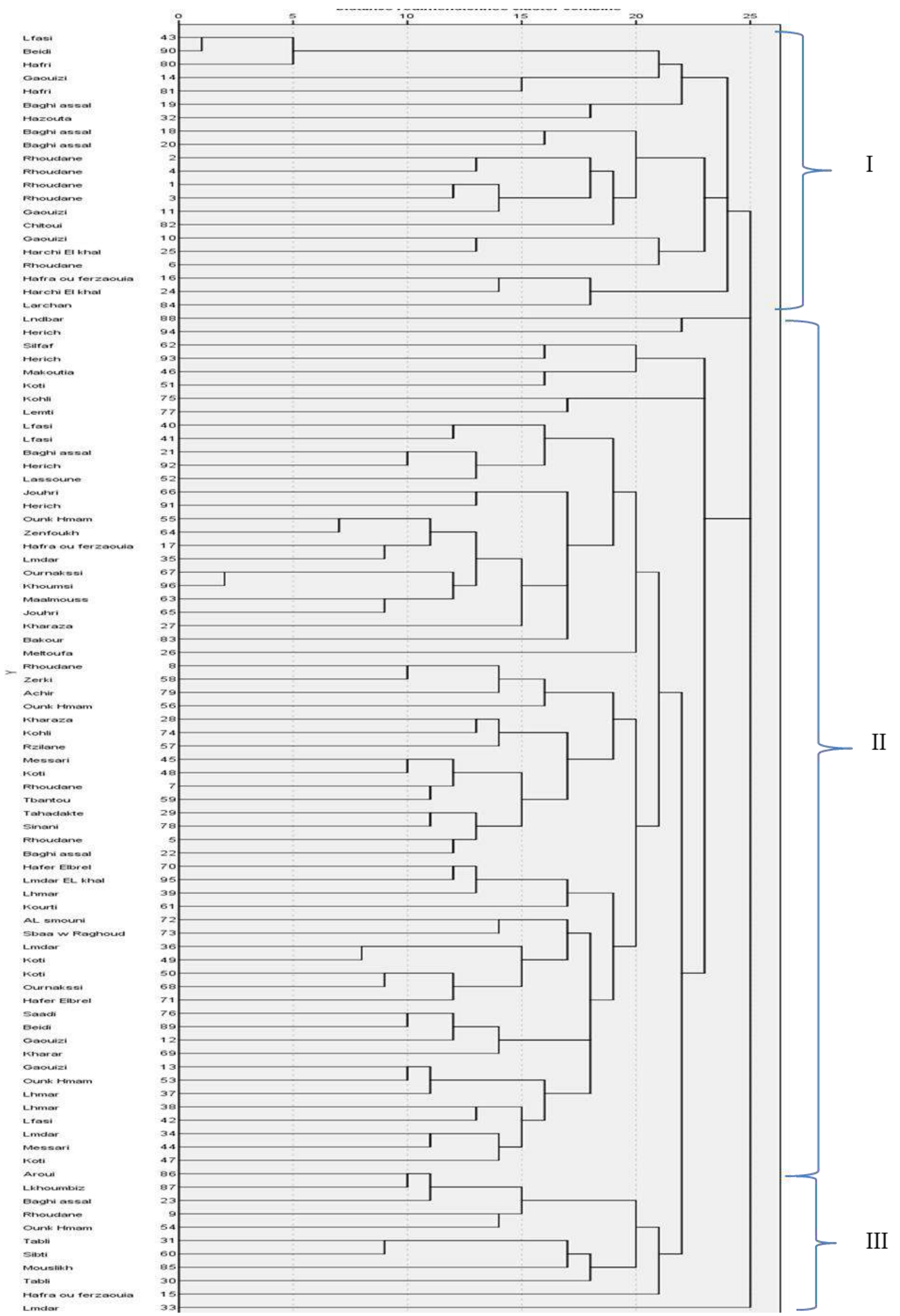

Fig. 5. Representation in cluster of individuals of the north region of Morocco.

IV. CONCLUSION

It could be concluded that there are a wide range of variability within the cultivated fig accessions under current study. This diversification could enrich the genetic base of this genus and required more studies to achieve the maximum usefulness from this diversification. Morphological results will be useful in characterizing and to create the first reference and catalogue of the fig accessions.

ACKNOWLEDGMENT

The authors are grateful to all farmers for providing necessary facilities for conducting this research work.

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Use of physiological criteria to differentiation of indigenous figs from northern Morocco

El Oualkadi A*, Hajjaj B*

*INRA- Regional Agricultural Research Center of Tangier, Morocco
Corresponding author. E-mail: ai.oualkadi@gmail.com

Abstract*— This work is a contribution to the differentiation of cultivars of autochthones figs prospected in Northwestern of the Morocco. 96 ecotypes of fig trees prospected in northern Morocco and about 49 indigenous figs well identified. Hierarchical classification showed that the different accessions were a great variability. This diversification could enrich the genetic base of this genus and required more studies to achieve the maximum usefulness from this diversification. Physiological results will be useful in characterizing and to create the first reference and catalogue of the fig accessions.

Keywords*— Ficus carica L; Northen of Morocco, physiolgical parameters, Hierarchical classification.

I. INTRODUCTION

The fig is the oldest fruit in the world [1]. It is probably native to the Middle East and naturalized in several regions and especially those around the Mediterranean basin. The latter supplies the bulk of world production, estimated at one million tones, of which 27% is produced by Turkey. In Morocco, F. carica is present in all regions under diverse environmental conditions. However, its cultivation remains sporadic, despite the richness of this plant genetic heritage and its age [2]. The fig tree plays a significant economic role in the diet of the population of the northern region of Morocco. However, the marketing of the product is little developed in our time and this after having had a much greater importance in the past. In addition, the introduction of plum in the Zoumi and Moukrisset areas and the olive grove in the Beni Ahmed area has driven the fig tree out of the main irrigated and fresh land. This rapid extension of the plum tree and the olive tree shows that they have met the climatic and edaphic conditions favorable to their development. Thus the fig tree has been eliminated from these perimeters and now only survives on rough and dry terrain.

The varietal heritage of the fig tree (*Ficus carica L*) at the Mediterranean scale consists of a few hundred varieties whose genetic diversity has been characterized essentially morphologically [3], [4]. Several studies have reported the use of morphometric and pomological parameters as well as isozyme markers to discriminate fig cultivars [5], [6], [7]. In Morocco, the first work on the pomological description of fig tree varieties was made by [8] but from limited surveys in the Chefchaouen region. Agro-morphological markers, with immense interest, fall under the characterization. However, they vary according to the phenological stages of the tree and interfere with environmental factors. The analysis of phenotypic traits is also more complex in fig trees than other fruit species because of the particularities of the presence of two types of production in the year (figs-flowers and figs of autumn). To overcome these constraints, the choice of phenotypic markers in limited numbers, but rigorously selected for their discrimination performance, as well as the need for periods of repeated observations at specific periods (fruiting, vegetative rest, age of the plant, organ studied) may suffice for the characterization of the tree. The use of this type of markers is undeniable and must be included in any project for the identification and use of genetic resources. About 80 phenotypic descriptors have been identified in this species, including 23 for the tree, 21 for the leaf and 34 for the fruit [9].

The purpose of this work is to make known the physiological characteristics of the local plant material prospected in northern Morocco, by proposing a genetic knowledge base necessary for the selection of efficient genotypes of domestic fig tree.

II. MATERIAL AND METHODS

Plant Material

- The study looked at 96 ecotypes of fig trees prospected in Northwestern of Morocco. It is about 49 indigenous figs, well spread in the orchards of northern Morocco (Table.1). The work was based mainly on surveys carried out in 14 stations in four large areas in the north - west of the country. These stations were chosen according to the importance of fig orchards in agrosystems (Fig. 1).

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◆ Béni Ahmed area: characterized by its richness in fig and caprifigue as well as a good knowledge of cultivation techniques in particular caprification.

◆ - Areas of Moukrisset, Zoumi and Oued Laou: areas rich in figs with very diversified varieties, but with a lack of knowledge of caprification techniques.

◆ - Khmiss anjra zone: constitutes a new and very diversified variety of resources especially in Douar Tafza, this zone is also characterized by a neglect of caprification

Table.1: List of varieties studied

The main varieties		
Rhoudane	Saadi	Jouhri
Gaouizi	Lemti	Ournakssi
Ferzaoui	Sinani	Kharar
Baghi assal	Achir	Hafer elbrel
Harchi el khal	Hafri	Sbaa ou rhgoud
Meltoufa	Chitoui	Kohli
Kharaza	Bakour	Zerki
Tahadakte	Larchan	Tbantou
Tabli	Mouslikh	Sibti
Hazouta	Aroui	Kourti
Lmdar	L'khoumbiz	Silfaf
L'hmar	Lndbar	Smouni
Fassi	L'beidi	Khoumsi
El messari	harchi lbeid	Ounk Hmam
Makoutia	L'mdar eL khal	Ozilane
Qouti	Maalmouss	
Lassoune	Zenfoukh	

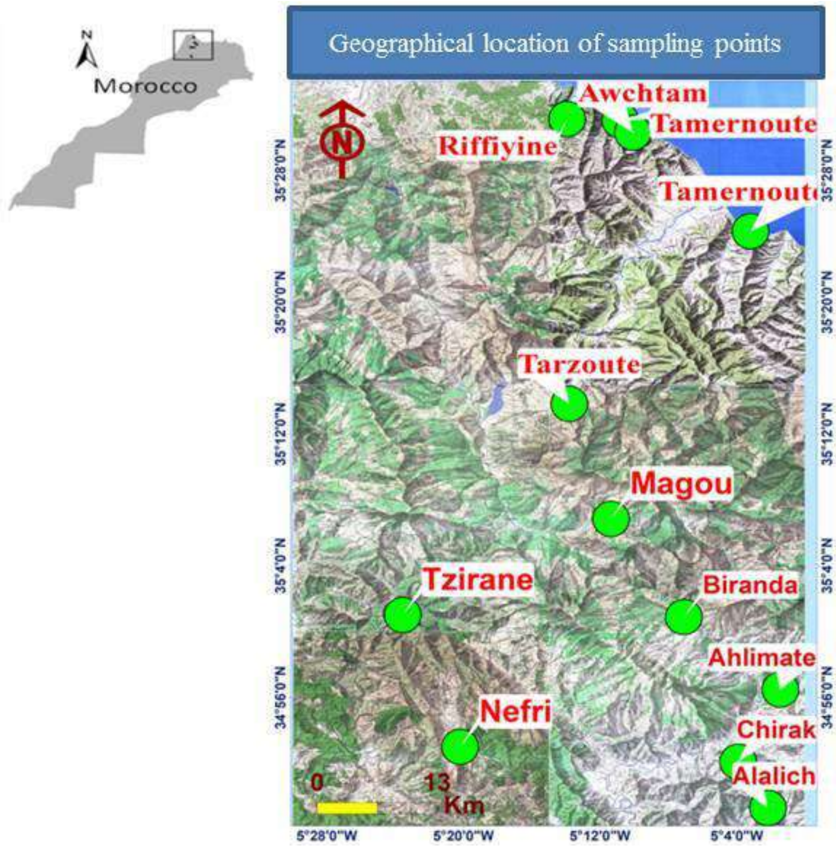

Fig. 1: Localization of the sites prospected

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Physiological characters:

Five physiological characters were taken into account: type of fruiting, precocity, yield, production and capacity. For each character, the different modalities are envisaged. To facilitate the acquisition and processing of data during surveys, each category was assigned a code (Table 2).

Fruiting type:

From the point of view of fruiting, fig varieties can belong to five different types:

- Common type: corresponds to figs which are parthenocarpic, and which generally give a harvest of figs per year,
- Smyrna type: figs one crops that require pollination by the blastophage, and have only one harvest at the end of summer,
- San pedro type: which are two crops and therefore give two harvests in the year, the first being the harvest of fig-flowers in July and the second, mid-August beginning of September requiring pollination by the blastophage,
- Type of one crop: varieties whose maturity occurs annually. Only once, usually in August,
- Type of two crop: varieties whose fruit maturity occurs in two distinct periods. The fruits of the first season, called "figs flowers", ripen in late June-August. The second season usually takes place in late August- October. (Simonet, Chopinet & Baccialone, 1945) Dans certains cas, la variété est presque strictement one crop; dans d'autres, presque strictement two crop. Tous les intermédiaires existent (Table 1.)

Precocity:

- The precocity or maturity of the figs is the moment of full maturity of the variety, the one where the greatest number of fruits is ripe on the trees. There are 5 cases:
- Very early: when the maturity will be end of July,
- Early: the maturity of the figs is from August 1st to 10th,
- Mid-season: maturity is between August 11th and 30th,
- Late: the maturity of the figs takes place from 1st to 30th September,
- Very late: maturity is after October 1st

Yield:

- The yield is the quantity in Kg of figs produced by the tree, there are 3 cases:
- Low yield: when the production is 20 Kg / tree,
- Average yield: the production is between 20 and 60 Kg / tree,
- High yield: when the production is 60 Kg / tree.

Production :

The production reflects the alternation of figs per year, so we distinguish:

Regular production: when you have a good fig production in two years,

Very irregular production: when production is good for one year and average for the year after.

Capacity :

Corresponds to the ability to yield mature fruits:

Autumn figs are carried by the growth of the year. In the first year of nursery, so the first shoot, the varieties carry, for the most part, fruits that fall before maturity. The notion of "speed of fruiting", widely used in the case of other fruit trees (especially pips and stone rosaceae), must be replaced by that of "capacity to yield mature fruits". This capacity can be: fast (before 3 years), average (3 to 5 years), long (more than 5 years).

Table.2 : Physiological characters

character	Modality
Type	Common type
	Type Smyrna
	Type San Pedro
	One crop
	Slightly two crop
	Has a tendency two crop
	Intermediate
	Frankly two crop
Precocity	Very Early
	precocious
	Mid-season
	Late
	Very late
yield	Low efficiency
	Average yield
	High efficiency
Production	Regular production
	Very irregular production
Capacity	Long capacity
	Average capacity
	Quick capacity

III. RESULT AND DISCUSSION

Physiological characters:

According to farmers in the region, the type of fruit is represented by three sub-characters (Fig.2); of which the Smyrna type represents 18.36% (effective 9), the San Pedro type represents 32.65% (effective size 16) whereas most of the varieties are of the Common type, that is to say, not requiring caprification (48.97%, effective 24). So, it is clear that the practice of caprification is not widespread among farmers. Half of the varieties are all Common, ie parthenocarpic figs that usually give figs without pollination. While this is not the case for all the figs prospected, that is to say figs that need to be pollinated to give mature figs, this shows the ignorance of farmers about the carprification technique.

On the other hand, survey results show that most autumn figs yield two crops per year (Fig. 3), that is, they are frankly two crops and more than half of varieties identified (63.26%, strength 31). The figs one crops represent 34.69%, (effective 17), while only one variety "harchi lkhal" is sometimes one crop and sometimes two crop and therefore can be considered as an exceptional case and classified as weakly two crop.

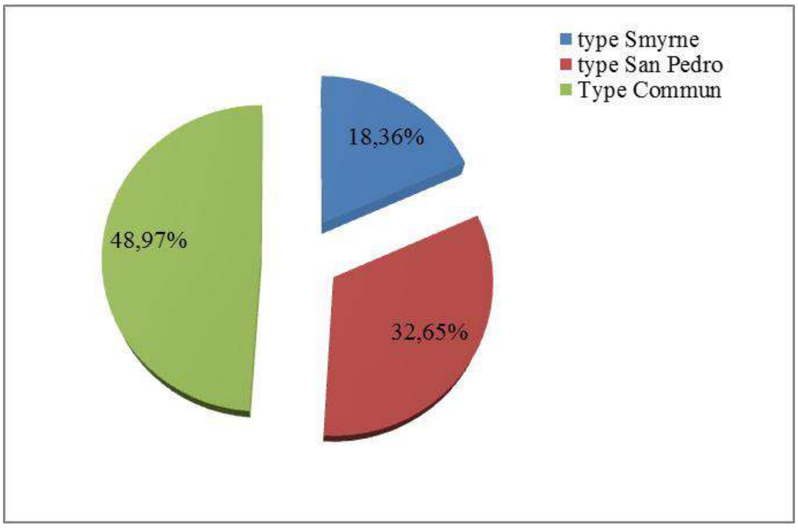

Fig.2: Character type of fruit

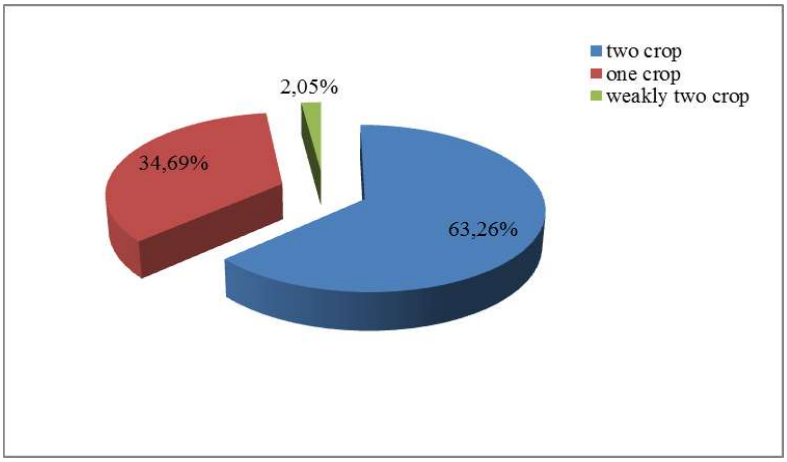

Fig. 3: typical type of harvest

Precocity:

The maturity of the figs is triggered when the color of the fruit changes, its softened pulp and skin begins to crack. In general, fresh figs must reach a certain stage of maturity before being harvested, as they remain immature if picked early [10]. An unripe fig is also less rich in sugars and has not yet developed its organoleptic properties. On the other hand, a late harvest leads to major handling difficulties (harvesting, storage, transport). In fact, few figs are harvested at full maturity on a tree [11].

Most varieties are early-maturing (90.38%, effective 34) (Fig.4), that is, fruit maturity is from August 1st to August 10th, while % have a mid-season production (effective 11), that is to say that their maturity starts from the 1st to the 30th of August, and only 4.08% have a production either very late or very early (effective 2). Early maturity may be favored by climatic conditions, especially high spring and summer temperatures [12].

The periods of harvest of the recorded figs are generally long (15 to 57 days) and are close (21 to 60 days) of those observed by [13]. These do not change according to the color of the skin of the fruits.

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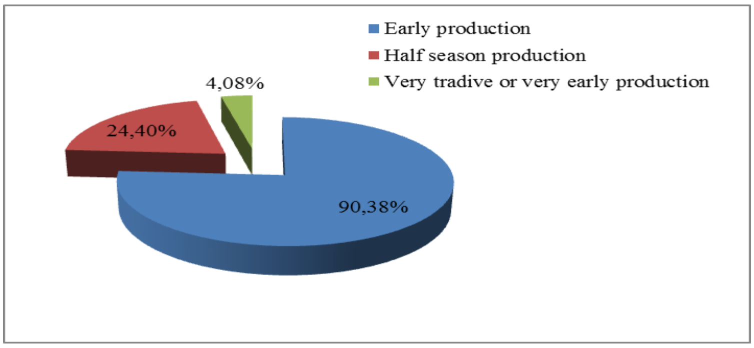

Fig.4: Production Precocity

Yield:

For yield, farmers say that most varieties have an average yield (75.51%, effective 37), while only 22.4% have a high yield (effective 12) (Fig.5). For example, in the Béni Ahmed area, it has been found that Rhouddane trees have a high yield that exceeds 64 kg / tree, whereas for an average yield the quantity varies between 38 and 40 kg / tree. Farmers say that these values are only approximate and vary from year to year depending on variety and climatic conditions.

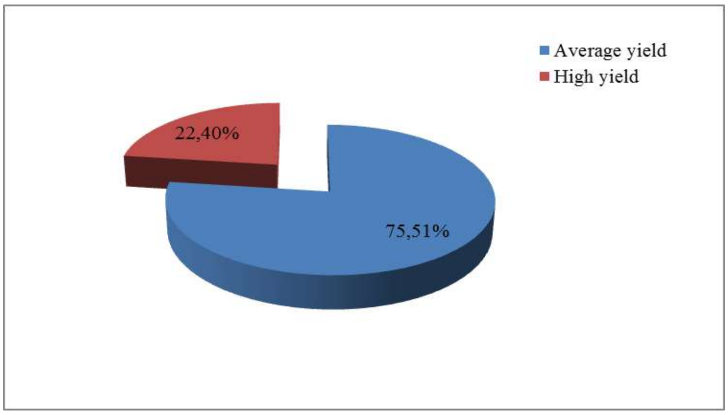

Fig 5. : Variety yield.

Production :

The production of figs varies from year to year depending on climatic conditions, varieties and cultural techniques. Surveys show that for all varieties studied, more than half have a regular production of 57.51%, while in the second half production is irregular representing a percentage of 42.85% (Fig.6).

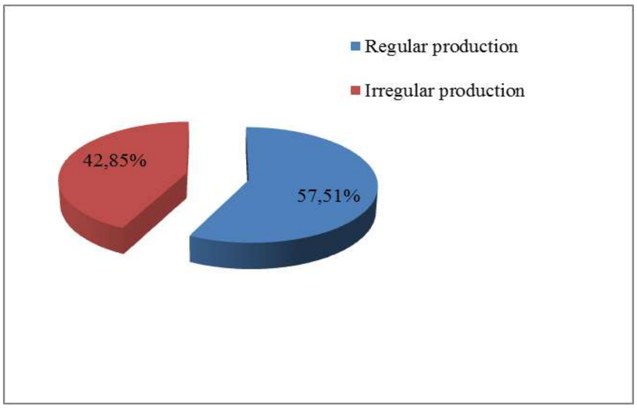

Fig.6: Type of production

Capacity:

Surveys show that most varieties have an average capacity to yield mature fruits, ie 3 to 5 years, representing 93.87% (number 46) of the varieties surveyed (Fig.7). While only 6.12% have a long capacity corresponding to more than 5 years, however no variety has a fast capacity (before 3 years).

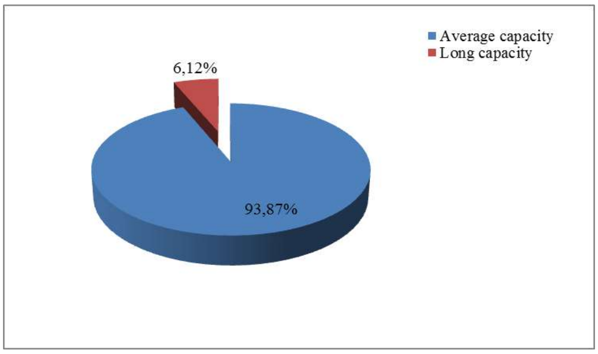

Fig.7: Ability to give fruit

Hierarchical classification

The hierarchical clustering shows that the fig accessions were distributed into two main groups. The first group includes two clusters separate also under two clusters. The second group also includes two clusters separate also under two clusters (Fig.8).

The first group included Rhouddane accessions, Lmdar and Baghi assal, the second group include principally Koti accessions. Generally all the groups were heterogenous. These differences might be due to the genotypic diversity or environmental effects on fruit characters. The varieties which bear the same name, with the same physiological characters, but it is a general case. Indeed, of the noticeable differences can be observed within the same range of variables. We think that they may be in certain cases different varieties but bearing the same name (homonymy) or on the contrary (synonymy). It is also possible that the same name was given to several genetically different fig cultivars with similar morphological characteristics in this region. As they can be in other cases polymorphic varieties, polyclonal nature of the varieties is shown in the evaluation of the national collection [20]. It is thus necessary to record the importance of the problems of denomination of the species listed in the prospected area. These problems are due to local names which vary from one locality to another and the absence of the syntheses and varietal characterization.

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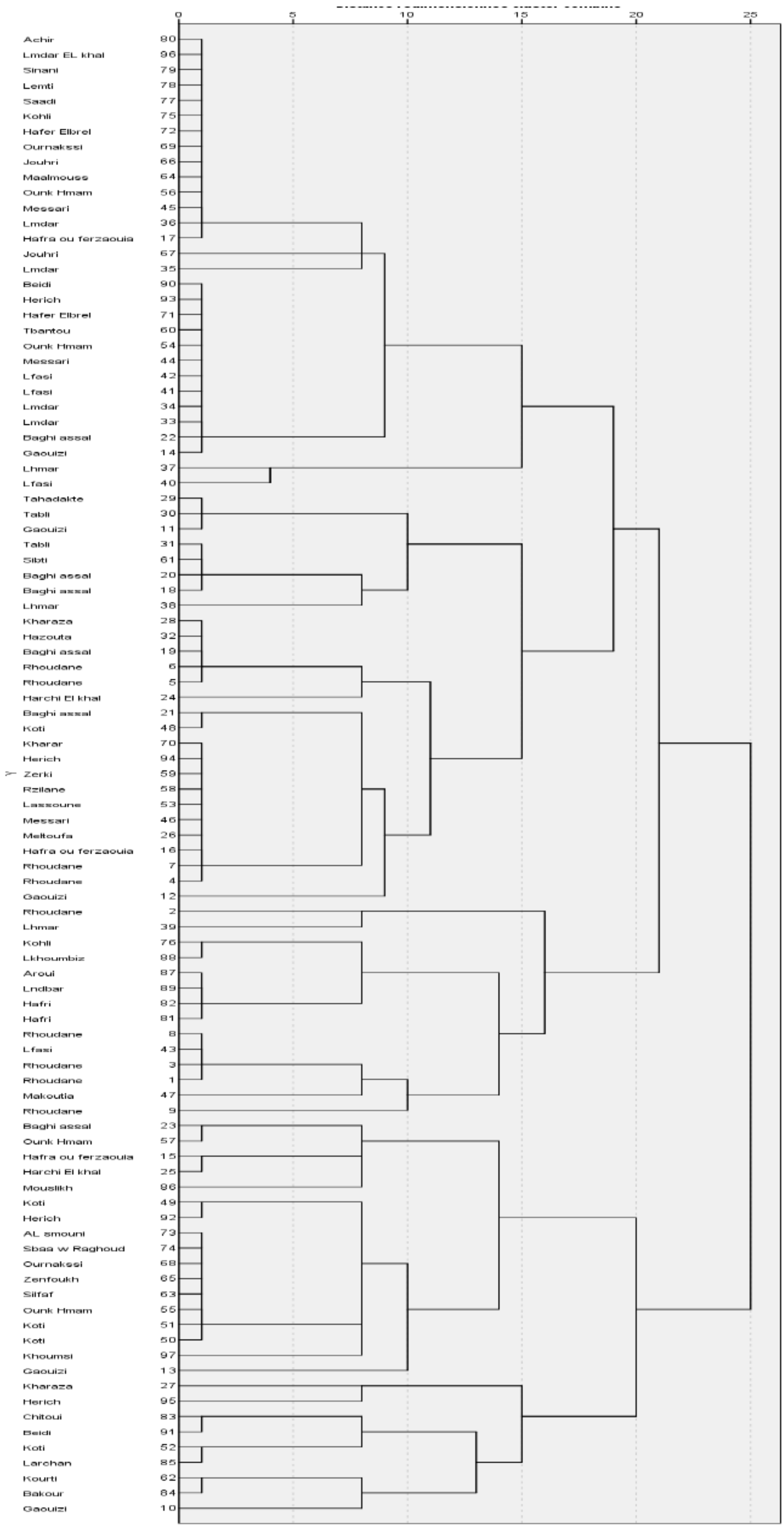

Fig. 8. Representation in cluster of individuals of the north region of Morocco.

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IV. CONCLUSION

The present study revealed that the physiological parameters vary according to the genotypes. This variability has been noted in similar studies and may also change with environmental conditions and farming techniques. It has been shown that fig cultivars are highly diverse and provide a large collection of genotypes. However, because of many cases of synonyms (several denominations for the same genotype) and homonyms (several genotypes for the same denomination), the physiological characterization is insufficient for the establishment of reference genotypes of Moroccan figs.

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The authors are grateful to all farmers for providing necessary facilities for conducting this research work.

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Effects of container shape on seedling growth of *Hevea brasiliensis*

Chen Xianhong[1], Wang Jun[2], Lin Weifu[3*]

[1]Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan 571737, China
E-mail: chenxhongjane@163.com
[2]Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan 571737, China
E-mail: wangjuncatas@163.com
[3*]Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan 571737, China
E-mail: RubberL@163.com

***Abstract*— *Effects of container shape on seedling growth of Hevea brasiliensis at different growth stages were observed to provide reference for rubber nursing. Rubber seedlings were planted in different shape containers with same height and volume. Then leaf whorl, stem diameter, plant height, tap root length, amount of first lateral root, root weight, shoot weight and root- shoot ratio were investigated at 90, 180 and 360 days after transplantation, respectively. The results showed that the optimal selection of container shape is the prism at 90 days, while the cube at 180 and 360 days. At 90 days, leaf whorls and root weight in prisms were significantly 10.3%, 11.3% more than in cylinders, plant height 13.4%, 20.6% higher than in cubes and cuboids, shoot weight 67.1%, 58.8%, 14.5% heavier than in cubes, cuboids and cylinders, respectively. At 180 days, stem diameter in cubes was significantly 16.4%, 10.9% bigger than in cuboids and prisms, plant height 16.5% higher than in cuboids, tap root length 18.5% longer than in cylinders, numbers of first lateral root 22%, 25%, 56.5% more than in cylinders, prisms and cuboids, shoot weight 23.5%, 40.4% and root weight 19.9%, 19.5% heavier than in prisms and cuboids, respectively. At 360 days, plant height in cubes was significantly 11.7% higher than in prisms and tap root length 14.7% longer than in cylinders. Based on specific surface area of containers and the growth at all days, the optimal selection of container shape is the cube and then the cylinder.*

***Keywords*— Container shape, Hevea brasiliensis, seedlings, growth.**

I. INTRODUCTION

Rubber seedling production in China mainly uses plastic bags to raise seedlings and nursery for 6-22 months, which usually causes seedling roots twisted, and some serious recover slowly after planting, resulting in irregular rubber forest phase. Mauer et al. investigated the relationship between root distribution, degree of deformity and growth of aboveground parts in 90 container seedlings plantations in Czech Republic，which showed that the adverse effects of root deformity caused by cylindrical container seedlings on growth could not disappear until 15 years, the adverse effects of peat and bottomless container could disappear after 10 years, root malformations of polyethylene bags and Koparfors porous container seedlings remained unchanged after 20 years of afforestation (Deng, 2008). Therefore, it is of great significance to study the effect of container shape on the root system and growth of rubber seedlings.

Container shape has great influence on root growth and development of Pinus tabulaeformis seedlings (Gao, 1983) and Picea spruce seedlings (Lesterw *et al.* ,1988). Container size has significant effects on seedling height, ground diameter and biomass of Quercus suber L. seedlings (Chirino *et al.*, 2008), Pingyi sweet tea seedlings (An *et al.*, 2013), Castanea mollissima grafted seedlings (Tan, 2016), Osmanthus fragrans seedlings (Qiu *et al.*, 2018), Chipi Qinggang (Wang *et al.*, 2019). The growth of Platycladus orientalis (Dong *et al.*, 2006), Fraxinus mandshurica seedlings (Wei *et al.*, 2016) and one-year-old white wax (Du *et al*., 2019) in non-woven bags were significantly better than that in plastic containers. Different ratio of nursery substrate components also have a significant impact plant height, ground diameter and biomass in container seedlings of Manglietia grandis (Zheng *et al*., 2018), Osmanthus fragrans seedlings (Qiu *et al*., 2018), Parashorea chinesis container seedlings (Pang *et al*., 2018), Millennium Tung container seedlings (Hong, 2019), Pinus tabulaeformis (Dong *et al*., 2019).

At present, container seedling research mainly focuses on nursery substrate, container material and sizes, but there are few studies on container shape of seedling which are based on experience and lack of relevant theoretical basis. This study is aimed at comparing the growth of rubber seedlings planted in containers of different shapes at different stages to understand the effect of container shape on the root system and growth of rubber seedlings and further recommend suitable rubber seedling container shape.

II. MATERIALS AND METHODS

2.1. Experimental materials

Rubber seedlings of two weeks-old were assembled into air pruning containers (without base) with same height and volume and different shapes (cylinder, prism, cube and cuboid), which were made of recycled HDPE plastic. The specific surface area of the container was cuboid > prism > cube > cylinder, and the seedling-raising medium was coconut bran.

2.2. Experimental method

The experiment was conducted from May 2017 to May 2018 in the protective cultivation base of natural rubber of Rubber Research Institute of Chinese Academy of Tropical Agricultural Sciences, Danzhou City, Hainan Province, China. The coconut bran of culture medium was put into containers with the same volume and different shapes and placed at the same density. Rubber seedlings with the same growth from the sand bed were transplanted into the containers. In order to ensure that the roots were not penetrated, the containers were placed on the cement ground. The water, fertilizer and pesticide management were carried out accordingly. Numbers of leaf canopy, stem diameter, plant height, main root length, numbers of primary lateral roots, root weight, shoot weight and root-shoot ratio of each treatment were observed at 90, 180 and 360 days after transplantation, respectively. Ten plants were observed each time in three replicates. Vernier caliper (0.02 mm) was used to measure stem diameter, and steel ruler (0.5 mm) was used to measure plant height and main root length. After detaching the containers and shaking off the coconut bran, the whole seedlings were separated immediately. The shoot and root weights were measured by the analytical balance.

2.3. Data analysis

Statistical analyses were performed with Data Processing System (DPS) statistical software package version 16.5 (Tang, 2013) using one-way ANOVA followed by the Duncan's Multiple Range Test (SSR) to evaluate significant difference among seedlings from different container shapes at $P<0.05$ and Topsis (technology for order preference by similarity to ideal solution) method for comprehensive evaluation analysis.

III. RESULTS

3.1 Effect of container shapes on the aboveground part growth of rubber seedlings

3.1.1 **Effect on numbers of leaf whorls** As shown in Fig.1, at 90 days, the number of leaf whorls of seedlings in prisms is 10.3% more than that in cylinders. There was no significant difference in the number of seedling leaf whorls among cylinders, cubes and cuboids, and among cubes, cuboids and prisms. At 180 days, the number of leaf whorls of seedlings in cuboids was significantly less than that in cylinders by 10% and there was no significant difference among cylinders, cubes and triangular prisms and among cuboids, cubes and prisms. At 360 days, the number of seedlings leaf whorls in prisms was 5.2% and 4.76% less than that in cubes and cuboids, respectively. There was no significant difference between the number of seedlings leaf whorls in cylinders and prisms, and among cylinders, cubes and cuboids.

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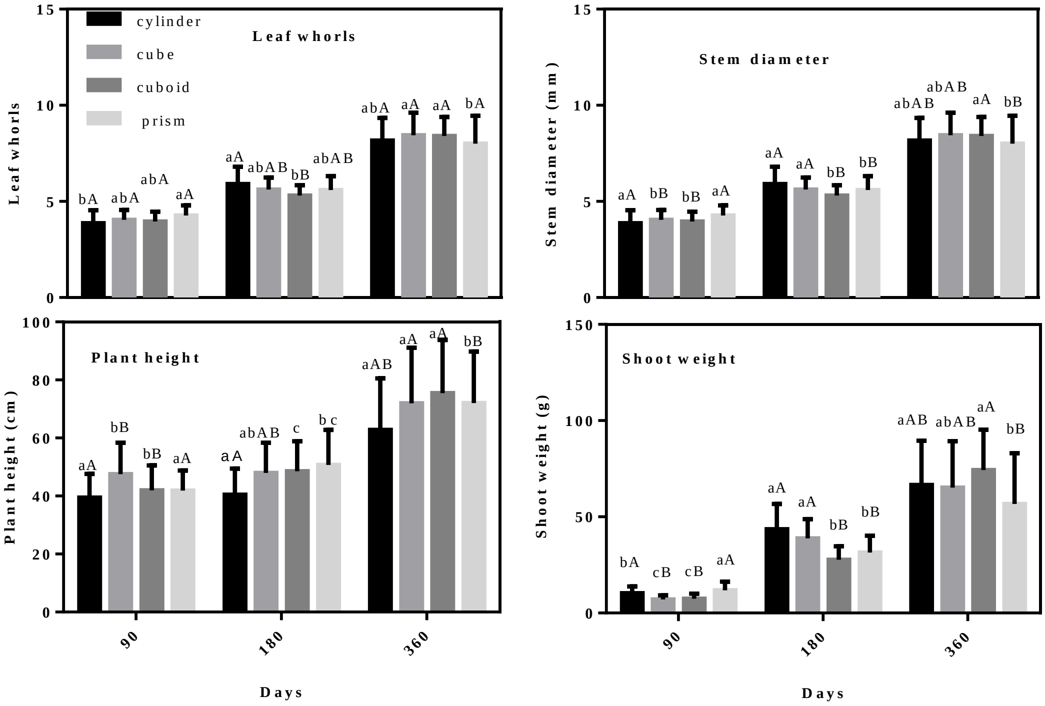

Fig.1 Comparison of leaf whorls, stem diameter, plant height, shoot weight of rubber seedlings raised in four container shapes at different growth days. Values are means ± SD (n = 10). Different lowercase or capital letters indicate significant at 0.05 and 0.01, respectively. The same letter is not significantly different.

3.1.2 **Effect on stem diameter** As shown in Fig.1, at 90 days, stem diameter of cubes and cuboids was 13% and 10.3% smaller than that in cylinders, 15.7% and 13.1% smaller than that in prisms, respectively. There was no significant difference between stem diameter of seedlings in cylinders and prisms, and between stem diameter of seedlings in cuboids and cubes. At 180 days, the stem diameter of seedlings in cuboids was 13.7% and 14.1% smaller than those in cylinders and cubes, respectively. The stem diameter of seedlings in prisms was 9.4% and 9.8% smaller than those in cylinders and cubes, respectively. There was no significant difference between stem diameter of seedlings in cylinders and cube, and between stem diameter of seedlings in cuboids and prisms. At 360 days, there was no significant difference among stem diameter of seedlings in cylinders, cuboids and cubes. The stem diameter of seedlings in prisms was 11.2% smaller than that in cuboids.

3.1.3 **Effect on plant height** As shown in Fig.1, at 90 days, there was no significant difference between plant height of seedlings in cylinders and that in prisms, and between plant height of seedlings cuboids and cubes. The plant height of seedlings in prisms was 13.4% and 20.6% higher than those in cubes and cuboids, respectively. The plant height of seedlings in cubes and cuboids was significantly higher than that in cylinders by 14.5% and 8.5%, respectively. At 180 days, the plant height of seedlings in cubes was 16.5% higher than that in cuboids; the plant height of seedlings in cuboids and prisms was 19% and 11.7% lower than that in cylinders, respectively; the plant height of seedlings in cylinders and cubes, cubes and prisms, cuboids and prisms, were not significant. At 360 days, there was no significant difference among plant height of seedlings in cylinders, cuboids and cubes. The height of seedlings in prisms was 7.8%, 10.5% and 12.1% smaller than those in cylinders, cubes and cuboids, respectively.

3.1.4 **Effect on shoot weight** As shown in Fig.1, at 90 days, shoot weight of seedlings in cube, cuboid and cylinder were 40.1%, 37% and 12.7% lighter than those in prisms, respectively. The shoot weight of seedlings in cubes and cuboids were 31.5% and 28% lighter than those in cylinders, respectively. There was no significant difference between shoot weight of seedlings in cuboids and cubes. At 180 days, there was no significant difference between shoot weight of seedlings in cylinders and cubes, and between shoot weight of seedlings in cuboids and prisms. The shoot weight of seedlings in cubes was 40.4% and 23.5% more than those in cuboids and prisms, and the shoot weight of seedlings in cuboids and prisms was 36.5% and 27.9% less than that of seedlings in cylinders, respectively. At 360 days, there was no significant difference among shoot weight of seedlings in cylinders, cuboids and cubes, and between shoot weight of seedlings in cubes and prisms. The shoot weight of seedlings in prisms was 14.8% and 23.6% lighter than those in cylinders and cuboids, respectively.

3.2 Effect of container shapes on the underground part growth of rubber seedlings

3.2.1 **Effect on tap root length** As shown in Fig.2, at 90 days, the tap root length of seedlings in cylinders, cuboids and prisms was significantly shorter than that of seedlings in cubes by 16.9%, 11.6% and 11.8%, respectively, while there was no significant difference in the tap root length of seedlings in cylinders, cuboids and prisms. At 180 days, the tap root length of seedlings in cubes, cuboids and prisms was significantly shorter than that in cylinders by 18.5, 20% and 25.3%, respectively, while there was no significant difference among the tap root length of seedlings in cubes, cuboids and prisms. At 360 days, there was no significant difference among the tap root length of seedlings in cubes, cuboids and prisms, and the tap root length of seedlings in cubes, cuboids and prisms was significantly longer than that in cylinders by 14.7%, 20.2% and 14.8%, respectively.

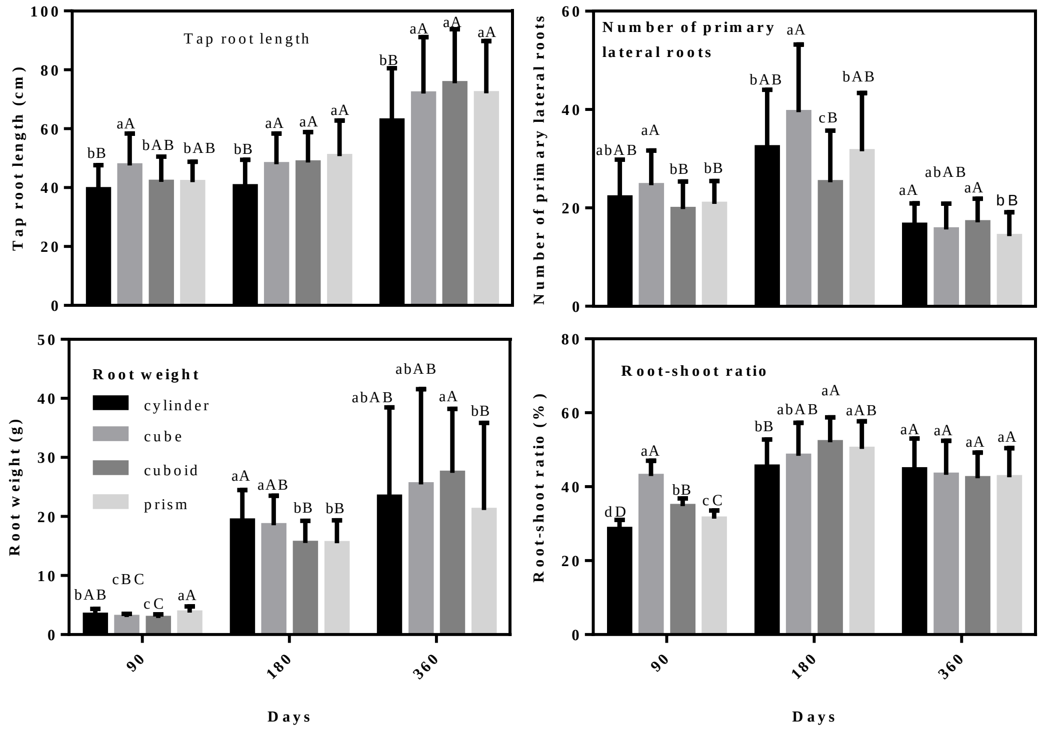

Fig.2 Comparison of tap root length, the first lateral root length, root weight, root and shoot ratio of rubber seedlings raised in four container shapes at different growth days. Values are means ± SD (n = 10). Different lowercase or capital letters indicate significant at 0.05 and 0.01, respectively. The same letter is not significantly different.

3.2.2 **Effect on the number of primary lateral roots** As shown in Fig.2, at 90 days, the number of primary lateral roots of seedlings in prisms was not significantly different from that of seedlings in cylinders and cubes. The number of primary lateral roots of seedlings in prisms and cubes was not significantly different. The number of primary lateral roots of seedlings in prisms and cuboids was 15.4% and 19.7% less than that in cube, respectively. At 180 days, there was no significant difference in the number of primary lateral roots between the seedlings in cylinders and prisms.

The number of primary lateral roots in the cube was 18% and 25% more than those in the cylinder and the prism, respectively. The number of primary lateral roots in the cuboids was 10%, 36.1% and 20% less than those in cylinders, cubes and prisms respectively. At 360 days, there was no significant difference among the number of primary lateral roots of seedlings in cylinders, cuboids and cubes, and between the number of primary lateral roots of seedlings in cubes and prisms. The number of primary lateral roots of seedlings in prisms was 13.8% and 16.4% less than those in cylinders and cuboids, respectively.

2.2.3 **Effect on root weight** As shown in Fig.2, at 90 days, the root weight of seedlings in prisms was 11.3% heavier than that in cylinders. The root weight of seedlings in cuboids and cubes was 15.5% and 11.6% lighter than that of seedlings in cylinders, 24.1% and 20.6% lighter than that of seedlings in prisms, respectively. There was no significant difference between root weight of seedlings in cuboids and cubes. At 180 days, there was no significant difference between the root weight of seedlings in cylinders and cubes, and between the root weight of seedlings in cuboids and prisms. The root weight of seedlings in prisms and cuboids was 20% and 19.8% lighter than that in cylinders, 16.6% and 16.4% lighter than that in cubes. At 360 days, the root weight of seedlings in cylinders, cuboids and cubes had no significant difference. The root weight of seedlings in cylinders, cubes and prisms had no significant difference. The root weight of seedlings in prisms was 23% lighter than that in cuboids.

3.3 Effect of container shapes on root-shoot ratio

As shown in Fig.2, at 90 days, the root-shoot ratios of seedlings in cuboids, cubes and prisms were 50.2%, 21.9% and 9.8% more than that in cylinders, respectively. The root-shoot ratios of seedlings in cuboids and prisms were 18.9% and 26.9% less than that in cubes, respectively. The root-shoot ratios of seedlings in prisms were 9.9% less than that in cubes. At 180 days, the root-shoot ratio of seedlings in cylinders and cubes had no significant difference and the root-shoot ratio of seedlings in cubes, cuboids and prisms had no significant difference either. The root-shoot ratio of seedlings in cubes and prisms was 14.6% and 10.6% more than that in cylinders, respectively. At 360 days, there was no significant difference in root-shoot ratio of seedlings in cylinders, cubes, cuboids and prisms.

3.4 Comprehensive evaluation of the effect of container shape on the growth of rubber seedlings

Based on the specific surface area, aboveground and underground observation indexes, and the size of CI value (approximation to the optimal vector), the comprehensive evaluation results of different container shapes on the growth of rubber seedlings are shown in Table 1. The best container shape at 90 days is the prism, followed by the cylinder. At 180 days and 360 days, the best container shape is the cube, followed by the cylinder. Combining the specific surface area and the observation indexes of three growth periods (90 days, 180 days, 360 days), we find that the best container shape is the cube, followed by the cylinder.

Table 1 Comprehensive evaluation of different container shapes on rubber seedling growth

Days	Container shapes	Cuboid	Triangle prism	Cube	Cylinder
	D+	0.3711	0.2271	0.312	0.255
	D-	0.1081	0.3249	0.2749	0.2763
	CI	0.2256	0.5886	0.4683	0.5201
90	Rank	4	1	3	2
	D+	0.3883	0.2714	0.1082	0.1765
	D-	0.1213	0.1804	0.3311	0.338
	CI	0.238	0.3994	0.7537	0.657
180	Rank	4	3	1	2
	D+	0.2154	0.1995	0.1189	0.157
	D-	0.1946	0.1729	0.1816	0.1928

	CI	0.4746	0.4644	0.6043	0.5513
360	Rank	3	4	1	2
	D+	0.5177	0.4049	0.341	0.3423
	D-	0.2855	0.377	0.4443	0.4261
	CI	0.3554	0.4822	0.5658	0.5545
Total	Rank	4	3	1	2

D+, distance to optimal vector. D-, distance to inferior vector. CI, approximation to the Optimal Vectors.

IV. DISCUSSION

The container shape has a great influence on the growth of seedlings. Growth tends to be stimulated when there is a mutual matching between the natural growth pattern of roots and the shape of their container (Biran, I and Eliassaf, A,1980). In this study, according to the aboveground and underground observation indexes, it is suggested that the prism container should be selected first for rubber seedling propagation at 90 days, the cube container at 180 days and 360 days, followed by the cylinder. The container shape has a greater impact on the spruce seedling (Lesterw *et al*. 1988), which suggested that the use of different shape containers should be flexible according to specific needs. The root system of the container seedlings of Pinus tabulaeformis with 60 degree prism is the best, followed by the regular triangle prism and the cylinder (Gao, 1983). It is further confirmed that the shape of the container has a great influence on the seedlings growth. In this study, the effects of container shape on stem diameter, plant height and main root length of rubber seedlings varied with the growth time. Among them, the biggest stem diameter of rubber seedlings was found in prisms at 90 days, cylinders at 180 days, cubes at 360 days, respectively. The highest plant height was found in cubes at 90 days, prisms at 180 days and cuboids at 360 days, respectively. The tap root length of rubber seedlings was the longest in cubes at 90 days, prisms at 180 days and cuboids at 360 days, respectively. Gao (1983) found that container shape had no significant effect on the root diameter, seedling height and main root length of seedlings, which may be related to the time of observation, the shape of the container, and the genus of the seedlings.

V. CONCLUSION

In this study, when the height and volume of each container with different shape are the same, the specific surface area, the aboveground and underground observation indexes of rubber seedlings in the different shape containers are synthetically evaluated. At 90 days, the prism is preferred as the nursery container shape of the rubber seedlings, followed by the cylinder; at 180 days and 360 days, the cube is preferred, followed by the cylinder. Based on the comprehensive evaluation of the specific surface area of each nursery container shape, the aboveground and underground observation indexes in three growth periods, the cube was the first choice as the container shape to nursery rubber seedlings, followed by the cylinder.

ACKNOWLEDGEMENTS

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Increasing Yield Components of Several Promising Lines of Red Rice through Application of Mycorrhiza Bio-Fertilizer and Additive Intercropping with Soybean in Aerobic Irrigation System

Wayan Wangiyana*, I Gusti Putu Muliarta Aryana, Ni Wayan Dwiani Dulur

Faculty of Agriculture, University of Mataram (Unram), Mataram, Lombok, NTB, Indonesia
*Corresponding author Email: w.wangiyana@unram.ac.id

***Abstract*— *This study aimed to examine the effect of application of a bio-fertilizer containing arbuscular mycorrhizal fungi (AMF) and additive intercropping with soybean relay-planted between double rows of rice on growth and yield components of various promising lines of red rice grown in aerobic irrigation systems on permanent raised-beds. The field experiment, carried out on farmers' riceland in Beleke village of Gerung district in West Lombok, Indonesia, from May to September 2018, was designed according to Split Split-Plot design with three blocks and three treatment factors, i.e. intercropping (T0= without; T1= with soybean) as the main plot factor, red rice promising lines with 4 genotypes (G1= MG4, G2= MG10, G3= AM4, G4= AM10) as the sub-plot factor, and AMF bio-fertilizer (M0= without; M1= with AMF) as the sub-sub-plot factor. The pre-germinated red rice seeds were planted on permanent raised-beds, with a base spacing of 25x20 cm, which was then modified into a double-row pattern of 20x20 cm within the double-row and 30 cm between double-rows. The results indicated that application of the bio-fertilizer "Technofert" containing AMF significantly increased growth and yield components of various promising lines of red rice, especially in relation to filled grain number and grain yield per clump, but significantly reduced percentage of unfilled grain number. Additive intercropping with soybean that was relay planted between double-rows of rice (one week after seeding rice) also significantly increased grain yield of the red rice genotypes. Among the four selected genotypes, grain yield of AM10 was the highest, especially when bio-fertilized with AMF and intercropped with soybean.*

***Keywords*— *red rice, aerobic rice system, arbuscular mycorrhiza, intercropping, soybean*.**

I. INTRODUCTION

In general, rice is grown on irrigated rice fields with a flooded system (conventional technique of growing rice), and even farmers in Indonesia in fully irrigated areas generally provide water for their rice crop by continuous flow of irrigation water. Because the land is flooded, and the irrigation water even flows between rice paddocks, then the need for irrigation water for rice production using the conventional technique is very high. For example, from the results of Yaligar *et al*. [1], in rice cultivation with conventional techniques, i.e. the inundated irrigation system commonly practiced by farmers, the total use of irrigation water reached 20260 m^3/ha, while with dry seeded direct planting it was only 4260 m^3/ha.

In addition to inefficient or high use of irrigation water in rice production systems under flooded irrigation or conventional cultivation techniques, the inundated system could cause a lot of disadvantages. These could include pollution of water bodies in the downstream area due to seepage, runoff and percolation from paddy water during the maintenance process for the rice plants, such as after spraying pesticides to control pests / diseases / weeds, and after applying fertilizers. Because of the anaerobic condition due to flooding, rice fields of the conventional cultivation techniques also result in emissions of methane and N_2O gasses [2]. Nitrogen fertilization, which is generally done using Urea fertilizer, is also inefficient in flooded rice because of high N loss through ammonia volatilization, denitrification, and leaching, while most rice soils are acute N deficiency [3]. According to Buresh *et al.* [4], the total gaseous N loss from Urea broadcasted to flooded rice was 34% in Thailand and 31% in Indonesia after 10 days of application, and the majority of the N loss from Urea applied was through ammonia volatilization, which reached up to 88% of the total N loss [5]. The conventional technique of rice cultivation is also a source of P leaching in addition to N leaching, which all contributes to pollution of downstream areas [6].

To avoid those disadvantages of flooding in growing rice, a less water-consuming cultivation technique of growing rice has been developed, i.e. the aerobic rice systems (ARS), which term was coined at the IRRI; and with the ARS, rice is grown under nonflooded, nonpuddled and nonsaturated soil conditions [7]. Unfortunately, when using irrigated rice varieties, grain yields are normally reduced under the ARS; therefore, specific varieties must be developed to have high yielding rice varieties under the aerobic rice systems, such as those developed in North China, which yield up to 6-7.5 t/ha [7]. However, when rice is grown in monoculture under aerobic rice systems, grain yields normally declined; so that it is suggested to have some rotations with legume crops [8].

Because rice is grown under nonpuddled and nonflooded soil conditions in aerobic rice systems, then it is also possible to grow rice plants in intercropping with legume crops. Many have reported that intercropping cereal crops with legume crops can provide some benefits for both crops. Inal *et al.* [9] reported that intercropping maize with peanut significantly increased chlorophyll and P content of maize. Chu *et al.* [10] also reported that chlorophyll and N contents as well as weights of dry matter per plant, spikelet per plant and 1000 seeds were higher in rice plants intercropped with peanut compared with rice plants in monocrop, and they measured significant transfer of N from peanut to rice in the intercropping systems.

In addition to intercropping with legume crops, there could be much better opportunities of using crop symbiosis with beneficial soil microbes such as arbuscular mycorrhizal fungi (AMF) and *Rhizobium* sp to manage plant nutrients for better crop nutrition under aerobic rice systems, in which soil is not flooded, when compared with under the conventional technique of growing rice, in which soil is normally flooded. Solaiman and Hirata [11] reported that AMF development (infection, colonization, and sporulation) was much better in rice inoculated under dry nursery than under wet nursery, which resulted in higher grain number and grain yield per clump on AMF inoculated rice plants than the uninoculated ones, especially those inoculated in the dry nursery. Wangiyana *et al.* [12] also reported that AMF inoculation significantly increased filled panicle number and grain yield of a promising red rice line grown together with several varieties of peanut in pot under aerobic rice system, and the magnitudes of the AMF inoculation effects were different between different varieties of peanut growing together with the red rice plant.

The aim of this research was to examine the effects of AMF inoculation on several promising lines of red rice additively intercropped with soybean plants inserted between double rows of rice plants grown on permanent raised beds under aerobic irrigation system.

II. MATERIALS AND METHOD

The field experiment was conducted on farmers' rice-land in Beleke Village of Gerung District of West Lombok Regency (Indonesia), from May to September 2018. The experiment was designed according to Split Split-Plot design with 3 blocks, to test three treatment factors, namely intercropping (T) with soybeans as the main plot factor, consisting of two levels of treatment (T0 = without, and T1 = relay planting of one soybean row planted between the double-rows of rice plants one week after planting pre-germinated rice seeds); promising lines of red rice (G) as the subplot factor, consisting of 4 genotypes selected from previous research (G04= MG4, G10= MG10, G15= AM4, and G21= AM10); and application of "Technofert" bio-fertilizer containing mixed species of AMF (M) as the sub-sub-plot factor, consisting of 2 levels of treatment (M0 = without, and M1 = with "Technofert" application at the time of planting the pre-germinated rice seeds). Red rice plants were grown on permanent raised-beds, with a base plant spacing of 25 x 20 cm, which was then modified into double-row patterns, as also has been explained in Wangiyana *et al.* [13], and the spatial arrangement of the row patterns for the double rows of rice plants and inserted single rows of soybean is as sown in Figure 1.

Formation of raised-beds, planting of pre-germinated red rice seeds, fertilizer application, mycorrhiza application, application of irrigation water, and harvest of the red rice were done exactly the same as those explained in Wangiyana *et al*. [13], except for the dose of Phonska (NPK 15-15-15) fertilizer, which was full doses in this experiment. The red rice genotypes used were also the same as those explained in Wangiyana *et al.* [13], which were previously selected from 11 promising lines of upland red rice genotypes and 12 promising lines of amphibious red rice genotypes [14]. The other difference was that there was an intercropping treatment in this experiment, i.e. additive intercropping those red rice genotypes with soybean var. Dering-1, which seeds were supplied by "Balitkabi" research institute in Malang, East Java, Indonesia. Soybean was relay-planted 7 days after

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sowing (DAS) of red rice, between double-rows of rice plants as sown in Fig. 1.C. After emergence, soybean plants were tinned by leaving only two soybean plants per planting hole, and after that soybean was fertilized with Phonska by dibbling the fertilizer beside the soybean plants within the soybean row with a recommended dose of 200 kg/ha.

Observation variables included the growth of rice plants consisting of plant height, number of tillers and panicles per clump, percentage of panicle number and weight of dry straw per clump, and rice yield components consisting of number of spikelet and filled grains per clump, percentage of unfilled grain number, weight of 100 filled grains, and filled grain weight (or grain yield) per clump. Data were analyzed with Analysis of Variance (ANOVA) and Tukey's HSD at 5% level of significance, using the statistical software CoStat for Windows ver. 6.303. In addition, correlation and regression analyses were also conducted to examine the degree of relationship between selected observation variables, using Minitab for Windows Rel. 13.0.

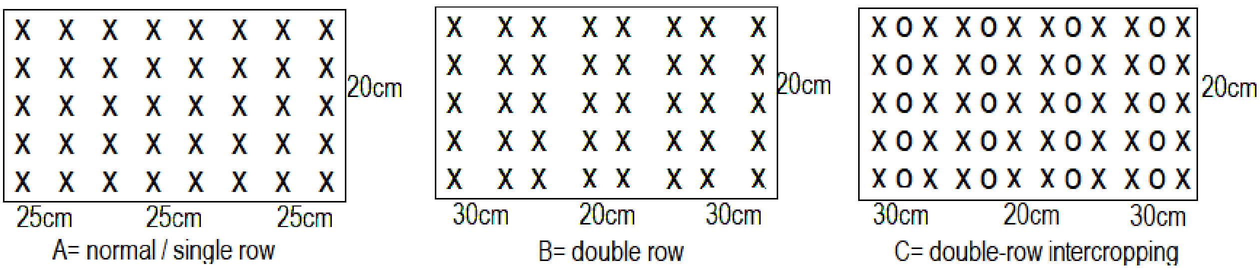

Fig.1. The schematic planting geometry of rice and soybean in the additive relay intercropping treatment (x = rice plants; o = soybean plants)

III. RESULTS AND DISCUSSION

Results of data analysis of growth variables (Table 1) and yield components (Table 2) of the red rice using ANOVA indicate that application the "Technofert" bio-fertilizer containing arbuscular mycorrhizal fungi (AMF) had the strongest effects, in which this treatment factor shows significant effects on all observation variables. Intercropping treatment also shows significant effects but only on straw dry weight, grain number, filled grain number, and grain yield per clump, while genotypes of the red rice show differences only in panicle number and dry straw weight per clump of the growth variables (Table 1), and in all yield components (Table 2). However, the interaction effects among the treatment factors were significant only on some observation variables, including three-way interaction effects on weight of dry straw, %-unfilled grain number, and grain yield per clump.

Table 1. Summary of ANOVA results on the effects of intercropping with soybean and mycorrhiza application on growth of red rice (plant height and tiller number per clump at anthesis; panicle number, %-panicle number, and weight of dry straw per clump)

Treatment factors and their interactions	Plant height at anthesis	Tiller number per clump at anthesis	Panicle number per clump	%-panicle number per clump	Weight of dry straw (g/clump)
Main effects:					
Intercropping	ns	ns	ns	ns	*
Genotypes	ns	ns	***	ns	***
Mycorrhiza	**	**	***	***	***
Interaction effects:					
Genotype x Intercrop	ns	ns	ns	ns	*
Mycorrhiza x Intercrop	ns	ns	*	ns	**
Mycorrhiza x Genotype	ns	ns	ns	ns	***
Myc x Geno x Intercrop	ns	ns	ns	ns	***

Remarks: ns= non-significant; *, **, *** = significant at p-value <0.05, p-value <0.01 and p-value <0.001, respectively

Table 2. Summary of ANOVA results on the effects of intercropping with soybean and mycorrhiza application on yield components of red rice (spikelet number, filled grain number, %-unfilled grain number, weight of 100 grains, and grain yield per clump)

Treatment factors and their interactions	Spikelet number per clump	Filled grain number per clump	%_Unfilled grain number	Weight of 100 filled grains (g)	Grain yield (g/clump)
Main effects:					
Intercropping	*	*	ns	ns	*
Genotypes	**	*	*	***	**
Mycorrhiza	***	***	***	***	***
Interaction effects:					
Genotype x Intercrop	ns	ns	ns	ns	ns
Mycorrhiza x Intercrop	**	**	ns	ns	**
Mycorrhiza x Genotype	ns	ns	ns	ns	ns
Myc x Geno x Intercrop	ns	ns	**	ns	*

Remarks: ns= non-significant; *, **, *** = significant at p-value <0.05, p-value <0.01 and p-value <0.001, respectively

Based on the main effects on the mean values of the observation variables, it can be seen from Table 3 and Table 4 that application of the AMF bio-fertilizer significantly increased growth and yield components of the red rice while reducing percentage of unfilled grains. This can occur because of the ability of AMF in helping their host plants, which in this case the red rice genotypes, to increase nutrient uptake and absorption of water from the soil [15], [16], [17]. Solaiman and Hirata [16] also indicated that rice plants inoculated with AMF increased dry matter partitioning from straw to grains, which increased filled grain number, when compared with uninoculated rice plants, as can also be seen from our results (Table 4) that AMF inoculated red rice resulted in higher number of spikelet and filled grains per clump but lower percentage of unfilled grain number, compared with those uninoculated with AMF bio-fertilizer.

In terms of the effect of intercropping red rice with legume crop by inserting one row of soybean plants (additive series) between double-rows of red rice one week after seeding rice, it can be seen that additive intercropping with soybean of Dering-1 variety increased dry matter production of the red rice plants (straw dry weight) (Table 3) and several yield components such as spikelet number and filled grain number, as well as grain yield per clump (Table 4), when compared with those of red rice plants grown in monocrop. From a previous pot experiment [18], it was clear that leaves of red rice plants that were planted together with soybean plants in one pot were much greener than those of red rice plants in monocrop. This indicated N contribution by soybean plants to red rice plants grown together in an intercropping system. Some researchers have also reported N contribution from legume crops to cereal crops in intercropping systems, such as from peanut to maize [9], from peanut to rice [10], and from soybean to sorghum [19], and the rates of N transfer from legumes to non-legumes will be greater when there is an involvement of AMF hyphae infecting roots of both legume and non-legume crops grown in an intercropping system [20], [21].

In relation to the interaction effects between the treatment factors, Fig. 2 shows the three-way interaction effect of AMF application, intercropping with soybean, and different genotypes of rice on grain yield of the red rice tested. It can be seen from Fig. 2 that grain yield of the G21 per clump was highest when the rice plants were bio-fertilized with AMF and additive intercropped with soybean, but when it was neither bio-fertilized nor intercropped with soybean, its grain yields were low. This indicates the interaction effects of application of AMF bio-fertilizer and intercropping with soybean, which was significant on grain yield of the red rice tested (Table 2). This could be due to the ability of soybean in establishing tripartite symbiosis, i.e. with AMF and *Rhizobium* bacteria, due to the application of AMF bio-fertilizer to the rice plants adjacent to the soybean plants, with a distance of only 15 cm between soybean and rice plant. According to the results obtained by Fujita *et al.* [19], the closer the distance between sorghum and soybean plants in intercropping, i.e. among four levels of planting distances tested (12.5 cm, 17.7 cm, 25 cm, and 50 cm), then the higher the rates of N transfer from soybean to sorghum. According to other previous research, this kind of N transfer is facilitated by the involvement of AMF that infect roots of both legume and non-legume crops in the intercropping system [20], [21].

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Table 3. Average plant height, tiller number per clump at anthesis, panicle number, %-panicle number, and weight of dry straw per clump for each level of the treatment factors

Treatments	Plant height (cm) at anthesis	Tiller number per clump at anthesis	Panicle number per clump	%-panicle number per clump (%)	Weight of dry straw (g/clump)
M0: no AMF	89.90 b	25.93 b	21.23 b	87.40 b	30.00 b [1)]
M1: with AMF	95.23 a	27.63 a	24.88 a	93.87 a	39.05 a
HSD 0.05	3.33	1.09	0.99	3.23	1.36
G04	93.12 a	25.54 a	21.13 c	88.40 a	33.09 bc
G10	91.10 a	27.55 a	24.04 ab	91.73 a	35.35 ab
G15	90.03 a	26.47 a	22.71 b	90.59 a	32.15 c
G21	96.00 a	27.55 a	24.33 a	91.83 a	37.51 a
HSD 0.05	6.57	2.63	1.52	5.22	2.41
T0: monocrop	93.33 a	26.28 a	22.19 a	89.04 a	33.17 b
T1: intercropping	91.79 a	27.28 a	23.92 a	92.23 a	35.88 a
HSD 0.05	6.46	0.11	3.42	6.19	2.15

[1)] Mean values followed in each column by the same letters are not significantly different between levels of a treatment factor based on its Tukey's HSD at 5% level of significance

Table 4. Average grain number per clump, filled grain number per clump, %-unfilled grain number, weight of 100 grains, and grain yield per clump for each level of the treatment factors

Treatments	Spikelet number per clump	Filled grain number per clump	%_Unfilled grain number	Weight of 100 filled grains (g)	Grain yield (g/clump)
M0: no AMF	1774.2 b	1622.6 b	8.52 a	2.55 b	41.34 b [1)]
M1: with AMF	2120.7 a	2027.5 a	4.34 b	2.69 a	54.64 a
HSD 0.05	124.6	127.2	1.28	0.07	3.35
G04	1810.6 b	1710.1 ab	5.76 a	2.54 b	43.62 b
G10	2074.3 ab	1962.5 a	5.53 a	2.44 b	48.01 ab
G15	1805.3 b	1688.4 b	6.47 a	2.74 a	46.29 b
G21	2099.5 a	1939.3 ab	7.96 a	2.77 a	54.04 a
HSD 0.05	273.9	265.7	2.44	0.12	7.39
T0: monocrop	1678.4 b	1581.8 b	5.87 a	2.62 a	41.48 b
T1: intercropping	2216.4 a	2068.3 a	6.99 a	2.62 a	54.49 a
HSD 0.05	282.5	312.9	5.04	0.07	7.97

[1)] Mean values followed in each column by the same letters are not significantly different between levels of a treatment factor based on its Tukey's HSD at 5% level of significance

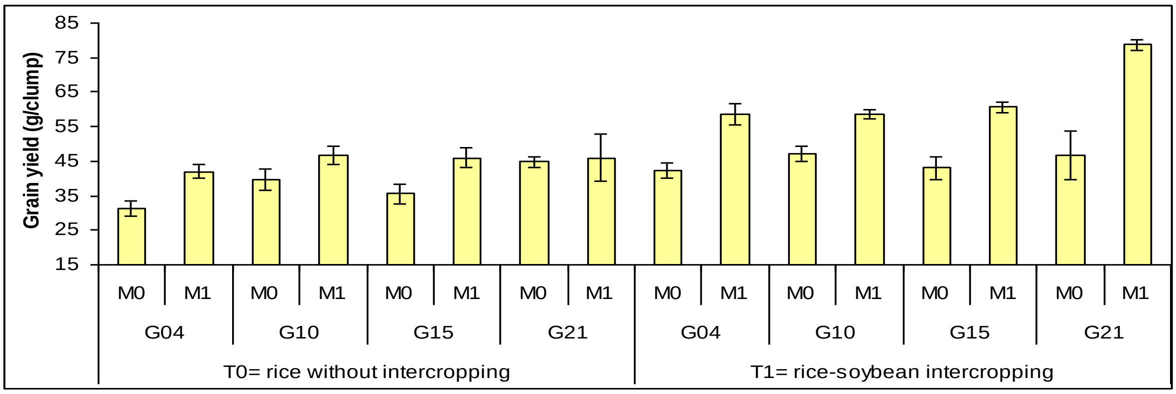

Fig. 2. Average (Mean ± SE) grain yield (gram/clump) of several promising lines of red rice as affected by application of bio-fertilizer containing AMF and additive intercropping with soybean in aerobic system

IV. CONCLUSION

It can be concluded that application of the bio-fertilizer "Technofert" containing AMF significantly increased growth and yield components of various promising lines of red rice, especially in relation to increased grain yield and decreased percentage of unfilled grain number. Relay intercropping of soybean between double-rows of red rice plants also significantly increased grain yield of red rice. Among the four selected genotypes, grain yield of AM-10 was the highest, especially when inoculated with AMF and intercropped with soybean.

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Bacterial Degradation of Contaminated Soil using Organic Manure

Fadina Olubunmi[1], Solaja Olatunde[2], Abiodun Abiodun[2], Adetoyi Helen[2]

[1]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria.
[2]Department of Environmental Health Technology, Ogun State College of Health Technology, Ilese-Ijebu, Nigeria.
Correspondence author –tolaja2004@yahoo.com.hk

***Abstract*— *Soil contamination is the presence in soil of unwanted impure materials from human activities. Many techniques of remediation of contaminated soils have been developed such as physical, chemical, photo degradation etc. Biological treatments offer the best environmental friendly method for remediating hydrocarbons and other contaminated soils because it utilized the capability of the indigenious micro-organisms in the environment to break down the hydrocarbons and heavy metals into innocuous substances. This study investigated the abilities of bacterial isolated, identified from organic manure and determine the total petroleum hydrocarbon degradative potentials. 2kg of soil was thoroughly mixed with 200ml and 400ml of spent oil to give 5% and 10% (w/w) each of organic manure ,poultry litter(PL),cow dung(CD) and mixed poultry litter and cow dung(MPLCD) was individually introduced into each spent oil contaminated soil and the rate of biodegradation was observed for a period of 12weeks.The percentage of total petroleum hydrocarbon (TPH) loss was significantly higher in the soil contaminated with 5% spent oil amended with MPLCD(40.46%) followed by PL (35.53%) and CD(27.70%) while 32.42% loss only was recorded in the soil contaminated with 10% spent oil and amended with MPLCD while PL was 30.04% and 25.60% for CD. The hydrocarbon-utilizing bacteria isolated band identified includes Bacillus, Pseudomonas, Micrococcus and Staphyloccus. The amendment spent oil contaminated soil with organic manure enhances the rate of degradation of petroleum hydrocarbon. These additives can best be suited to remove or neutralize the contaminants in the soil.*

***Keywords*— Bacteria, Total Petroleum Hydrocarbon, degradation, contaminated soil, organic manure.**

I. INTRODUCTION

Soil contamination is the presence in soil of unwanted impure materials from human activities. It can also be the distortion of the soil environment by human activities. Soil is the habitat for variety of organisms, including fungi, bacteria, protozoa, insects, nematodes, worms, and many other animals. Viruses are also present in soils Contamination of soil environment by hydrocarbons (mostly petroleum hydrocarbons) is becoming prevalent across the globe. This is probably due to heavy dependence on petroleum as a major, source of energy throughout the world, rapid industrialization population growth and complete disregard for the environmental health. Leaks and accidental spills occur regularly during the exploration, production, refining, transport and storage of petroleum and petroleum products. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution (Holliger *et al*., 1997). Also, (Fadina and Mbong 1998) reported that the contamination of soil with spent lubricating oil adversely affected micro and macro elements in soil, resulting in reduced growth and productivity patterns in two soybeans varieties.

Many techniques of remediation of contaminated soils have been developed, such as physical, chemical, degradation, photo degradation. However, most of these methods have some drawbacks in completely remediating hydrocarbon contaminated soil. Some of these methods leave behind daughter compounds which are more toxic to the environment than the parent compounds. Biological treatment offers the best environmental friendly method for remediating hydrocarbon and heavy metals contaminated soil because it utilized the capability of the indigenous micro-organisms in the soil environment to break down the hydrocarbons and heavy metals into the innocuous substances. In Nigeria, most of the terrestrial ecosystem and shore lines in oil producing communities are important agricultural land under continuous cultivation. Any contact with crude oil usually results in damage to the soil, microorganisms and plants (Adedokun and Ataga, 2007). In urban areas of Nigeria, most mechanic workshops are poorly managed and can be sources of constant release of used spent oil discharged from the crank cases of vehicles, motorcycles, generators which can be aesthetically unsightly and cause serious environmental pollution. Biodegradation is favored as a good option for the remediation of polluted sites mainly because it uses inexpensive equipments, it is simple and environmentally friendly.

This method has been investigated by several research studies to remediate petroleum polluted soil using various nutrient sources such as inorganic fertilizer, Urea, sawdust, compost manure, and bio-solids (Namhoong *et al*., 2002). Mushroom compost and Spent Mushroom Compost (SMC) have been applied in treating organic pollutants contaminated soils (Eggen, 1999, Trejo-Hernandez *et al*., 2006). Organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent but they take longer periods of time to grow when compared to their bacterial counterpart (Lee*et al*., 2007). Organic waste like banana skin, spent mushroom compost and brewery spent grain were found to enhance the biodegradation of used lubricating oil up to 90% within the period of 3 months (Abioye *et al*., 2009b, 2010).

Therefore, the main objective of this study is to isolate, identify the bacterial from degraded contaminated soil and determine the total petroleum hydrocarbon degradative potential of the intrinsic bacterial.

II. METHODOLOGY

2.1 STUDY AREA:

The experiment was carried out at the roof to garden of the Department of Crop Protection and Environmental Biology.

2.2 COLLECTION AND PROCESSING OF SAMPLES.

2.2.1 Soil sampling: The soil sample was collected randomly at a depth of 0-30cm from the fallow field. They were bulked to form a composite sample and transported in polythene bags to the laboratory, air dried and sieved through a 2mm mesh.

2.2.2 Spent Motor Oil: The spent motor oil used for the experiment was collected from a freshly drained motor car engine.

2.2.3 Collection of Poultry Litter and Cow Dung: The poultry litter (PL) and cow dung (CD) that was used was obtained from the Teaching and Research farm, University of Ibadan. The freshly collected manure was sun dried for 72 hours to allow moisture removal and accelerate the distribution of nutrients to the microbes.

2.2.4 Preparation of soil for Bioremediation - 2 Kg of sieved (2mm) soil was contaminated with 5% and 10% of spent lubricating oil and thoroughly mixed and left for 24 hours for homogenization. 10% (w/w) of each organic manure, poultry litter (PL), cow dung (CD), mixed poultry litter and cow dung(MPLCD) was individually introduced into each spent oil contaminated soil and thoroughly mixed.

The experimental pots were filled with the soil-oil – organic manure mixture. The control pots consist of soil-oil mixture without organic manure was also set up. The experiment was set up in four replicates. Periodic sampling from each experimental pot was carried out on day 0 and subsequently 4 weeks interval for 12 weeks of post contamination. Composite samples were obtained by mixing 5g of soil collected from four different areas of the pots for isolation and enumeration of bacterial and also the determination of Total Petroleum Hydrocarbon (TPH).

Table.1: Experimental Design

Treatment	Details of Treatment
1s (control)	2kg soil + 5% spent oil.
2(control)	2kg soil + 10% spent oil.
3	2kg soil + 5% spent oil + 10% PL
4	2kg soil + 5% spent oil + 10% CD
5	2kg soil + 5% spent oil + 10% MPLCD
6	2kg soil + 10% spent oil + 10% PL
7	2kg soil + 10% spent oil + 10% CD
8	2kg soil + 10% spent oil + 10%MPLCD

Key:

PL = Poultry litter
CD = Cow dung
MPLCD = Mixed poultry litter and cow dung

2.3 ENUMERATION AND IDENTIFICATION OF BACTERIAL POPULATION

Four replicate samples from each oil- contaminated soil were withdrawn in every four weeks for the enumeration and identification of bacterial. 1g of oil-contaminated samples were weighed and poured into 9ml of sterile distilled water and mixed thoroughly. Concentration of dilutions were made at 10^{1} to 10^{10} for bacterial. 0.1ml of dilution levels 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} for bacterial was cultured using pour plate method on nutrient agar (NA), King B medium to determine the loads of Total Heterotrophic Bacteria (THB). All media, sterile distilled water were sterilized by autoclaving at 121^0C for 15mi\nutes.

The Nutrient Agar plates were incubated at 30^0C for 48 hours, thus enumerating for only bacteria. The isolates from different plates were purified by repeated streaking on fresh agar medium.The characterization of isolates was based on the colonial, cell morphology and biochemical tests.The data obtained were compared with standards obtainable in Bergey's manual of the determinative bacteriology (Holt *et al*, 1994).

2.4 PHYSICO – CHEMICAL PARAMETERS OF THE SOIL.

The physical and chemical characteristics of the contaminated soil and the organic manure were determined.

2.4.1` Soil pH determination --The pH was measured using Jenway 3510 pH meter (Hendershot *et al;* 1993).

2.4.2 Determination of Organic Carbon (Walkley – Black Method)

2.4.3 Determination of Total Nitrogen (Kjeldahl Method)

2.4.4 Determination of Available Phosphorus (Bray11 method)

2.4.5 Hydrometer method of Soil Mechanical Analysis

2.5 Total Petroleum Hydrocarbon (TPH) Analysis

This was carried out using the method of Adesodun and Mbagwa (2008). Ten grammes (10g) of soil samples were weighed into 50ml flask and 20ml Toluene (Analar Grade) was added. After shaking for 30 minutes on an orbital shaker ,the liquid phase of the extract was measured at 420nm (nanometer) absorbance using DR/4000 Spectrophotometer. The Total Petroleum Hydrocarbon in the soil was estimated with the reference to a standard curve derived from fresh used engine oil diluted with Toluene.

2.6 Procedure for Preparing Standard Curve for TPH Analysis

Preparation of the standard solution was by diluting 0.2ml of fresh spent oil in 100mls of Toluene to give 2000ppm. It was then filled into the 100ml flask to meet the required mark using Toluene.

Preparation of 100ppm, 200ppm, 300ppm, 400ppm, 500ppm and 600ppm from the stock solution using the formula $C_1V_1 = C_2V_2$,

Where, C_1 = Concentration of stock solution

V_1 = Unknown volume

C_2 = Concentration of desired solution e.g. 100ppm

V_2 = Desired volume e.g. 10ml

The concentration of each volume was put in a 10ml flask and add more Toluene till it reaches the desired mark. The absorbance for each concentration was taken using the spectrophotometer and plotting the calibration curve of Absorbance value against concentration

III. RESULTS

Physico-chemical Parameters: The physicochemical properties of the contaminated soil and the organic manure are shown below in Table 2. The soil had a pH of 6.31±0.55 and a low concentration of total nitrogen, organic carbon, organic matter and available phosphorus as 3.65±0.51%, 10.35±0.28%; 17.89±0.38% and 18.34±0.36mg/kg respectively. The organic manure used comprised of Poultry litter, cow dung and the mixed Poultry litter and cow dung which had a pH of 8.60±0.25; 8.20±0.28 and 9.60±0.36 respectively. The mixed Poultry litter and cow dung had a higher percentage of total nitrogen of 6.50±0.64% followed by Poultry litter of 6.00±0.95% and cow dung 5.20±0.64%. Whereas, the available phosphorus of Poultry litter is 25.00±0.24 mg/kg; cow dung is 22.00±0.25mg/kg while the phosphorus of mixed poultry litter and cow dung was 24.00±0.12mg/kg. The poultry litter, cow dung and the mixed poultry litter and cow dung had an organic carbon (%) of 17.17±0.16; 16.92±0.67 ad 17.20±0.35 respectively while the total organic matter (%) was higher 29.74±0.16% in the mixed poultry litter and cow dung when compared with the low percentage recorded in poultry litter 29.69±0.28% and cow dung litter 29.69±0.28% and cow dung 29.25±0.46%. The moisture content was higher in contaminated soil (39.00±0.20%) compared to the organic manure of poultry litter of 17.20±0.06%; cow dung 16.81±0.68% and mixed poultry litter and cow dung of 11.35±0.24%.

2: Physicochemical Parameters of Contaminated soil and Organic manure.

Properties	Contaminated soil	PL	CD	MPLCD
pH	6.31±0.55	8.60±0.25	8.20±0.28	9.60±0.36
Total Nitrogen (%)	3.65±0.51	6.00±0.95	5.20±0.64	6.50±0.64
Available phosphorus (%)	18.34±0.36	25.00±0.24	22.00±0.25	24.00±0.12

Total Organic carbon (%)	10.35±0.28	17.17±0.16	16.92±0.67	17.20±0.35
Total organic matter (%)	17.89±0.38	29.69±0.28	29.25±0.46	29.74±0.16
Moisture content (%)	39.00±0.20	17.20±0.06	16.81±0.68	11.35±0.24
Sand (%)	87.50±0.70			
Silt (%)	5.15±0.64			
Clay (%)	7.35±0.07			

Means of triplicate ± standard deviation
PL: Poultry litter
CD: Cow Dung
MPLCD: Mixed Poultry litter and cow dung.

Baseline total Heterotrophic microbial population counts in the contaminated soil and organic manure.

The result in table 3 shows the total heterotrophic bacteria in the contaminated soil and the organic manure. The total heterotrophic bacteria counts in the contaminated soil of 5% and 10% was 0.76±0.28x 10^3 cfu/g and 0.32±0.35 x 10^3 cfu/g Whereas the total heterotrophic counts of bacteria in poultry litter, cow dung and the mixed poultry litter and cow dung was 1.32±0.15 x 10^6 cfu/g; 2.00±0.11 x 10^6 cfu/g and 2.68±0.15 x 10^6 cfu/g respectively.

3: Baseline Total Heterotrophic microbial population counts in the contaminated soil and organic manure.

Samples	Total heterotrophic bacteria
(Control)Contaminated soil with 5% spent oil	0.76±0.28 x 10^3
(Control)Contaminated soil with 10% spent oil	0.32±0.35 x 10^3
Poultry Litter (PL)	1.32±0.15 x 10^6
Cow dung (CD)	2.00±0.11 x 10^6
Mixed Poultry Litter and Cow Dung	2.68±0.15 x 10^6

4: Cultural and Biochemical characteristic of pure bacteria species isolated from degrading contaminated soil.

Test	*Bacillus sp.*	*Staph sp.*	*Micrococcus sp.*	*Pseudomonas sp.*
Colony shape	Circular	Irregular	Circular	Circular
Elevation	Raised	Flat	Raised	Flat
Cell shape	Rod	Cocci	Cocci	Rod
Pigmentation on Nutrient agar	Yellow	Yellow	Cream	Greenish
Gram stain	+	+	+	-
Catalase	-	+	_	+
Motility	+	-	-	+
Oxidase	+	-	-	-
Spore stain	+	-	-	_
King-B-medium	None	None	None	Greenish
Temperature: 28.5±2^{0}C.	+	+	+	+

+ = Positive reaction
- = Negative reaction

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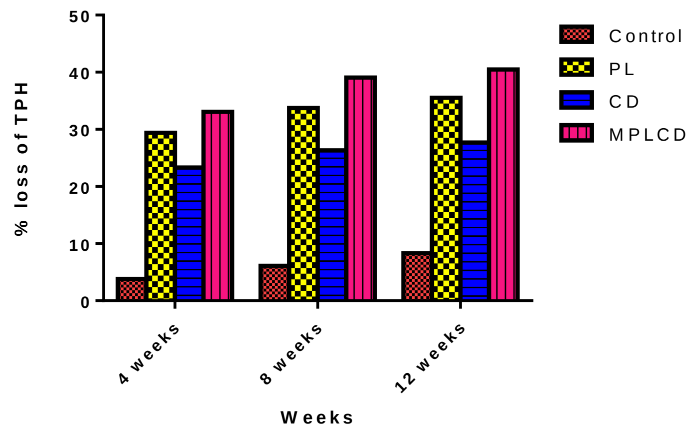

Fig 1. - Percentage loss of Total Petroleum Hydrocarbon in a contaminated soil with 5% spent oil.

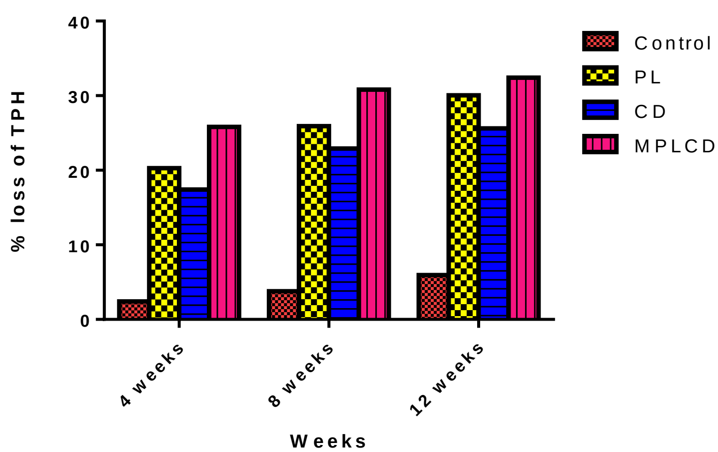

Fig 2. - Percentage loss of Total Petroleum Hydrocarbon in a contaminated Soil with 10% Spent Oil.

Figure 1 and 2 shows marked percentage loss of Total Petroleum Hydrocarbon content during the period of study with the addition of different organic manures (Poultry litter, cow dung and mixed poultry litter and cow dung). At the end of the first 4 weeks of the study, the contaminated soil with 5% spent oil (50000mg/kg-1) showed a significant in total petroleum hydrocarbon loss of 29.38%; 23.32% and 33.07% in the soil amended with PL, CD and MPLCD respectively compared with the unamended control contaminated soil of 3.83% while there was low degradation of hydrocarbon in the soil contaminated with 5%. The contaminated soil with 10% spent oil (100000mg/kg-1) showed a significant loss in total petroleum hydrocarbon content of 20.27%; 17.41% and 25.83% in to contaminated soil amended with poultry litter, cow dung and mixed poultry litter and cow dung whereas 2.4% reduction from the control contaminated soil.

AT 8 weeks of the study, there was a reduction in the degradation of contaminated soil with 5% spent oil amended with poultry litter was 33.73%, cow dung was 26.32% while mixed poultry litter and cow dung loss 39.07% and the unamended control soil loss 6.10%. Also, the contaminated soil with 10% spent oil, loss of total petroleum hydrocarbon content in the soil amended with poultry litter was 25.93%, cow dung 22.93% and for mixed poultry litter and cow dung was 30.83% while the unamended control soil loss 3.80%.

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AT the end of 12 weeks, there was a significant loss of total petroleum hydrocarbon content in the amended contaminated soil with 5% and 10%.

The level of total petroleum hydrocarbon loss in the amended contaminated soil with 5% spent oil was for poultry litter 35.53%,cow dung 27.70% and mixed poultry litter and cow dung was 40.46% while unamended control soil was 8.32%. However, for contaminated soil with 10% of spent oil, a significant reduction of 30.04%, 25.60% and 32.42% respectively for poultry litter,cow dung and mixed poultry litter and cow dung whereas the total petroleum hydrocarbon content loss in the unamended control soil was 5.95%.

AT the end of the study, it was observed that the organic manure applied showed a tremendous degradation in the contaminated soil with spent oil. The high degradation of contaminated soil occurred in the soil amended with mixed poultry litter and cow dung. This can be due to the presence of consortium of microbes which enhances the biodegradation of spent oil from the soil.

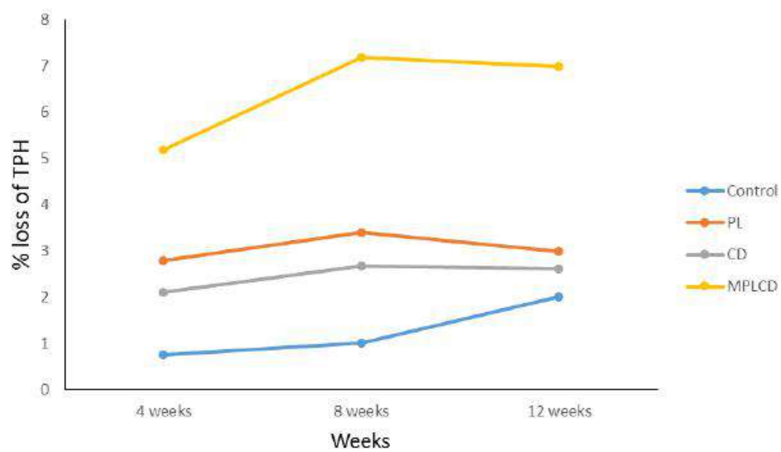

Fig 3:- Counts of Hydrocarbon – utilizing bacteria population in soil contaminated with 5% spent oil (cfu/g).

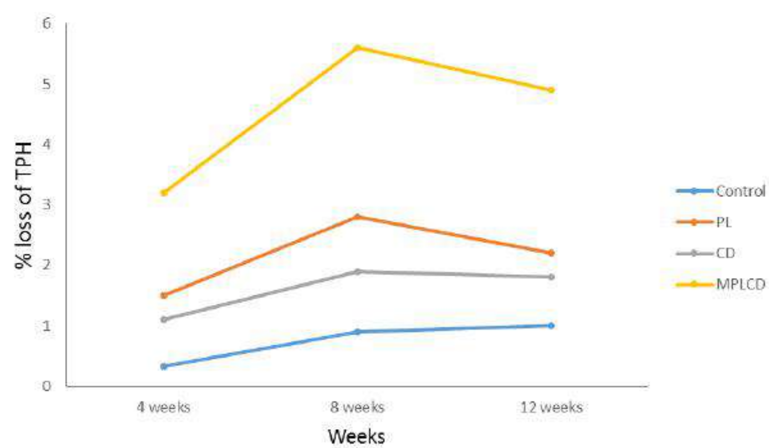

Fig 4:- Counts of Hydrocarbon – utilizing bacteria population in soil contaminated with 10% spent oil (cfu/g).

Figure 3 and 4 presented the heterotrophic counts of hydrocarbon – utilizing bacteria population in the soil contaminated with 5% and 10% spent oil. The hydrocarbon – utilizing bacteria population counted in the amended soils with 5% spent oil contaminated at the end of first 4 weeks. $2.80 \pm 0.16 \times 10^6$ for poultry litter; $2.10 \pm 0.95 \times 10^6$ for cow dung and the mixed poultry litter and cow dung was

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5.20±1.55 x 10^6 while the unamended control soil was 0.76±0.16 x 10^6 cfu/g.

Also the contaminated soil with 10% spent oil showed hydrocarbon – utilizing bacteria population in the amended soil contaminated as poultry litter was 1.50±0.16 x 10^6, cow dung was 1.10±0.74 x 10^6 and mixed poultry litter and cow dung was 3.20±1.11 x 10^6 while in an unamended control was 0.32±0.06x10^6

AT 8 weeks of treatment application, the level of hydrocarbon – utilizing bacteria counts from the contaminated soil with 5% spent oil for poultry litter was 3.40 ± 0.67 x 10^6, cow dung was 2.68 ± 0.45 x 10^6 and the mixed poultry litter and cow dung was 7.20±1.11 x 10^6 while that of the unamended soil was 1.01±0.12 x 10^6 cfu/g. The hydrocarbon – utilizing bacteria counted for the soil contaminated with 10% spent oil was 2.80±0.15 x 10^6 for poultry litter, 1.90±0.10 x 10^6 for cow dung and 5.60±0.64 x 10^6 for mixed poultry litter and cow dung while the value for unamended control soil was 0.90±0.06 x 10^6 cfu/g.

At the end of 12 weeks, of the study, the counts of hydrocarbon – utilizing bacteria in the soil contaminated with 5% spent oil was 2.00±1.19 x 10^6 for the unamended control soil, the amended contaminated soil with poultry litter, cow dung and the mixed poultry litter and cow dung was 3.00±0.15 x 10^6, 2.62±0.60 x 10^6 and 7.00±0.10 x 10^6 cfu/g respectively. While with 10% spent oil contaminated soil, the amended contamination soil was 2.20±1.60 x 10^6 for poultry litter, 1.80±0.10 x 10^6 for cow dung and the mixed poultry litter and cow dung was 4.90±1.22 x 10^6 while the uamended control soil was 1.00±0.25x 10^5.

It was observed that the organic manure added had a greater microbial population in the amended contaminated soil. However towards the end of the study, there was a microbial population drop in the treatment which is also similar to the 10% spent oil contaminated soil. Microbial counts was significantly higher in soil amended with different organic manures when compared to those of the unamended control.

Contaminated soil at the 0.05% probability level, indicating the role of nutrients in the enhancement of microbial population. There was a significantly higher hydrocarbon – utilizing microbial population in the mixed poultry litter and cow dung than those amended with cow dung and poultry litter while the microbial population in the poultry litter was significantly higher ($P < 0.05$) than the cow dung. The hydrocarbon – utilizing microbial population in the contaminated soil with 10% spent oil exhibited a similar trend as observed in 5% concentration of hydrocarbon – utilizing bacteria at 0.05% probability level in the soil amended with organic manures. The result of the microbial population obtained demonstrated microbial population in the different organic manures:

Plate 1: Isolated Rod of Pseudomonas specie

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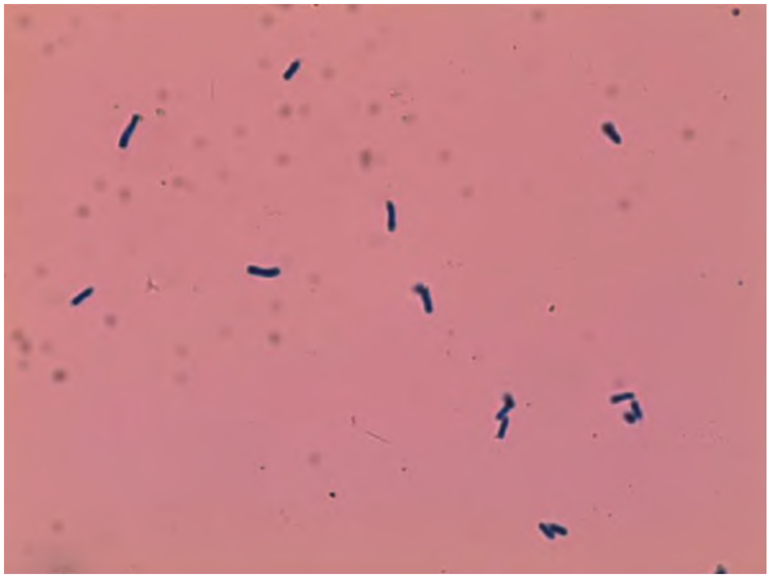

Plate 2: Isolated Rod of Bacillus specie

IV. DISCUSSION

The findings in this research indicated that the pH value obtained for poultry litter, cow dung and the mixed poultry litter and cow dung was 8.60 $\pm$ 0.25, 8.20 $\pm$ 0.28 and 9.60 $\pm$ 0.35 respectively. This was an indicative of the alkaline nature of the organic manure samples used. This condition of alkalinity may be responsible for the high counts of total heterotrophic and hydrocarbon-utilizing bacteria. This difference was supported by the reports of Ijah and Abioye, (2003); Njoku *et al*. (2009) that bacteria thrive better in alkaline medium than acidic medium.

The particles size analysis of the soil samples indicated that the soil was sandy. This is because the sand percentage of the soil samples was 87.50%.

The nitrate content was higher in the organic manure used compared to the contaminated soil. This may be due to the presence of high nitrogenous compounds in the poultry litter, cow dung and the mixed poultry litter and cow dung.sA similar result was reported by Atagana (2008) who worked on compost manure. This result also agrees with the findings of Okwule and Ijah (2014) who observed higher nitrate contents in palm oil mill effluent soil (POME) amended with poultry droppings added to the contaminated soil enhances the growth of bacterial and fungal in the biodegradation process.

The presence of moisture content may also support the growth and survival of these microbes and providing moisture necessary for their bioactivity. This may arise from the rain since this study was carried out during the early rainy season (March-May, 2016). This is in agreement with Stephen and Egene, (2012) who observed high moisture content in the spent lubricating oil polluted soil during the rainy season.

The organic carbon and organic matter contents were higher in the organic manure used, carbon and organic matters were utilized by the microorganisms for their growth and metabolic activities. Carbon also serves as a source of nutrients and also required for biodegradation. Lee *et al*., (2003), reported that the addition of carbon in the form of pyruvate stimulates the microbial growth and enhances the rate of polycyclic aromatic hydrocarbons (PAHs)

Phosphorus content of the poultry litter, cow dung, and mixed poultry litter and cow dung was higher compared to that of the contaminated soil. The presence of these organic manures contributed to the higher content of phosphorus which was used by micro-organisms during the biodegradation process and this was reported by Ijah and Abioye (2003).Also, Thieman and Palladino (2009) reported that high level of Nitrogen and Phosphorus in the organic manure added to the contaminated soil enhances the growth of bacterial and fungal in the biodegradation process. Microorganisms play an important role in the degradation of environmental pollutants. Microbial growth and metabolism in the impacted areas can be mitigated by a number of factors such as pH, temperature, concentration of pollutant, moisture content, conductivity, oxygen content, nutrient availability and bioavailability and the property of the impacted soil medium (Rahman *et al*.,2001).

The organic manure used (poultry litter, cow dung and the mixed poultry litter and cow dung) contained considerable numbers of heterotrophic and hydrocarbon degrading bacteria. The counts of total heterotrophic microbes were higher compared to the hydrocarbon degrading microbial population. The reason for higher counts of total heterotrophic bacteria may be attributed to the fact that hydrocarbon degrading bacteria are also heterotrophic. Naturally, they are part of the heterotrophic community. The high counts of petroleum hydrocarbon degrading microbes in the sample is therefore suggestive of previous exposure to oil contamination this will ultimately boost the supply of carbon and hence, favours the growth of these microbes (Ijah and Abioye, 2003).

The microbial population of hydrocarbon degrading micro-organisms in an ecosystem quantitatively reflects the degree or extent of exposure of that ecosystem to hydrocarbon contamination.Ijah and Antai (2007) reported that the high counts of the total heterotrophic bacteria and fungi in organic manure may be the result of the presence of appreciable quantities of Nitrogen and phosphorus which can enhances the multiplication of bacteria and fungi in the soil.

The result of the investigation has shown that four bacterial genera were tested for petroleum hydrocarbon degradative potentials. This was revealed that these microbes exhibited responses and potential to breakdown petroleum hydrocarbon and utilize as source of energy and carbon. All the bacteria exhibited the highest degradative potentiality during the period of study. Onuoha *et al*., 2014 suggested that the differences in the rate of hydrocarbon degradation may be due to the natural ability of the microbes in the hydrocarbon degradation. The petroleum hydrocarbon- utilizing bacteria isolated and identified were to be the species of *Pseudomonas, Bacillus, Micrococcus* and *Staphylococcus*. These bacteria species has been implicated in the hydrocarbon degradation by the authors (Onuoha *et al*., 2011; Ijah, 1998) Okpokwasili and Okorie (1990) isolated similar hydrocarbon- utilizing bacteria from the Niger- Delta aquatic systems. Chikere and Okpokwasili (2004) also made similar findings on petroleum effluents. It has also been observed that some microorganisms are more abundant in areas of high concentration of hydrocarbons. These micro floras are actively oxidizing the hydrocarbons and this is considered as another source of carbon for use in the ecosystem. This study revealed that, the indigenous microbial populations in the oil contaminated soils are capable of mineralizing these pollutants in the environment to the safe and acceptable level. There was a general increase in the hydrocarbon utilizing microbial population in the treatment for both 5% and 10% spent oil contaminated soil. Whereas the un-amended control soil exhibited lowest rate in the microbial population. This relatively lowest rate obtained may have resulted from the toxicity of spent oil to the soil micro-organisms, brought about by the high concentration of the spent oil before the remediation treatments. This was also observed by Odokuma and Dickson, 2003.

The first 8 weeks exhibited significant increase in the hydrocarbon- utilizing microbial population, and towards the end of the research, there was a decrease in the microbial population which may be due to the decline in the availability of readily metabolizable components of hydrocarbon for the organisms.

The counts of microbial population of hydrocarbon- utilizing bacteria in the 5% and 10% spent oil contaminated soil amended with different organic manure in the study were significantly higher ($p<0.05$) when compared with those of the unamended control soil. These counts are similar to those of Ijah and Antai (2003b), who observed counts of hydrocarbon degraders in oil polluted soil to be $x10^6$ cfu/g.

The reasons for the significant higher counts of bacteria in the amended contaminated soil may be due to the availability of appreciable quantities of nitrogen and phosphorus from the organic manure added to the contaminated soil which enhances the multiplication of bacteria. (Thieman and Palladino, 2009; Adesodun and Mbagwu, 2008). Also, it was observed that the decrease in the hydrocarbon- utilizing bacteria towards the end of the study was as a result of the decline in the availability of readily nutrients for the growth of the microbes which have been reported also by Vasudevan and Rajaram, 2001.

This study also revealed that the biodegradation of oil breakdown increases with time. The different organic manure added to the contaminated soil significantly enhanced the degradation of the contaminated soil in comparison with the control soil without the addition of organic manures. This might be as a result of released nutrients from the organic manure into the contaminated soil for use by the oil degraders. This finding is in consistent with the work done by Abioye *et al.,* 2009b who used banana skin (BS), brewery spent grains (BSG) and spent mushroom compost (SMC) as organic waste amendments in the biodegradation of used motor oil in soil.

It was observed that the high concentration of oil contamination has a reduced effect on the rate of microbial reduction of total petroleum hydrocarbon. The ineffectiveness of the organic manure with high concentration of oil could be attributed to the reduction in the activity of the soil microbes at that level of oil pollution. Ijah and Antai (2003) reported high degradation of hydrocarbon in soil contaminated with 10% and 20% crude oil compared to those contaminated with 30% and 40% crude oil which experienced partial degradation within a period of 12months. Also, it is important that the reduction or loss in total petroleum hydrocarbon in the contaminated soil may not only be due to the biodegradation process included by nutrient additions, but other processes such as volatilization, adsorption to organic compounds and other abiotic factors are equally implicated in the reduction process. This is the case in the two un-amended control soils where there were reductions in total petroleum hydrocarbon without any organic manure amendment.

V. CONCLUSION

The amendment of spent oil contaminated soil with organic manure enhances significantly the rate of biodegradation of petroleum hydrocarbon. The spent oil contaminated soil amended with mixed poultry litter and cow dung with 5% and 10% spent oil contamination exhibited highest rate of oil biodegradation and counts of hydrocarbon- utilizing bacteria compared to the soil amended with poultry litter and cow dung.

The un-amended control soil also showed that the remediation of the contaminated soil can be achieved through natural processes of biodegradation, photo-oxidation, evaporation and volatilization without external interferences.

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Bacterial wilt resistant gene searching in Eggplant (*Solanum melongena*) and its two wild relatives

Fatema Tuz Zohura[1] and Md Ekramul Hoque[2*]

[1]MS Student, Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.
Email: tamannafatema75@gmail.com
[2*]Professor, Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.
Email: ekramul.sau@gmail.com

***Abstract*— Eggplant is an important vegetable in all over the world. Bacterial wilt caused by Ralstonia solanacearum is a major disease of eggplant. Near about 32% crop loss occurred due to this disease. The wild relatives of eggplant viz. Solanum villosum and Solanum sisymbriifolium showed resistant against bacterial wilt disease. Hence, attempted was made to search the resistant gene of bacterial wilt in three Solanum spp. Some total of 16 different bacterial wilt resistant molecular markers RAPD (OPB-17, OPG-05, OPH-07) UBC (176, 205, 317) SSR (STM0007, emh01J23, emb01N07) SCAR (me1, me2, me4, me5) and SRAP (SCU176-534, SCU176-1190F1R1, SCU176-1190F2R2) were screened to identified the resistant DNA fragment. Only one primer UBC#176 showed amplification in target DNA. The primer UBC#176 gave DNA amplification in target position at 350 bp in the species of Solanum villosum. The obtained DNA fragment showed maximum 79% homology with Solanum lycopersicum cultivar I-3 chromosome 8 which has GeneBank Accession No. CP023764.1. Alternative approach was made to identify resistant gene of bacterial wilt disease. Total RNA was extracted and cDNA was synthesized. The synthesized cDNA was used as template to find out the resistant gene. But none of the gene specific primer was amplified using cDNA as template. Further study was needed to find out the bacterial wilt resistant DNA fragment in wild type.*

***Keywords*— Bacterial wilt, eggplant, eggplant wild relatives, resistant gene, sequencing.**

I. INTRODUCTION

Eggplant or brinjal (*Solanum melongena* L. 2n = 2x = 24) is an important solanaceous crop grown widely in Asia, Africa, and the subtropical region including the southern USA. It is the second most important solanaceous fruit crop after tomato *(Solanum lycopersicum* L.) [1]. In respect of total acreage production eggplant is the second most important vegetable crop next to potato in Bangladesh [2]. A large number of eggplant cultivars are grown in Bangladesh. In Bangladesh it is cultivated in an area of about 1,25,860 acres with productivity of 5,07,432 MT (BBS 2017). Eggplant has a number of health benefits such as it has ayurvedic medicinal properties and is good for diabetic patients. It has been recommended as an excellent remedy for those suffering from liver complaints [3]. It is an important source of fiber, potassium, manganese, as well as vitamins C, K, and B6. Phenolic compounds in eggplant contain significant amounts of chlorogenic acid, one of the most powerful free radical scavengers found in plants. Chlorogenic acid has been shown to decrease low density lipid (LDL) levels, and also serves as an antimicrobial, antiviral, and anti-carcinogenic agent.

Successful cultivation of eggplant crop has been hindered due to infestation of many insect pests and diseases. Among these, bacterial wilt disease is the most devastating and a limiting factor caused by *Ralstonia solanacearum* throughout the tropical, sub-tropical and temperate regions of the world [4, 5]. Bacterial wilt is an important disease of many plant species especially solanaceae [6]; causing enormous economic losses, which limits eggplant production from 4.24 to 86.14 percent [7]. It perpetuates in the soil, enters the plant through the roots, progressively invades the stem vascular tissues and blocking of the vessels by bacteria, and finally it leads to partial or complete wilting of plant [8]. Understanding the host and pathogen is a pre requisite for devising proper strategies for control of disease. Control of wilt diseases is also complicated by the scarcity of sources of disease-resistant host germplasm, and the soil and vascular habitats of the pathogen [4, 9]. Host resistance appears to be the main strategy for the control of bacterial wilt because of the resistant imposed by the lack of effective chemical controls, the soil-borne nature of the pathogens, and the wide range of hosts [10].

In the Solanaceae, most cultivated species (*Solanum melongena* L.) is highly susceptible to bacterial wilt. The wild species *Solanum phureja* [11, 12] and *Solanum stenotomum* [11] were identified as possible sources of resistance for potato (*Solanum tuberosum*), *Lycopersicon pimpinelifolium* for tomato (*L. esculentum*) [13] and *S. villosum, S. torvum, S. sisymbriifolium, S. gilo* and *S. aethiopicum* [14] for eggplant (*S. melongena*). *Solanum villosum* Mill. has been reported to be useful for introgression of disease resistance. *Solanum villosum* is (2n= 48) believed to have originated in Eurasia, and is sometimes considered to be resistant to bacterial wilt. Interestingly *Solanum villosum* has been identified to carry traits of resistance to most serious diseases of eggplant, particularly bacterial and fungal wilts [15].

Despite of its valuable potential for disease resistance, little is known about *S. villosum* regarding variability in morphology, fertility and levels of resistance to both bacterial and fungal wilts, as well as genetic diversity. The molecular technique is applied to identify disease resistant gene from wild relatives of eggplant. *Solanum villosum* and *Solanum sisymbriifolium* are two important wild relatives of eggplant. Sequence-related amplified polymorphism (SRAP) technology has been recognized as a new and useful molecular marker system for mapping and gene tagging in many crops plants [16]. Sequence characterized amplified region (SCAR) markers are more reproducible and easier to manipulate in marker-assisted selection (MAS) programs than other markers. Due to the co-dominant or dominant nature, SCAR marker can provide a valid tool for the accurate assessment of genotype at the linked locus. SCAR can be considered to be an ideal marker for plant breeding programs. It was screened that two SCAR markers linked to *Fusarium* wilt resistance gene in eggplant [16]. Hence, three *Solanum spp*. *viz. Solanum melongena, Solanum villosum* and *Solanum sisymbriifolium* were used for screening of bacterial wilt resistant gene through SCAR marker. In addition, other categories of molecular markers *viz.* RAPD, UBC, SSR and SRAP were used to identify the target amplification. Therefore, the research work was carried out to investigate the source of bacterial wilt resistant gene in three *Solanum spp*. including cultivated eggplant.

II. MATERIALS AND METHODS

One variety (BARI Begun-01) of eggplant (*Solanum melangena*) and two wild relatives *viz*. *Solanum villosum* and *Solanum sisymbriifolium* were used as experimental materials. DNA extractions from young leaves were performed according to a modified Doyle and Doyle (1990) [17] method by using CTAB protocol. On the basis of literature five different set of molecular markers were synthesized which showed linkage with bacterial wilt resistant in some other crops. The types of molecular markers are (a) RAPD (b) UBC (c) SSR (d) SCAR and (e) SRAP. Some total of 16 different primers were synthesized from all groups of primer. The list of primers is given in "Table- 01".

1.1. DNA isolation and purification

Genomic DNA was extracted using CTAB method from three species of *Solanum*. The RNase and proteinase treatment was given to the extracted DNA for purification from RNA and protein. The purified DNA was visualized by 2% agarose gel. DNA concentration was measured by DNA Nanodropper. Working DNA sample was prepared (20-25 nm/µl) for PCR reaction on the basis of concentration of main stock.

1.2. PCR amplification and elution of DNA fragment from gel

PCR amplification was carried out with purified genomic DNA of three *Solanum spp*. and the above gene specific primers. Ten microliter (10 µl) reaction mixture (Bio Basic, Canada, GeneON, Taiwan) was used for target amplification. The annealing temperature was adjusted on the basis of Tm of the primer. The PCR product was visualized on 2% agarose gel and save in gel documentation system. For further study the target fragment of DNA was cut from agarose gel with fine blade and taken in an eppondrof tube. Then the gel fragment was eluted by QIAquick Gel Extraction Kit. The eluted DNA fragment was stored at -20ºC and it was rechecked in 2% agarose gel.

1.3. DNA sequencing and alignment of nucleotide

The primer UBC- 176 produced approximate 350bp DNA fragment. The amplified 350bp fragment of DNA was sequenced by Applied Bio-system, DNA. The obtained DNA sequences were used in NCBI, BLAST program to carry out the sequence homology with other organism through computer software. Another molecular approach was carried out to findout the bacterial wilt resistant gene. The methodological steps are given below.

1.4. RNA extraction and cDNA synthesis

The total RNA was extracted from three *Solanum spp*. by QIAGEN RNA extraction kit. Near about 50-100 mg fresh leaf sample were collected and it was soaked in liquid nitrogen and then grind with mortar and pestle. In the successive step QIAGEN RNA extraction protocol was used to isolate total RNA from the leaf sample. Finally the RNeasy spin column was used to isolate the total RNA. Approximate 30-40 µl RNase-free water was added directly to the spin column membrane and the RNA elution was done by 10000 rpm for 1 min. The extracted RNA was visualized by 1% agarose gel.

The total isolated RNA was used for cDNA synthesis. cDNA Synthesis was done by QIAGEN Quantitect Reverse Transcription Kit. Template RNA was thaw on ice. gDNA Wipe out Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-free water were thaw at room temperature (15–25°C). Each solution mixed by flicking the tubes. It was centrifuged briefly to collect residual liquid from the sides of the tubes, and then stored on ice. The genomic DNA elimination reaction was prepared on ice according to QIAGEN Kit mixed and then stored on ice. Incubation was done for 2 min at 42°C. Then immediately placed on ice. The reverse-transcription master mix was prepared on ice according to same protocol mixed and then stored on ice. The reverse-transcription master mix contains all components required for first-strand cDNA synthesis except template RNA. Template RNA was added to each tube containing reverse-transcription master mix. Mixed and then stored on ice. Incubation was done for 15 min at 42°C. Incubation was done for 3 min at 95°C to inactivate Quantiscript Reverse Transcriptase. Reverse-transcription reactions were stored on ice and proceed directly for long-term storage, store reverse-transcription reactions at –20°C temperature.

1.5. PCR amplification using cDNA as a template

All five categories primers ('Table no.1') were used for amplification of target DNA band. Different thermal conditions were used for different primer to get specific DNA fragment.

III. RESULTS AND DISCUSSION

Solanum melangena (eggplant) is highly susceptible to bacterial wilt disease. *Solanum villosum* and *Solanum sisymbriifolium* are two wild relatives of *Solanum melangena* which showed considerable resistance to bacterial wilt. Hence, attempt was made to screen these two *spp*. with different molecular markers to identify any DNA fragments which are linked with bacterial wilt resistance gene. The major finding of these experiments was given in following sub-heading.

1.6. PCR amplification with different gene specific primers

The three species *viz. Solanum melangena* (BARI begun-01), *Solanum villosum, Solanum sisymriifolium* template DNA were used for amplification of DNA fragment which may linked with bacterial wilt resistant gene. Some total of 16 primers ('Table No. 1') were used for the same. It was showed that, only one primer UBC#176 was given amplification at 350 bp DNA fragment in the species *S. villosum* ('Fig. No.1'). It was not amplified in *S. melangena* (BARI begun- 01), *S. sisymriifolium*. It was reported that a total of 800 RAPD primers were screened and only six primers (UBC#176, 205, 287, 317, 350, and 676) showed polymorphism between resistant pool and susceptible pools of bacterial wilt in the Tomato Line Hawaii 7996 [19] . Of these, only two markers UBC#176 and 317 revealed a 100% linkage in the individual plants comprising the contrasting bulks. The marker UBC#176 was converted into a co-dominant SCAR marker and designated as SCU176-534. The marker SCU176-534 was confirmed by genotyping the individual of the resistant pool and susceptible pools and gave the same result as UBC#176 [19]. The above mentioned reference was a good evidence of our research finding. Hence, there is a probability to identify bacterial wilt resistance DNA sequence in this 350 bp DNA fragment. Hence investigation was carried out with the specific 350 bp fragment.

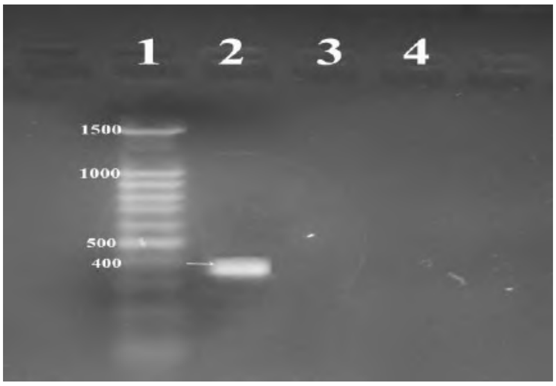

Fig. 1: DNA amplification in Solanum villosum at 350 bp position, Line 1: Ladder, lane 2: Solanum villosum, lane 3: Solanum sisymriifolium,lane 4: Solanum melangena

Table 1: List of molecular markers which are tightly linked or linked with bacterial wilt resistant gene

Sl No.	Types of Marker	Marker Name	Forward Primer(5′-3′)	Reverse Primer (5′-3′)	Reference List
1.	RAPD	OPB-17	AGGGAACGAG	----	[19]
2.		OPG-05	CTGAGACGGA	----	
3.		OPH-07	CTGCATCGTG	----	
4.	UBC	UBC#176	CAAGGGAGGT		[20]
5.		UBC#205	CGGTTTGGAA		
6.		UBC#317	CTAGGGGCTG		
7.	SSR	STM0007	GACAAGCTGTGAAGTTTA T	AATTGAGAAAGAGTGTGTGTG	[19]
8.		emh01J23	ATGCAGCTCCCATAAACC CTAAAA	GTTTCCAAGACCAGCACTCCAAA C	[21]
9.		emb01N07	TGATAAGAAGGGCAAGCT CAGTCC	GTTTCGAGCTTATGGCTACACTG GACCT	
10.	SRAP	me1	TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAT	[22]
11.		me2	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGC	
12.		me4	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTGA	
13.		me5	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCGA	
14.	SCAR	SCU176-534:	TTGAACCAAGAATCTATT CG	GAACTTGAATGCCTACCAAA	[20]
15.		SCU176-1190F1R1	TGCGGATACTATCGGAAA TA	CAACTCATTTCAGTCCGATT	
16.		SCU176-1190F2R2	TCACTCGGTGAGTCAATA GAT	TTTGCCGATGTTATCATGT	

1.7. Gel elution, DNA purification and sequencing of target DNA band

The species *Solanum villosum* produced 350 bp DNA fragment with UBC#176 primer. The amplified fragment was eluted from agarose gel and taken into eppendorf tube. It was purified by QIAGEN DNA purification Kit. The purified DNA fragment was again loaded in the 2% agarose gel for confirmation of purification. It showed exact, DNA band in the same 350 bp position. Hence, it proved that, purification of DNA was successfully completed. The concentration of purified DNA was done by DNA nanodropper. It was 20-25 ng/µl. This amount is sufficient for sequencing works. The required amount of DNA was sent to abroad for sequencing of nucleotide. The sequence of nucleotide was given below. It was 341 bp fragments.

GCAGGAAAAAATGCGGGAATTCCTATTGGCGCCA
GCTCGTTCACGCCGGAAAACCCCTTTTTCAATCGT
GGGAGCTTGACGTACACCTCCCTTGAAAGTCTG
ATCTATTTTGCTATTGTCCTTACGAATTTTATCGG
AAAATTGATGAAATATGATCGAAGAACCATCCCC
AAAAAAATTATGAAAACTGGGATAATTCCTCCTG
CACCGGGTTGGTTACACTCGAAAACCCCTCCTTAT
ATCGTGTATGCTTGTCCTTTGCCTCCCTTGGGGGG
GTCCGGCCCAGTTTTTGCGAACTCCAACAAATTCC
CCGGAATACCTGACCCCTCCCTGGACAATGGTGT
GGT = 341 bp

1.8. Alignment and homology searching

The obtained sequenced DNA was used for NCBI, BLAST to identify homology with other gene of interest. *Solanum villosum* sequence get 78% homology with *Solanum lycopersicum* cultivar I-3 chromosome 5 which has GeneBank Accession No. CP023761.1. It also showed 78% homology with *Solanum pennellii* chromosome ch05, complete genome which has GeneBank Accession No. HG975444.1 and 79% homology with *Solanum*

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lycopersicum cultivar I-3 chromosome 8 which has GeneBank Accession No. CP023764.1.
Another alternative approach was made to search the bacterial wilt resistance gene in *S. melangena, S. villosum* and *S. sisymriifolium*. The assumption is that, from total RNA we will synthesis cDNA and this cDNA will be used as template for amplification of target bacterial wilt resistant DNA fragment. Previous synthesized all linked gene specific primer will be used for the same. On the basis of this principle the following activities were done.

1.9. Total RNA extraction and cDNA synthesis

Total RNA was extracted from three species *viz. S. melangena* (BARI begun-01), *S. villosum, S. sisymriifolium* were used to check the RNA molecule. It was done on the basis of QIAGEN RNA purification kit. The 2% agarose gel (Fig. 2). The high quality RNA band was noticed from the three species. Near about 400-500 µg/µl RNA was generated from leaf tissue. Those total RNA used as a template for cDNA synthesis. QIAgen cDNA synthesis kit was used for the same. The reaction mixture and other protocol were mentioned in the methodology. The produced cDNA was stored at -20°C for further use.

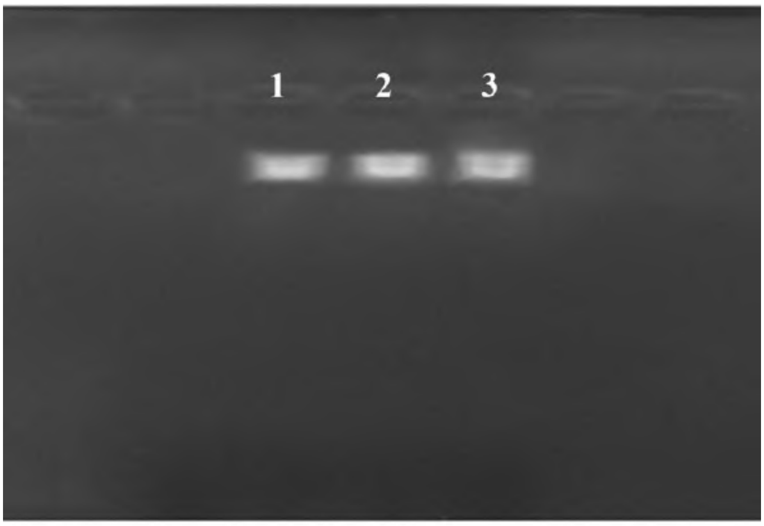

Fig. 2: RNA amplification in Lane 1- BARI Begun 1, Lane 3-Solanum villosum, Lane 4- Solanum sisymbriifolium

1.10. PCR amplification with cDNA as template

The previously synthesized all tightly linked gene specific primers were used for PCR amplification to identify bacterial wilt resistant gene. It was sorry to say that, none of the 16 primers gave amplification in any one of the three *Solanum spp.*

IV. CONCLUSION

Eggplant is an important vegetable in our country. It has multipurpose used in our daily consumption. It's production is seriously hampered due to different biotic diseases. Among them bacterial wilt disease is major one. Wild relatives of eggplants are resistant to bacterial wilt. *Solanum villosum* and *Solanum sisymriifolium* are two common wild relatives of eggplant which showed resistant to bacterial wilt. Hence, attempt was made to identify DNA fragment from wild species which will be resistant to bacterial wilt. Two different approaches was carried out to fulfill the objectives. Some total of 16 different bacterial wilt resistant molecular markers were used for the same. Out of them only one primer UBC#176 was gave DNA amplification in target position (350 bp) in the species *Solanum villosum.* The obtained DNA fragment was sequenced and homology was detected through NCBI, BLAST. It was showed maximum 79% homology with *Solanum lycopersicum* cultivar I-3 chromosome 8 which has GeneBank Accession No. CP023764.1.
Alternatively, Total RNA and cDNA was used to amplify the target link DNA fragment by using of previous primer. None of the primer was able to regenerate any DNA fragment which was linked to bacterial wilt. Hence further CODEHOP method, genome editing or any advanced molecular technique may be applied for searching of target bacterial wilt resistant gene.

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