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FOREWORD

I am pleased to put into the hands of readers Volume-4; Issue-3: May-Jun 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: July, 2019

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Protected Areas in the Brazilian Semi-Arid and the Management and Conservation of Ecosystems

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Abstract— *The use of biodiversity has not been done sustainably on the planet, especially in South America, which has caused irreversible environmental impacts. Geodiversity is an important component of the existing natural heritage, but it lacks studies that mainly exploit the characteristics of the landscape and its forms of conservation. In this way, the aim is to discuss the relevance of the sustainable use of natural resources of protected areas of the Brazilian semi-arid, especially of the paleodunate areas, as well as to present a proposal for the management and conservation of these areas in accordance with the realities investigated, based on renowned theorists, in the existing legislations and above all, based on the practical investigations carried out. Therefore, it is crucial to understand the relevance of the adequate management of Brazil's protected areas, especially those that have environmental indicators from other geological eras and that, once destroyed, will never be recomposed, since it is a dynamic complex, thus requiring greater attention and a coherent management for its actual conservation.*

Keywords — Conservation, Brazilian semi-arid, Management, Protected areas.

I. INTRODUCTION

The political framework related to the creation and management of conservation units in Brazil is established in Law No. 9,985, which in July 2000 established the System of Conservation Unit (SNUC). The preparation of a system of conservation units in Brazil began in 1976, in a paper called "An analysis of priorities in nature conservation in the Amazon", which based the preparation of the National System of Conservation Units Plan of Brazil, published between 1979 and 1982 (MERCADANTE, 2001).

This document proposed the immediate creation of National Parks, Biological Reserves and National Forests in the region, invariably in areas rejected by the planned economic projects. In fact, between 1979 and 1985, 10 (ten) National Parks were created, 04 (four) of which in the Amazon; 13 Biological Reserves, five of them in the same region, and 15 (fifteen) Ecological Stations, 11 (eleven) in the Amazon, totaling 20 (twenty) conservation units of integral protection in that part of the country, or 9.7 million of hectares (BARRETTO, 2003).

In 1988, the year of the promulgation of the current Constitution, the federal government commissioned a critical evaluation of the categories of conservation units existing in the country and the preparation of a preliminary bill to establish a national system of conservation units. In May 1992, a proposal was sent as a Bill to the National Congress, finally being approved. On July 19, Law No. 9,985/2000 was published, which instituted the SNUC (MERCADANTE 2001).

Based on these premises, it can be affirmed that the National Strategic Plan for Protected Areas (PNAP) in Brazil was created in 2005 with a Ministerial Working Group composed of specialists, managers of conservation units and leaders of organizations of the civil society and social movements, being that only in April of the following year, the PNAP was officially instituted through Decree No. 5,758. The PNAP defined principles, guidelines, objectives and strategies for Brazil to establish a comprehensive system of protected areas, ecologically representative and effectively managed, integrating broader terrestrial and marine landscapes by 2015.

However, from 2015 to the present, the index of environmental impacts has grown in Brazil, especially in the Semi-arid region, even though it has numerous legal documents that protect natural areas. In this context, this research aimed to discuss the relevance of the sustainable use of natural resources of protected areas of the Brazilian semi-arid, especially of the paleodunares fluvial areas, as well as present a proposal for the management and conservation of those areas in accordance with the realities investigated, based on in the theories, in the existing legislations and above all, based on the practical investigations carried out.

The methodology is based essentially on the Geosystemic Theory (SOTCHAVA, 1977), the Ecodynamic Method (TRICART, 1977) and, in the Geosystem - Territory - Landscape Theory (GTP) of Bertrand and Bertrand (2007). Therefore, it is crucial to develop conservation measures for protected environments, since landscapes are products and records of the geological evolution of the planet and integrate the planet (LOPES, ARAÚJO, 2011), as well as corroborate for the sustainable use of the biome. Thus, it is essential to understand the urgency of the adequate management of protected areas in Brazil, essentially those that protect environmental indicators of paleoeras, paleoclimates and paleoventos.

The article is divided into four iten, such as: 1. Introduction, 2. Theoretical contribution that deals with management of ecosystems in conservation units, where it is approached conservation units of Caatinga - semiarid brasileiro, conservation unit dunas do São Francisco and categorization of dune areas. 3. Methodology, disjunction the path traveled by research and theories of support. 4. Resulted, which addresses relevance of conservation units for the environment, and environmental conservation plan for the dunes and landscapes of the middle São Francisco. Finally, we have the conclusions and bibliographical references.

II. THEORETICAL FRAMEWORK

1.1 LEGAL FRAMEWORK FOR PROTECTED AREAS

Although a relevant part of the current normative framework regarding protected areas in Brazil has been instituted in the last ten years, there are structuring norms of the national policy that came into force before that period. It is a specific case of Law No. 6,938, which in 1981 established the National Environmental Policy; of the Federal Constitution, enacted in October 1988, and other important laws for the planning and management of protected areas (BRASIL, 1998).

Among the many environmental protection laws, the laws that make up the legal framework for the current policy of protected areas in the country are described in chronological order below:

i. Law N° 4.771/1965 (Forest Code): when the Forest Code was instituted, Law Nº. 4.771, of September 15, 1965 and its subsequent amendments, brought to the Brazilian legislation the figures of the areas of permanent preservation (APP) legal reserve, whose vegetation must be kept free of low cut depending on the role they play for the maintenance of ecological services. According to the Forest Code, the forests that make up the indigenous patrimony are subject to the regime dispensed to the PPA areas (BRASIL, 1965).

ii. Decree n ° 84,017/1979: published on 09/21/79, approves the regulation for National Parks (BRASIL, 1979).

iii. Law nº 6,938/1981: approved on 08/31/1981, instituted the National Environmental Policy, establishing as a principle "the governmental action in the maintenance of the ecological balance, considering the environment as a public patrimony to be necessarily insured and protected, taking into account the collective use "(article 2, paragraph I) (BRASIL, 1981).

iv. Federal Constitution of 1988: enacted in 1988, establishes that "everyone has the right to an ecologically balanced environment, well-used by the people and essential to a healthy quality of life, imposing the duty of defending public authority and the community, and preserve it for present and future generations "(article 225) (BRASIL, 1988).

v. Decree No. 99.274/1990: that decree, of 06/06/90, regulates the law of the National Environmental Policy, specifying that its execution will be carried out when the Public Power, in the different levels of government, "protect the areas representative of ecosystems through the implementation of conservation units [...] "(article 1, paragraph II) (BRASIL, 1990).

vi. Decree No. 1,298/1994: published on October 27, 1994, approves the National Forest Regulation (BRASIL, 1994).

vii. Convention on Biological Diversity (CBD): the CBD was signed by the President of the Republic during the United Nations Conference on Environment and Development in June 1992 and was ratified by the National Congress through Legislative Decree No. 2/94, In accordance with Decree No. 2.519, on 03/17/98, and promulgated through Decree No. 2.519. Brazil, in its capacity as signatory, has followed its principles and determinations, with special attention to Article 8, which deals with in situ conservation, and Article 6, which defines measures for the conservation and sustainable use of biodiversity. The PNAP meets the commitments assumed by Brazil under the CBD, in particular, those derived from Decision VII / 28 and VII/5 (BRASIL, 1998).

viii. Law nº 9.985/2000: created the SNUC, composed of defined territorial spaces and their respective environmental resources that have relevant natural characteristics, legally established by the Public

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Power, with conservation objectives and under a special administration regime, called conservation units (BRASIL, 2000).

ix. Decree No. 4,340/2002: regulated articles of Law No. 9,985/2000 (SNUC) relating to the following topics: creation of conservation unit, definition of subsoil limits and airspace of the conservation unit, creation and operation of mosaic of management units, operation of advisory and deliberative councils, shared management with civil society organizations of public interest, authorization for the exploitation of goods and services in conservation units, compensation for significant environmental impact, resettlement of traditional resident populations in units of conservation, reevaluation of unit of conservation of nonexistent category in the SNUC, operation of the Biosphere Reserves (BRASIL, 2002).

x. Decree No. 4.339/2002: published on 08/22/2002, established the principles and guidelines for the implementation of the National Biodiversity Policy, considering the commitments assumed by Brazil in the CBD to develop strategies, policies, plans and programs of biodiversity and the other existing norms related to the subject (BRASIL, 2002).

xi. Decree No. 4,703/2003 and amendments: published on 05/21/2003, provides for the National Program for Biological Diversity (Pronabio) and the National Commission for Biodiversity (Conabio). The Pronabio's main objective is to promote collaboration between the Public Power and civil society in the conservation of biodiversity, the sustainable use of its components and in the fair and equitable sharing of the benefits derived from such use (BRASIL, 2003).

xii. Decree No. 5.092/2004: this Decree, of 21/05/2004, defines rules for the identification of priority areas for the conservation, sustainable use and distribution of the benefits of biodiversity, within the framework of the powers of the Ministry of Environment Ambient (BRASIL, 2004).

xiii. Decree n. 5.746/2006: published on 04/05/06, approves regulations for the Particular Reserves of the National Patrimony (BRASIL, 2006).

xiv. Decree n ° 5,758/2006: published on 04/13/06, institutes the National Strategic Plan for Protected Areas - PNAP, its principles, guidelines, objectives and strategies, and provides other measures (BRASIL, 2006).

xv. Law N ° 11.428/2006 (Atlantic Forest Law): when establishing the use and protection of the native vegetation of the Atlantic Forest biome, this law establishes the definition of the biome and conditions for its conservation, economic use and recovery, and that it is the most dramatically reduced Brazilian biome, with less than 8% of its original vegetation (BRASIL, 2006).

xvi. Law No. 11,516/2007: published on 08/28/07, created the Chico Mendes Institute for the Conservation of Biodiversity - Chico Mendes Institute, a federal autarchy linked to the Ministry of the Environment (BRASIL, 2007).

Regarding this history of laws and regulations built in Brazil over decades, it is possible to affirm that, it is not for lack of legislation that environmental areas are not being protected as they should be, but because of the absence of a strong control and by the lack of political will, since currently, all legislation that must be strengthened is being revised to "loosen up" and simplify processes, thus leaving the natural environment even more unprotected and accessible to the exploitation of all nature.

1. MANAGEMENT OF ECOSYSTEMS IN CONSERVATION UNITS

2.1 CONSERVATION UNITS OF CAATINGA - SEMIÁRIDO BRASILEIRO

The Caatinga is the only exclusively Brazilian biome and covers a territory of 734,478 km2 and although it is an exclusive Brazilian biome, much is left in the background in relation to its conservation (MMA, 2003). Although little known and/or studied, the Caatinga is rich in the diversity of endemic species, because of its climatic and soil conditions (MMA, 2002). However, much is known for wood and furniture exploitations, because of human actions.

The creation of Conservation Units (Figure 1) is today one of the main instruments for the conservation of biodiversity (BENSUASAN, 2006), aiming at the preservation in situ, which consists of the conservation of ecosystems and natural habitat and the maintenance and recovery of viable populations of species in their environments "(BRASIL, 2000).

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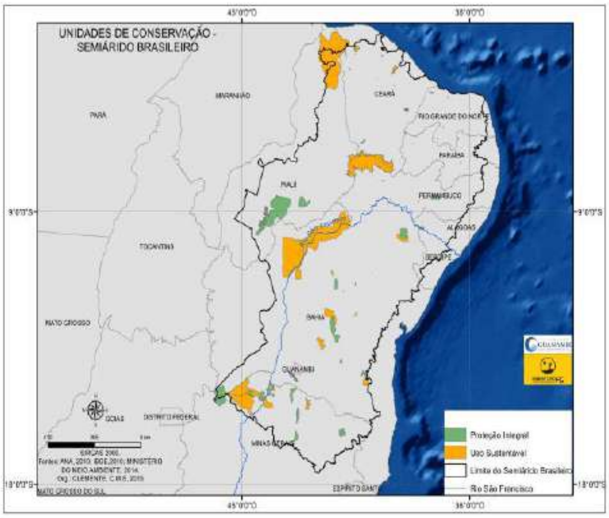

Fig. 1 - Conservation Units of the Brazilian Semi-Arid

Source: ANA; IBGE (2010); MMA (2014)

It can be seen then that the current network of Conservation Units in the Caatinga biome is insufficient to guarantee the maintenance of its biological diversity, being urgent the creation of new units, in addition to actions that enable the effective implementation and operation of the units existing.

The Caatinga biome is inserted in the morphoclimatic domain of the semiarid, which according to Souza (2000) has the sertões as typical geographic environments. In that region, erosion surfaces developed on crystalline rocks, originating from the pre-Cambrian period. One of the main characteristics of the semiarid sertões are the high temperatures and the rainfall irregularity, being the irregularity in the space and in the time the main mark of the semi-arid climate.

Historically, the exploitation of natural resources in the Brazilian Northeast (NEB), is practiced in an unsustainable way. Since the beginning of Brazilian colonization in the sixteenth century, the natural resources of the Brazilian semi-arid have been thoroughly exploited. It can then be verified that the current network of Conservation Units in the Caatinga biome is insufficient to guarantee the maintenance of its biological diversity, being urgent the creation of new units, in addition to actions that enable the effective implementation of existing units.

Table 1 presents the area and percentage of ecoregions of the Caatinga protected by public conservation units of federal and state administration, according to the protection group (integral protection, sustainable use).

Table 1 - Caatinga ecoregions protected by conservation units

Ecoregion		Public Protected Areas of Integral Protection		Public Conservation Units for Sustainable Use		Federal Public Protected Areas of Integrated Protection		State Conservation Units of Comprehensive Protection		Federal Public Conservation Units for Sustainable Use		State Conservation Units of Sustainable Use	
Name	Area (km²)	Area (ha)	%	Area (ha)	%	Area (ha)	%	Area (ha)	%	Area (ha)	%	Area (ha)	%
Complex of Greater Complex	41.420	7.700	0,2	852.753	20,6	7.700	0,2	0	0,0	849.690	20,5	3.063	0,1
Complex of Ibiapaba - Araripe	69.510	306.803	4,4	1.252.870	18,0	280.212	4,0	26.591	0,4	1.249.920	18,0	2.950	0,0
Northern Country Depression	206.700	66.971	0,3	1.386.780	6,7	27.798	0,1	39.163	0,2	413.807	2,0	972.973	4,7
Borborema Plateau	41940	1.001	0,0	46.768	1,1	177	0,0	824	0,0	0	0	46.768	1,1
Southern Country Depression	373.900	140.996	0,4	752.433	2,0	81.928	0,2	59.068	0,2	198.332	0,5	554.101	1,5
Dunes of São Francisco	36.170	0	0,0	1.752.162	48,4	0	0,0	0	0,0	0	0,0	1.752.162	48,4
Chapada Diamantina Complex	50.610	200.305	4,0	121.278	2,4	152.000	3,0	48.305	0,1	0	0,0	121.278	2,4
Catherine's Shrug	30.800	162.995	5,3	67.503	2,2	162.995	5,3	0	0,0	3.043	0,1	64.480	2,1
Total	851.050	886.761	1,0	6.232.546	7,3	712.810	0,8	173.951	0,2	2.714.791	3,2	3.517.755	4,1

Source: Adapted PNUD (2010)

"Private Reserves of Natural Heritage" (RPPN) protect less than 50,000 hectares or 0.1% of the territory of ecoregions, while indigenous lands and other protected areas considered as priority areas by mapping 2007 contribute little more than 630 thousand hectares, representing less than 1% of ecoregions.

Research shows that protection rates are very low, except for those related to Environmental Protection Areas (APA), a category of management responsible for the high rates of the sustainable use group. Private reserves contribute to the protection of small areas and are distributed throughout all ecoregions, with Catherine's short having the smallest area and percentage of protection by these units.

Indigenous lands are found mainly in the ecoregions of Catherine's short, Plateau da Borborema and Campo Maior Complex. In the general context, the Borborema Plateau is the least protected ecoregion, both by indices and by the type of protection, which is essentially carried out by indigenous lands, whose primary objective is cultural, being that the conservation of nature in these areas, under current conditions, is not fully guaranteed.

For this reason, it is recommended to prioritize the creation of integral protection conservation units in the ecoregions of the Borborema Plateau, Dunas of São Francisco, Campo Maior complex, Northern Sertaneja Depression, Southern Sertaneja Depression.

2.2 CONSERVATION UNIT DUNAS DO SÃO FRANCISCO

The Dunes and Sidewalks of Bajo-Medio San Francisco has an area of State Environmental Protection, belonging to the group of sustainable use, with an area of 1.085,000 hectares, created by Decree n. 6.547 of 07/18/1997, issued by the government of the State of Bahia, having as its managing body the Secretariat of Environment and Resources (D.O.E/BA, 1997).

The APA Dunes and low-middle trails São Francisco is located in a region of semi-arid climate, with low rainfall, but the outcrop of the water table occurs at some points. The vegetation is rich, diversified and unique, composed of a transition between the Cerrado and the Caatinga. In this context, angico and aroeira bushes, lagoons, heather and extensive trails of buritis emerge. As well as the flora, the fauna of the APA also deserves highlight, even presenting some species of endemic reptiles and rodents. The region is a great attraction for scientists.

The São Francisco Dunes, despite the large area protected by environmental protection areas (APA), is the only ecoregion with no comprehensive protection unit. The Borborema Planalto is protected by four integral protection units, being a federal (PN Catimbau) and a state (PE del Pau Ferro) the main responsible.

The Ecoregion of the Campo Mayor Complex is barely protected by a comprehensive protection unit, the Seven Cities PN. Three federal ecological stations and three state parks are the units that protect the great Northern Sertaneja Depression ecoregion. The largest of them, the Depression Sertaneja Meridional, has its integral protection carried out mainly by four federal units and six state ones, of the park category in its majority.

According to Pacheco and Oliveira (2016) the dunes of the San Francisco are considered important ecosystems for hosting an odd biological diversity, composed of a flora rich in species and a fauna made up of insects, reptiles, amphibians, small mammals and some species of birds that They use the dunes to build their nests. Due to their importance, they are considered areas of environmental preservation, and then law, which forces the managing body to adopt land use and occupation plans and environmental management and conservation, protects them.

This geographical space suffers considerable remodeling by virtue of anthropogenic origins, causing modifications, in some cases, irreversible. Among the conditioning factors originating from dune environments are distinguishing elements that contribute to the process of dune destructiveness that are incorporated both to factors of an anthropic nature and to those correlated with the dynamics of the coastal elements acting on the site, which are listed as examples: the existence of the vegetation and its state of degradation, the frequency of the wind sands in the dune composition, the wind direction speed influencing dune addition/erosion processes, the seasonal variation of the rains, in addition to anthropogenic derivations as an advance of agricultural practices , vehicle traffic, in vulnerable areas, among others (PACHECO, OLIVEIRA, 2016).

2.3 CATEGORIZATION OF DUNE AREAS

The natural areas can be categorized according to the theory of Tricart (1977) in: stable areas, intergrades areas and unstable areas, being:

a) Stable - where pedogenetic processes supplant the mechanical processes in the evolution of modeling. According to Tricart (1977, p. 36) "the conditions are close to what the phytoecologists designate by the term climax". The media considered to be morphodynamically stable have a sufficiently closed plant cover to avoid triggering the mechanical processes of morphogenesis.

b) Intergrades - that are areas of transition and in these, morphogenesis and pedogenesis act mutually in the dynamics of the landscape. These means, in effect, ensure the gradual passage between stable and unstable media. What characterizes these media is the permanent interference of morphogenesis and pedogenesis, exercising concurrently over the same space. On the other hand, Tricart (1977, p. 51) affirms that "the intergrades media are delicate and susceptible to phenomena of amplification, transforming themselves into unstable media whose exploitation is compromised".

c) Unstable - where there is a predominance of morphogenetic processes versus pedogenetic processes, either by factors of a natural nature or due to anthropic causes. There are several processes that contribute to a greater susceptibility of these media. One of them is the vegetation, because it intervenes introducing an indirect influence of the climate, being the greatest instability realized in the regions that present strong climatic instabilities. In that case, part of the vegetation is poorly adapted to climatic irregularities and biostatic influences are reduced to a minimum.

In this sense, anthropic degradation adds natural causes, particularly effective in rugged regions where the climate has severe limiting factors to the vegetation. Such difficult ecological conditions make degradation easier, preventing the reconstitution of vegetation in certain periods.

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III. METHODOLOGY

The present investigation is classified according to Gil (1999), Andrade (2006), and Cervo et al. (2007), according to its nature, its technical procedures, the approach to the problem and the objectives. Regarding the approach to the problem, it is a qualitative research, since it considers the existence of a dynamic relationship between the real world and the subject, being descriptive and using the inductive method, being that the obtained data are analyzed indutively.

This study was based methodologically on the morphodynamic approach elaborated by Tricart (1977) and on the GTP method (Geosystem - Territory - Landscape) of Bertrand and Bertrand (2007) to trace an analysis of the environmental dynamics of the study area.

Preliminarily a systematic bibliographic analysis was made, seeking to characterize in a general way the dune environment of the San Francisco lowlands and the processes of degradation and/or conservation of those environments.

The adopted method of Tricart (1977) allows identifying the morphodynamic processes responsible for the genesis of the relief to the environmental stability of that landscape, submitting to the analysis of crucial parameters, such as surface structure of the dune field, land use, vegetation and surface processes.

The GTP method (Geosystem - Territory - Landscape), which is relevant not only for the delimitation and cartographic representation of the areas, but essentially for the detection of the existing problems in the premises and the degree of responsibility of the anthropogenic action on them, as well as, the planning of strategies to contain, reverse or enliven the impacts already provoked in the environments studied. Thus, this methodology envisages the search for the sustainable management of natural resources, which seeks to lead sciences to the understanding of the functioning of landscape units, in their naturalistic/social/cultural whole.

IV. RESULTS

4.1 RELEVANCE OF CONSERVATION UNITS FOR THE ENVIRONMENT

The formation of preserved areas known as Units of Conservation (UC) is considered of extreme importance in the preservation of ecosystems, providing a concrete possibility of conservation and protection of the environment, being an environmental public policy, apparently, federal level. Through the defense of natural resources, these Units have the main objective of protecting biodiversity under special State protection (SILVA, et al, 2017).

The formation of preserved areas, currently known as the Conservation Unit (UC), is considered of significant importance in the preservation of ecosystems, providing a continuous search for conservation and protection of the environment. The creation of these spaces has been signed in Brazil and in the world, as the fundamental and most diffuse strategy of protecting nature and defending natural resources. The main objective of these Units is to protect biodiversity under special State protection (SILVA, et al, 2017).

According to the National Cadaster of Conservation Units (CNUC), there are currently about 1,113 Conservation Units in Brazil, and the growth of these territorial spaces is increasingly frequent, with the objective of preserving fauna, flora and abiotic factors, in that they have defined limits under a special administration regime, to which adequate guarantees of protection apply.

It is ensured and obligatory by the SNUC, in its article 27, that each UC has a management plan. According to the SNUC, the management plan is about one:

> Technical document through which, based on the general objectives of a Conservation Unit, is established its zoning and the rules that should preside over the use of the area and the management of natural resources, including the implementation of the physical structures necessary for the management of the Conservation Unit (Article 2, paragraph XVII).

In the case of integral protection units, this planning and management tool should contemplate a buffer zone and ecological corridors, which include measures that promote the protection of biodiversity and integrating the units to the economic and social life of the communities neighbors. By establishing standards, guidelines, programs and zoning of the UC, the document helps in the allocation and obtaining of resources for the implementation of proposed measures and interventions. As determined by CONAMA Resolution No. 347, of September 10, 2004, Conservation Units that present natural underground cavities destined for public visitation also need Speleological Management Plans (SMEs) (BRASIL/CONAMA, 2012).

The positioning of Silva stands out; Bonilla; (2011) when affirming that "the absence of management plans in the UCs, is the result of the difficulty in effecting the agrarian regularization of the areas, because they lack resources and the process is also quite slow" (p. 53). In addition to the extensions are not selected by the technical-scientific method, but by political-economic pressures, interfering in environmental monitoring activities (SILVA, BONILLA, BIRTH, 2011).

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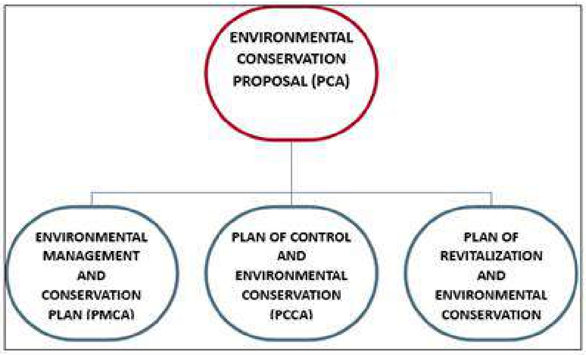
4.2 ENVIRONMENTAL CONSERVATION PLAN FOR THE DUNES AND LANDSCAPES OF THE LOWER MIDDLE SÃO FRANCISCO

The possible preservation proposals in the dune geosystem are totally based on the characteristics of the ecoregion, since it is inserted in an Environmental Protection Area. In this conviction, it is suggested the creation of three strategic plans, for the three environments and, according to the characteristics of the GTP. In this way an Environmental Conservation Plan (PCA) is proposed for the dune area, based on the application of plans for each area categorized from the conception of Tricart (1977), namely:

Table 1- Environmental Conservation Proposal



Suerce: Pacheco (2019)

The Environmental Management and Conservation Plan - oriented towards the areas that still present themselves as stable, so that these are not seen in the future totally damaged because it is a fragile and vulnerable environment due to climatic and socioeconomic conditions.

The Environmental Conservation and Control Plan - this would be applied to areas that are in transition from stable aspect to middle intergrades. It is necessary to create degradation control strategies in the areas in process and strategies to conserve what remains of some fragments.

The Plan of Revitalization and Environmental Conservation - in this one is primary by strategies of revitalization/reforestation of the areas considered as strongly unstable and, from the results would be a control of preservation, analyzing the resilience capacity of the respective environments.

The proposals suggested above should start with those responsible for the management of the PAs, in the case of the Dunes and Veredas of the low and medium São Francisco, by the Government of the State of Bahia, in association with the municipalities affected by the impacts, where are located the fields of dunes. In addition to the municipalities, a partnership with the community is essential, since it is these subjects who are living together in that context, and can contribute positively to this awareness.

V. CONCLUSIONS

It is evident that the caatinga, due to the historical processes of use and occupation and also by the fragility of its natural environments, has suffered during the last five centuries a deep process of alteration of its natural conditions.

This degradation has contributed to the advance of desertification in some regions of the Northeast of Brazil. However, despite the semiarid condition, the Caatinga biome presents a series of peculiar conditions, since among those of the semi-arid regions dispersed by the planet is only present in the brazilian territory. In addition, the caatinga shelters a great diversity of species of both the fauna and the flora, being in great part endemic of the Brazilian Northeast.

Therefore, the Conservation Units of the biome are still insufficient to guarantee the effective conservation of its natural aspects, these already in an advanced degree of alteration by the human activities.

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Biostimulants Enhanced Seedling Root Growth and Bulb Yields of True Seed Shallots (*Allium cepa* var *aggregatum* L.)

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***Abstract*— *The objective of this research was to investigate the effects of biostimulan (PGPR) on root growth of seedlings and bulb yields of true seeds shallots (TSS). The research was conducted in the glasshouse in Tangtu village, Badung regency,Bali province of Indonesia, from April until October 2018. Complete randomized block design was used with 4 replications. Three kinds of biostimulants (B, C, D) and one control, and two TSS varieties (Sunren F1 and Tuktuk) were the treatments imposed. Results of experiment showed that biostimulants as well as TSS variety significantly (P<0.05) increased germination percentage, root length of TSS seedlings, leaf chlorophyll content, bulb diameter, bulb number, leaf and bulb fresh weight per pot. However there was no significant different effect between types of biostimulant. Interaction effect between biostimulants and TSS variety was only significant on leaf chlorophyll content. Biostimulant application could be expected to increase growth and yields of TSS.*

***Keywords*—Biostimulants, bulbs, roots, true seed shallots.**

I. INTRODUCTION

The use of true shallot seeds (TSS) is increasing particularly in Indonesia due to its advantages compared to seed bulbs (Basuki, 2009). The benefits of TSS are free from pathogen, small amounts of planting materials, easier transporting and storaging, producing healthier crops and bigger bulbs (Ridwan *et al*., 1998; Permadi, 1993; Sumaratne *et al*., 2005. The use of TSS ia economically beneficial due to doubling the yields compared to seed bulb crops van den Brink and Basuki, 2011). However, the slow growth of seedlings in the nursery takes 21-25 days to be able to transplant into the field resulted in longer time in producing bulbs compared to seed bulb crops. Seed priming has been proved to increase seed germination and reduce abnormal seedlings of onions ((*Allium cepa cv. aggregatum L.*) (Caseiro *et al*., 2004; Sevarani and Umarani, 2011; Jagosz, 2015). Biostimulant or plant growth promoting rhizobacteria (PGPR), is a group of beneficial bacteria actively colonizing the rhizosphere and important in increasing plant growth (Rahni, 2012). Research results showed that bacteria of *Pseudomonas, Azotobacter, Bacillius dan Serratia* genus were identified as phytohormone producers such as auxin, cytokinin, gibberellin, ethylene and absisic acids that were able to enhance the growth and yield of plants (Rahni, 2012). GA3 significantly enhanced seed germination, index of seedling vigour and the speed of seedling emergence of true seed shallots variety Tuktuk compared to KNO3 (Agung and Diara, 2017). *Steptomyces griseoviridis* was also reported *to produce auksin and IAA invitro* which is able to stimulate plant growth (Fitrah-pratiwi *et al*., 2017). Biostimulants that contain *Pseudomonas fluorescens* colonize the rooting zones of bamboo were reported to increase the solubility of soil phosphorus. Some strains of *Pseudomonas fluorescens* can avoid the infection of soil pathogen fungi and is used as biocontrol agents commercially in the glasshouse and in the fields (Arshad and Frankenberger, 1997).

II. METHODOLOGY

The research was conducted in the glasshouse in Tangtu village, Badung regency,Bali province of Indonesia, from April until October 2018. The objective of this research was to investigate the effects of biostimulan (PGPR) on root growth of seedlings and bulb yields of true seeds shallots (TSS). Complete randomized block design was used with 4 replications. Three kinds of biostimulants (B, C, D) and one control, and two TSS varieties (Sunren F1 and Tuktuk) were the treatments imposed. Biostimulant B consisted of *Rhizobia*, *Azospirillum* sp., *Bacillus subtilis*, *Aspergilus niger*, *Lactobacillus* sp, *Pseudomonas putida*. Biostimulant C consisted of total bacteria, *Azosprillum* sp., *Azotobacter* sp. and N fixed bacteria. Microorganisms in *biostimulant D* were *Pseudomonans fluorescence, Trichoderma, Aspergillus niger, Azotobacter, Azospirilum and Rhizobium.* After soaking in each biostsimulant for 24 hours, 25 seeds were germinated in petridshes as well as on moistened tissues wrapped with transparent plastic sheets. Seedlings were then transplanted into 20 cm diam pots. Germination percentage of seeds, root length of seedlings, leaf chlorophyll content, bulb diameter, bulb number, leaf and bulb fresh weight per pot were the variable measured.

III. RESULTS AND DISCUSSION

3.1 Germination Percentage

Biostimulants as well as variety significantly ($P<0.05$) increased the germination percentage of TSS. Biostimulants resulted in 41.37% higher germination than control (Table 1). There was no difference effect between types of biostimulants. Sunren F1 variety had 41.67 % higher germination than Tuktuk variety (Table 1). There was no effects of interaction between types of biostimulant and TSS variety on germination percentage.

3.2 Seedling Root lengths

Seedling root lengths of both varieties were significantly ($P<0.05$) increased by biostimulants. Biostimulants resulted in 27.65% higher root longer rooth lengths compared to control (Table 1). Variety of Sunren F1 produced 64.44% longer roots than Tuktuk (Table 1). There was no effects of interaction between Biostimulant and TSS variety on seedling root lengths. Rhizobacteria promote better root development directly through production of phytohormones and indirectly by inhibiting pathogen infection with synthesis of different substances (Benizri *et al*., 2001).

3.3 Leaf Chlorophyll Content

There was significant ($P<0.05$) effect of interaction between types of biostimulant and variety on leaf chlorophyll content. Biostimulants significantly increased leaf chlorophyll both on variety of Sunren F1 and Tuktuk although the effects on Sunren F1 higher than on Tuktuk. Leaf chlorophylls were increased by 58%, 48% and 60% due to biostimulants A, B and C application respectively compared to without biostimulant (Table 2). The total chlorophyll content was also reported to be significantly stimulated by *P. agglomeranset Proteamaculansen on tomato leaves (*Moustaine *et al*., 2017) in comparison with other treatments.

3.4 Leaf and Bulb Fresh Weights and Bulb Number per Pot

Biostimulant significantly increased ($P<0.05$) leaf and bulb fresh weights (FW) and bulb number per pot (Table 1). These parameters were 38.05%, 48.09% and 38,43% respectively higher than control. The effects of biostimulant were not different among types of biostimulant. Variety of Sunren F1 produced significantly higher values of those variables than Tuktuk (Table 1). Better root growth caused by the effects of biostimulant enhanced the absorbtion of soil nutrients and moisture surrounding roots and finally increased plant growth. As it well known biostimulant is defined as formula that contains living microorganisms which have potential to colonize plant roots and enhance plant growth due to increasing the availability and the acquisition of soil nutrients (Packialakshmi and Aliya, 2014). Biostimulant or Plant growth promoting rhizobacteria (PGPR) is basic components of biofertilzer. Strains of PGPR such as *Burkkholderia*, *Azospirillium*, *Enterobacter*, *Azotobacter*, *Erwinia*, *Rhizobium* and *Flavobacterium* have proved that function (Rodriguez and Fraga, 1999). The biostimulants increased the availability of soil nutrients (N, P, Zn and Fe) as well as production of phytohormones which were categorized as phytosimulators) (Naveed *et al*., 2008). Representatives of *Bacillus* and *Pseudomonas* were reported as bio-inoculants with high potential on cereals (Talic *et al*., 2006).Better plant growth, due to biostimulant application resulted in increased leaf number and leaf fresh weights. Enhancement of leaf growth and other shoot parts contributed to increasing photosynthates and finally enhancing the growth of shallot bulbs compared to without biostimulants. Variety of Sunren F1 may be genetically better than Tuktuk so its growth and yield performance were higher (Table 1).

3.5 Bulb Diameter

Biostimulants significantly ($P<0.05$) increased diameter of shallot bulbs. Bulb diameter was 27.92% bigger due to biostimulant application compared to without biostimulant. Sunren F1 variety had 64.83% bigger bulb diameter compared to Tuktuk (Table 1). Cell division, cell elongation, cell formation, and the formation of new tissue required carbohydrates. Carbohydrate synthesis was heavily influenced by the ability of plants to perform photosynthesis (Husen, 2007), although Sumiyati *et al*. (2016) did not find any differences in bulb diameter of shallots due to PGPR application.

IV. CONCLUSION

Biostimulants significantly ($P<0.05$) increased germination percentage, root length, leaf and bulb fresh weights and bulb number of TSS per pot. Biostimulants increased root length, bulb fresh weights, bulb number, leaf chlorophyll content and bulb diameter. by 27.65%, 45.09%, 38.43%, 55.33% and 27.92% respectively. There was no different effect between types of biostimulant. Variety Sunren F1 performed higher values than Tuktuk in all parameters. There was significant interaction effect between types of biostimulant and variety only on leaf chlorophyll content.

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Table 1. Effects of biostimulants and variety on percentage of seed germination, root length of seedlings, leaf FW pot^{-1},bulb number pot^{-1}, bulb diameter, and bulb FW pot^{-1}.

Treatments	Percentage of seed germination (%)	Root length of seedlings (cm)	Leaf FW (g pot^{-1})	Bulb number (bulbs pot^{-1})	Bulb diameter (mm)	Bulb FW (g pot^{-1})
Biostimulan						
A(Control)	42,67^{b}	4,07^{b}	14.0^{b}	2.3^{b}	16.11^{b}	16.6^{b}
B	71,00^{a}	6,05^{a}	21.5^{a}	4.0^{a}	24.41^{a}	31.6^{a}
C	73,33^{a}	5,38^{a}	21.5^{a}	3.7^{a}	20.74^{a}	28.2^{a}
D	74,00^{a}	5,45^{a}	24.8^{a}	3.5^{ab}	21.91^{a}	30.9^{a}
LSD 5%	14,931	0,844	7.170	1.208	4.477	8.850
Variety						
Sunren F1	76,50^{a}	7,79^{a}	27.85^{a}	5.0^{a}	30.76a	40.6^{a}
Tuktuk	54,00^{b}	2,77^{b}	13.05^{b}	1.7^{b}	10.82b	13.0^{b}
LSD 5%	10,558	0.597	5.070	0.854	3.165	6.258

Notes: Figures followed by the same letters in the same colomn and treatment are not significantly different at 5% LSD.

Table 2. Interaction Eefects of Biostimulants and Variety on Leaf Chlorophyll Content.(SPAD)

Treatments	**Biostimulants**			
Variety	**A (control)**	**B**	**C**	**D**
Sunren F1	22.2 g	52.3 b	43.1 d	56.0 a
Tuktuk	23.1 fg	27.2 f	34.7 e	47.6 c

Notes: Figures followed by the same letter (s) are not significantly different at 5% Duncan Multiple Range Test.

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Meat Quality of Japanese Quail (*Coutonix Coutonix Japanica)* Fed Graded Levels of Fermented Mango Kernel Meal

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Abstract— *This study is aimed at evaluating the effect of replacing maize with Fermented Mango Kernel Seed Meal(FMKSM) on meat quality of Japanese Quails . A total of two hundred and twenty five (225) one week old unsexed Japanese quails purchased from National Research Institute Vom, Plateau State, Nigeria were used in a six weeks study. The quails were equally allotted in to five treatment group of 45 birds per treatments. The control diet which contains 0% FMSKM and four other experimental diet in which maize was replaced with FMSKM at 10% , 15%, 20% and 25% levels of inclusion thus; 5 treatments respectively. At the end of the experiments, cooking loss, drip loss, Colour and pH were analyzed. From the results, cooking loss, drip loss, Colour and pH showed no significant differences (p>0.05) across treatments. Only water holding capacity at 25% inclusion of FMSKM showed a significant difference (p<0.05) . The result showed that the ater holding capacity remained constant through treatment 1-4 (100%),it then decreased at treatment 5. It was concluded that 20% inclusion of FMSKM is recommended in the feeding of meat type Japanese quail without having any effect on these meat quality parameters.*

Keywords— ***Japanese Quail, Coutonix Coutonix Japanica, Mango Kernel Meal.***

I. INTRODUCTION

Quail rearing for meat and egg production is becoming an economically viable activity in some places of the world and has increasingly developed. From the technical and economic viewpoints, quail rearing is attractive due to their rapid growth and early onset of lay, high reproduction rates, and low feed intake (Murakami & Ariki, 1998; Albino & Barreto, 2003).

Physical properties and eating quality of meat are affected by cooking temperature and time. During cooking, the distinctive meat proteins are denatured and this reasons structural changes in the meat textural profile. These resulted in destruction of cell membranes, shrinkage of meat fibres, the aggregation and gel formation of myofibrillar and sarcoplasmic proteins, and shrinkage and solubilization of the connective tissue Tornberg E. (2005). Heat treatment can result to undesirable meat quality changes, such as nutritive value loss because of lipid oxidation and changes in a few segments of the protein fraction (Sa-adchom *et al*., 2011).

The most important poultry meat quality attributes are appearance and texture because they most influence consumers' initial selection and ultimate satisfaction with products. Moreover, one of the major contributing components of appearance is color (Fletcher *et al*., 2000). The author, taking into account the results of much research, concluded that the major factors affecting poultry meat color were heme pigment content of meat and also preslaughter, postslaughter, and slaughter factors. The best way to determine the texture of cooked meat is through the sensory analysis of experienced panelists, which is an expensive and demanding method. Warner-Bratzler (WB) shear test for toughness of cooked meat is the most widely used and primary method (Cavitt *et al*., 2005; Lee *et al*., 2008).

Meat quality is significantly affected by preslaughter factors. Atmospheric conditions in the pre-slaughter period, and especially those causing an additional stress for animals can be important. Seasonal changes in temperature can affect the level of glycogen in muscles after slaughter and the ultimate pH, and consequently the quality of meat. An increase in glycolysis results from excessive excitement, starving and stress caused by ambient temperature, which in turn leads to high post-mortem pH values and consequently meat colour is influenced (Kreikemeier *et al*., 1998; Abril *et al*., 2001; Honkavaara *et al*., 2003). The problem of seasonal changes in meat quality caused by temperature stress was studied by many authors (Fabiansson *et al*., 1984; Jones and Tong, 1989; Mitlöhner *et al.*, 2002; Kadim *et al.*, 2004).

Quail production for meat is becoming a viable activity in some places in the world therefore this study is done to ascertain the quality of quail meat fed graded level of fermented mango kernel meal.

Objectives of study

To determine the effect of graded levels of fermented mango seed kernel on the meat quality of Japanese quail.

Justification

This study is done to improve the meat quality of Japanese quail using grade levels of fermented mango seed kernel since the protein content of the fermented mango seed kernel is more than that in maize.

Objectives

The study is done to reduce the production cost in poultry farming and yet achieve a good meat quality since the energy and protein level of fermented mango kernel seed can be compared to that maize.

II. MATERIALS AND METHOD

Physical Parameters Measured

Physical analysis of the meat quality was done using 3 birds randomly picked from each treatment (1-5) to measure and study some qualities of their meat using the following parameters;

- Colour
- Ph
- Water holding capacity
- Driploss
- Cooking loss

PROCEDURES

1. Colour

A chunk of lean meat was cut from the slaughtered birds of the various treatments with knife and brought close to the CIE LAB colour chat. The reading wastakenbase on the similarity of the colour of the meat to that on the CIELAB colour chat following the model of the commission Iternationionale d'Eclairge L*a*b* system was used, where L* is the lightness of the meat, a* the redness and b* the yellowness (Swatland, 1985; Monnin, 1998). This was done at slaughter (0 hour), 4 hours, and 24 hours.

2. pH measurement

After slaughter, breast fillets were directly measured using pH meter with 0.01 precision (Sentro, model 1001).

3. **Water holding capacity**

Water holding capacity was evaluated using a method adopted from Hamm (1960), based on meat water loss when pressure is applied on the muscle. Meat cubes weighing 2g were cut and laid between two filter papers circles. The weight of 10kg was placed on it for 5minutes and afterwards, it was re-weighed. Water holding capacity was calculated as initial weight minus final weight and expressed as percentage. Statistically

(Initial weight - final weight)/1 x 100

4. Drip Loss

To determine the drip loss, breast fillet samples of 10g was remove from the carcass of the various treatments and stored in plastic trays covered with water proof foil paper and refrigerated for 24hours. After this period, exudates were discarded and the samples were weighed following the procedures of Northcut *et al.*, (1994) and Dirik *et al.*, (1996). Drip loss was calculated as initial weight minus final weight and expressed as percentage. Statistically;

(Initial weight - final weight)/1 X 100

5. Cooking loss

Samples were weighed and put in plastic bags and cooked in boiling water of about 82 - 85^{o}C for 10minutes and allowed to cool on absorbent paper at room temperature. Samples were re-weighed following the procedures of Honikel, (1989). Cooking loss was calculated as;

Initial weight - final weight.

III. RESULTS AND DISCUSSION

Table.1: Colour Measurement of Japanese Quail Meat fed Graded Levels of Fermented Mango Seed Kernel meal

TREATMENTS	0 Hour (at slaughter)	4 Hours	24 Hours
T1	+b*	+b*	+b*
T2	+a*	+a*	+a*
T3	+b*	+b*	+b*
T4	+b*	+b*	+b*
T5	+b	+b*	+b*

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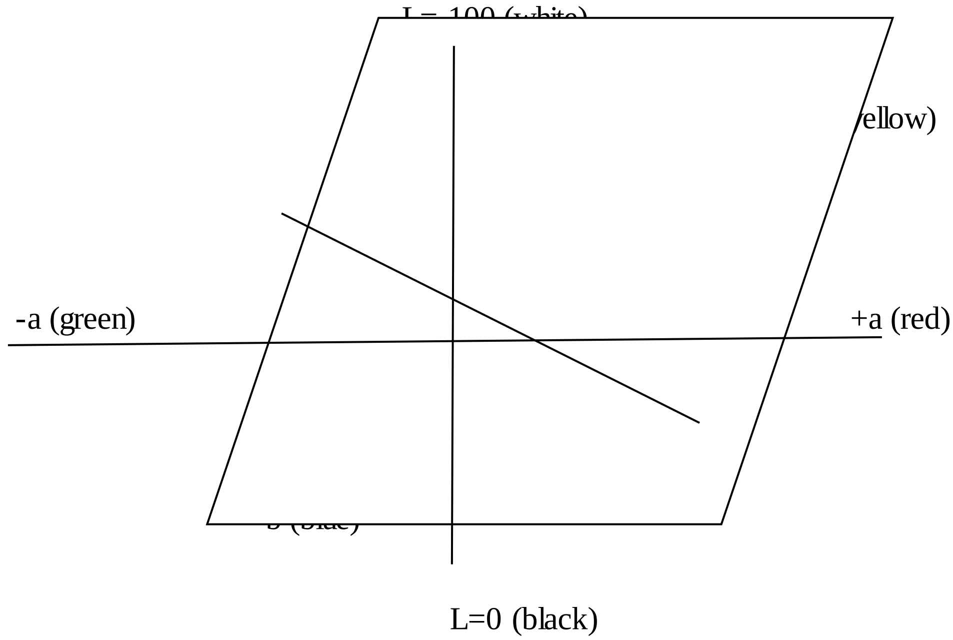

Diagram 1: Colour scale for determining meat colour

The colour of muscles was determined on post slaughter 0, 4, 24, on each muscle. The determination was done on the longitudinal out surface of each muscle. The CIELAB colour system was used considering l^{*}, a*, and b^{*} calorimetric coordinates as follows:

1. **L^{*} a value 100 corresponded to absolute white value – Absolute black**
2. **$a^{*}a^{+}$ corresponded to red spectrum.**
   a^{-} corresponded to green spectrum.
3. **b^{*}- b^{+} corresponded o yellow spectrum.**
   b^{-}Corresponded to blue spectrum.

Table 1 shows the result of evaluated meat parameter including lightness (L^{*}), redness (a^{*}) and yellowness (b^{*}). For treatment 1, 3, 4 and 5, the colour (+b^{*}) was constant through 0 hour, 4 hours and 24 hours. Whereas, treatment 2 shows a different shade of colour (+a^{*}) from the other treatments through the time of slaughter (0 hour), 4 hours and 24 hours.

Table.2: pH measurement of Japanese quail Meat fed graded levels of Mango Seed Kernel

Parameters (HOUR)	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**	**SEM**
24 hour (At Slaughter)	3.10	3.07	2.67	2.87	2.65	0.10
0 Hours	4.87	4.93	4.17	4.17	4.67	0.22
4 Hours	3.50	4.00	4.37	4.77	4.00	0.33

Table 2 shows the pH measurement for the various treatments from the time of slaughter, 4 hours and 24 hours. pH at 0 hour ranges from 2.65 to 3.10 with the highest in treatment 5. At 4 hours of slaughter the pH ranges from 4.67 – 4.87 while those in 24 hours ranges from 3.50 to 4.77 with the highest in treatment 4.

Table.3: Effect of feeding graded levels of fermented mango kernel seed on water holding capacity (WHC) and cooking loss of Japanese quail meat.

PARAMETERS	**Trt 1**	**Trt 2**	**Trt 3**	**Trt 4**	**Trt 5**	**SEM**
WHC	100	100	100	100	66.67	6.67
Cooking Loss	6.00	6.00	4.67	7.00	7.00	0.78
Drip Loss	33.33	66.67	100.00	66.67	100.00	18.17

Trt = treatment, WHC = water holding capacity

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IV. DISCUSSION

From this study, the meat colour of all the treatment (1-5) remained unchanged even during the process of transformation of muscle into meat. At slaughter, treatment 1,3,4 and 5 showed a yellowish colour (+b*) this might be as a result of the environmental temperature when the study was carried out (at midday). It is known that detoriation sets in faster at higher temperatures than at lower temperatures which increases the pH therefore altering the colour from reddish (+a*) to yellowish (+b*). This result is within the range observed by Owens *et al.*(2000), Woelfel*et al.*(2002), Woelfel and Sams (2001), Barbut (1998), and Vimini (1996), whom observed 2 to 50% yellowish colour in chicken breast fillets, depending on environmental conditions. Treatment 2 shows redness,this may be due to the 5% inclusion of the Fermented Mango Seed Kernel in the fed of the birds. However, the incidence observed in this present experiment is lower than that found by Lara (2003), who evaluate PSE percentage in the meat of broilers submitted to heat stress before slaughter and obtained incidences of 35.30% in non-stressed and 37.08% in heat-stressed birds.Some authors reported a significant effect of environmental temperature on meat colour (Owens *et al.*, 2000; Guarnieri*et al.*, 2002).

A pH of 4.93 at slaughter was observed and falls between the range of 5-5.9 recorded by several researchers Oguz*et al.*(2009); Gevrekci*et al.*(2009). Some authors (Genchev *et al.*, (2008)(2010) reported the value of 6.17 and 6.00 at slaughter which is higher than the 4.87 reported in this study. The difference might be due to different sanitary measures during slaughter which might increase the microbial load leading to a higher pH. The lower pH might indicate a higher microbial load in the meat samples after 4 hours of slaughter. It is an established fact that at room temperature, microbial load multiply as the keeping time is been lengthened which leads to a higher pH. So also karakaya *et al.* (2005) reported the pH of 5.59 at slaughter which is not far from the value obtained in this study. In a study carried out by Singh and Verma 1995, they recorded the pH of 5.8 to 5.9 at slaughter and a rapid decrease 2 hours after slaughter due to high microbial load at room temperature.

The water holding capacity as presented in Table 3, showed no significant difference (P>0.05) across the treatments. This stands to reason that quail meat has high water holding capacity regardless of it been fed the normal ration in treatment 1 which is the control nor been fed the experimental diet. From this study, it was observed that the water holding capacity remain constant (100%) in treatment 1-4 and decreased in treatment 5 (25% inclusion of experiment diet). This implies that the inclusion of fermented mango kernel up to 25% may affect the water holding capacity of the meat. Bower *et al.*, (2014) in his study gave a range of 74 – 92% which falls between the range of 66.67 – 100% obtained in this study. A lower value to that obtained in this study was recorded by Woelfel *et al.*, (2002) who found 47% of pale fillets out of a total of 3,554 fillets evaluated in commercial processing plant that could be potentially classified as PSE, and presented low water holding capacity. In line with this study, Garcia *et al.*, (2010) recorded and average value of 64.79 in the water holding capacity of normal fillets of chicken meat. This study is also consistent with the studyof Weeranantanaphan *et al.*, (2009) who stated that water holding capacity cannot be predicted in fresh meat to the same degree of accuracy as the chemical composition of the meat.

The cooking loss was observed to have increase at treatment 4 (20% inclusion of test ingredient) and treatment 5 (25% inclusion of test ingredient). This study achieve a similar cooking loss value (14.6%) in broiler chicken reported by Le Bihn-Duval *et al.*, (2008). Similarly, Garcial *et al.*, (2010) recorded 19.45 cooking loss value in chicken meat in a commercial processing plant. Dogan *et al.*, (2013), reported a cooking loss of 24% which is higher than that obtained from this study, the disparity can be attributed to difference in time, temperature, ultimate pH and the type of muscle cooked.

Drip loss in this experiment shows no significant difference (P>0.05) across the treatment, it increases across the treatments having its least value in treatment 1 which is the control. Treatment 3 and 5 having 15% and 25% inclusion of the experimental ingredient shows a higher drip loss of 100%, this may imply that 15% and 25% inclusion of fermented mango kernel seed is not advisable. This is consistent with the study of Kauffman *et al.*, (1992) who reported that an unacceptably high moisture loss from fresh product as purge or drip has been estimated to occur in as much as 50% of the pork produced. In contrary to this study, Garcia *et al.*, (2010) observed a little drip loss of 1.37 in chicken meat in a commercial processing plant.

V. CONCLUSION

The result obtained in this study shows that up to 20% fermented mango seed kernel can be included in the diet of quail birds without deleterious effect on their cooking loss, drip loss, colour and pH which are important factors in measuring meat quality.

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Re-Engineering on the Production of Surrogate Feeds for Broiler Chickens (Gallus–Gallus Domesticus): its Effects on Broilers' Live and Carcass Weights and Consumption Cost

Gener S. Subia, Jennilyn C. Mina, Rowell A. Diaz, Romeo B. Campos, Jr. and Gerald Quijano

Abstract— *This experimental study focused on the production of alternative feeds for broilers chickens using golden apple snail shell as the main ingredient. Three groups were compared and the characteristics of them are as follows: The commercial group was given pure commercial feeds, treatment one group (T_1) was given 25% pomaceacanaliculata's shell that was mixed with 37.5% of rice bran and 37.5% of corn, and the third group which was treatment (T_2) was given 50% pomaceacanaliculata's shell that was mixed with 25% of rice bran and 25% of corn as feed mix.The study found out that golden apple snail shell can be mixed with feeds for broiler production without negative effect on the carcass recovery. It can replace commercial feeds as food for broilers especially if the farm area or area near it were infested by golden apple snail. Although the consumption cost among the treatments and the commercial feed do not differ, it is practical to use T_2 as a substitute for commercial feeds since it is less than P3.00 to P5.00 per consumption cost.*

The findings of this study have valuable financial implications, particularly to the farmers and poultry owners.
Keywords— **Broiler chickens, consumption cost, feeds, re-engineering.**

I. INTRODUCTION

Rice is life, for most people living in Asia and has shaped the cultures, diets, and economies of thousands of millions of people [1]. One of the countries in Asia that rice is their most significant food crop in the Philippines. It is the 9th largest rice producer in the world, accounting for 2.8% of global rice production [2].

However, because of pests and insects, rice produces low income for many farmers in the country. In the study of [3], their respondent farmers rated pesticide application was perceived to be effective (73%) but not efficient in controlling rice insects. Moreover, farmers recognized the negative effects of pesticide applications in the environment (76%). Thus, efficient, safe, low-cost pest control strategies are needed to reduce the reliance of farmers to pesticides and to improve agricultural production and food security of smallholder farmers in the Philippines[3], thus, increasing their production and income.

One of the rice pests that needed to be controlled efficiently and safely is the "Golden Apple Snail" also known as "Golden Kuhol". The golden apple snail, (Pomacea Canaliculata) (Mesogastropoda Palidae) has been introduced to several Asian countries where it has unexpectedly developed into a pest of rice [4].According to several researchers as cited by [5], this species has invaded several European, American, and Asian countries and damages rice and aquatic organisms.

Maria Lizbeth Severa J. Baro of the Bureau of Agricultural Research (BAR), stated that "golden kuhol may be considered a threat in rice production, but many farmers are (again) looking at the golden kuhol at a different perspective" [6]. Since golden kuhol is nutritious and easy to digest and snail meat provides protein and energy-giving fat while the shell contains calcium, phosphorous, vitamins, and minerals, farmers used it as supplementary feed for their livestock and Chickens [6].

The chicken (Gallus gallusdomesticus), is a type of domesticated fowl, a subspecies of the red jungle fowl that is one of the most common and more in any other bird and in any domestic animals, with a total population of more than 19 billion as of 2011[7]. Since the number of chicken increases significantly, the need to feed them with food that could stimulate their fast growth and reproduction is necessary.

According to [8], "one of the common issues with regard to backyard flocks relates to poor or inadequate feeding programs that can lead to vitamin and mineral deficiencies for the birds. Vitamins and minerals are very important components of a chicken's diet and unless a formulated ration is feed, it is likely that deficiencies will occur."

As researchers and teacherswho have the knowledge and the sense of purpose that allows them to rise above casual or conventional approaches and to dothings others cannot [9] as cited in [10]and persons who are engaged in business enterprise particularly in farming and livestock production, the researchers borrowed the idea of using golden kuhol shellas an alternative feeds for chicken, especially broiler chickens. This study would help not only them but the farmers in their province to reduce the said pest in their farm and turn it into resources. This could also providethe researchers' additional savings since they will be using free alternative feeds for their broilers instead of buying commercial feeds.

The context of the problem gave the idea to the researchers to conduct a study onthe production of surrogate feeds for Broiler Chickens (Gallus–Gallus Domesticus) and looked on its effects on broilers' live and carcass weights and consumption cost in Barangay, San Roque, San Isidro, Nueva Ecija.

II. METHODOLOGY

This study utilized an experimental research design.The commercial group was given pure commercial feeds, treatment one (T_1) was given 25% *pomaceacanaliculata's* shell that was mixed with 37.5% of rice bran and 37.5% of corn, and the third group which was treatment (T_2) was given 50% *pomaceacanaliculata's* shell that was mixed with 25% of rice bran and 25% of corn as feed mix. There were two sampling procedures used in the broiler chickens. The first procedure was the complete enumeration sampling, a type of purposive sampling technique where all the live weight broiler chicken got their weight and measures. The second procedure is random sampling. The random sampling procedure was used in the carcass recovery of the broiler chicken. The researchers used Microsoft Excel and statistical package for social sciences (SPSS) in analyzing the gathered numerical data. The study was conducted from January 2018 to July 2018.

III. RESULTS AND DISCUSSION

1. Live Weight of Broiler Chicken

Table 1.1.Live Weight of Broiler Chicken

LIVE WEIGHT in Grams *(g)*			
	Treatment 1	***Treatment 2***	***Control Group***
Week 0	77.21	77.21	77.21
Week 1	153.85	168.85	154.75
Week 2	242.55	270.05	232.75
Week 3	587.55	625.05	680.05
Week 4	1169.05	1169.05	1227.05
Week 5	1419.05	1519.05	1553.05
Week 6*	1600.05	1669.05	1713.05
Sum	***5,172.10***	***5,421.10***	***5,560.70***
Average	***862.02***	***903.52***	***926.78***
Variance	***381,963.95***	***410,797.87***	***447,836.07***

** last five (5) days of observation to complete 40 days.*

Table 1.1 presents the sum, average, and variance of live weight of the broiler chicken from week 1 to week 6 after subjected to the feeding ofthe three different treatments. The weekly sum, average, and variance of the Treatment 1 ***(T1)***were 5,172.10; 862.02; and 381,963.95 respectively. For Treatment 2 ***(T2),*** the weekly total sum in grams was 5,421.10; 903.52 for the average; and 410,797.87 for a variance. For control (commercial) group the weekly total sum in grams was 5,560.70; 926.78 for the average; and 447,836.07 for variance.

The data revealed that the control group fed with commercialized feeds was heavier than other groups of chickens in terms of their live weight. Further testing to show if the commercial feeds are superior to the other treatments as to its effects on the live weight of the broilers chickens, Table 1.2 shows that it is not.

Table 1.2.Comparison of the Three Treatments as to their Effect to the Live Weights of Broiler Chickens

Source of Variation	*SS*	*Df*	*MS*	*F*	*p–value*	*Decision*
Between Groups	12916.62	2	6458.31	0.01562	0.9845	Ho Accepted
Within Groups	6202989.42	15	413532.63			
Total	6215906.04	17				

There is no significant difference on the live weight of the broilers that were subjected to T_1 (25% *pomaceacanaliculata's* shell that was mixed with 37.5% of rice bran and 37.5% of corn), T_2(50% *pomaceacanaliculata's* shell that was mixed with 25% of rice bran and 25% of corn as feed mix)and the control group (which were fed with commercial feeds), respectively. The computed p–value of 0.9845 using Analysis of Variance (ANOVA)was greater than 5% level of significance which means the Ho (Null hypothesis) is accepted implying that there is no significant difference in the live weights of the broiler chickens subjected in the three different treatments.

This result suggests that the broiler chickens have similar live weights regardless if they have eatenT_1, T_2orcommercial feeds.This implies that the commercial feeds can be replaced by the two treatments in feeding broiler chickens if the commercial feeds are not available.

2. Dressed (Carcass) Weight of Broiler Chickens

Table 2.1.Dressed (Carcass) Weight of Broiler Chicken

Dressed (Carcass) in Grams *(g)*			
Replication	***Treatment 1***	***Treatment 2***	***Control Group***
I	1,250	1,350	1,400
II	1,200	1,350	1,250
III	1,250	1,250	1,350
Sum	***3,700***	***3,950***	***4,000***
Average	***1,233.33***	***1,316.67***	***1,333.33***
Variance	833.33	3,333.33	5,833.33
** last five (5) days of observation to complete 40 days.*			

Table 2.1 presents the dressed (carcass) weight of the broiler chickens classified into varying levels of Treatments. The data divulged that the control group fed with commercialized feeds was heavier in their weight in grams than the other groups. The sum of carcass weight, average, and variance of the Treatment 1 ***(T1)*** were3,700; 1,233.33; and 833.33 respectively. For Treatment 2 ***(T2),*** the sum in carcass weight for each replication was 3,950; 1,316.67 for the average; and 3,333.33 for a variance. Lastly, for the control (commercial) group the sum of each replication for carcass weight in grams was 4,000; 1,333.33 for the average; and 5,833.33 for variance. Testing if the difference was significant, Table 2.2 shows the result.

Table 2.2.Comparison of the Three Treatments as to Dressed (Carcass) Weight of Broiler Chickens

Source of Variation	*SS*	*Df*	*MS*	*F*	*P–value*	*Decision*
Between Groups	17222.22	2	8611.11	2.5833	0.1551	Ho Rejected
Within Groups	20000	6	3333.33			
Total	37222.22	8				

The computed p–value of 0.1551 using Analysis of Variance (ANOVA)was greater than 5% level of significance which means that the Ho is accepted implying that there is no significant difference in the dressed (carcass) weights of the broiler chickens exposed in the three different treatments.

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The data suggests that the broiler chickens have the same dressed (carcass) weights irrespective if they have eaten25% *pomaceacanaliculata's* shell that was mixed with 37.5% of rice bran and 37.5% of corn,50% *pomaceacanaliculata's* shell that was mixed with 25% of rice bran and 25% of cornor which were fed with commercial feeds.

This means that the two treatments can substitute the commercial feeds especially if golden apple snails are prominent in the area.

3. Feed consumption weight

Table 3.1.Feed Consumption Weight of Broiler Chicken

Feed Consumption Weight in Grams (g)			
	Treatment 1	***Treatment 2***	***Control Group***
Week 1	1,436.50	1,436.50	2,693.27
Week 2	1,915.33	1,915.33	3,591.02
Week 3	2,872.98	2,872.98	5,386.52
Week 4	4,309.47	4,309.47	8,079.77
Week 5	5,027.71	5,027.71	9,426.40
Week 6*	3,589.86	3,589.86	6,733.15
Sum	**19,151.85**	**19,151.85**	**35,910.13**
Average	***3,191.98***	***3,191.98***	***5,985.02***
Variance	1452083.897	1452083.897	5104382.062
** last five (5) days of observation to complete 40 days.*			

Table 3.1 presents the feed consumption weight of broiler chickens classified into varying levels of Treatments.The weekly sum feed consumed weight, average and variance of the Treatment 1 ***(T1)*** with 25% level of shell were 19,151.85; 3,191.98 and 1,452,083.897 respectively. For Treatment 2 ***(T2),*** the weekly total sum feed consumed in grams was 19,151.85; 3,191.98 for the average; and 1,452,083.897 for a variance. For Control (commercial) group the weekly total sum of feed consumed weight in grams were35,910.13; 5,985.02 for the average; and 5,104,382.062 for variance.

The data presented revealed that the control group fed with commercialized feeds consumed more weightin grams of feeds. When compared to see if the difference of feeds consumed is significant, Table 3.2 shows the result.

Table 3.2.Comparison of the Three Treatments as to Feed Consumption Weight

Source of Variation	*SS*	*df*	*MS*	*F*	*P–value*	*F crit*
Between Groups	14078692.85	2	7039346.43	2.64	0.1507	Ho Accepted
Within Groups	16017099.71	6	2669516.62			
Total	30095792.56	8				

Table 3.2 shows the significant difference on the feed consumption weight of broiler chickenssubjected to the three treatments. The p–value of 0.1507is greater than 0.05 which means that the Ho is accepted. This means that there is no significant difference in the feed consumption weight regardless of the treatments.

4. Cost Analysis

Table 4.1 shows the cost analysis ofT_1 (25% *pomaceacanaliculata's* shell that was mixed with 37.5% of rice bran and 37.5% of corn), T_2(50% *pomaceacanaliculata's* shell that was mixed with 25% of rice bran and 25% of corn as feed mix)and the control group (which were fed with commercial feeds), respectively.

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T_2shows that the cost in every broiler chicken is much lesser than T_1 and in the control group.

Table 4.1.Cost Analysis of the Three Treatments

Variable Cost in Peso	Treatment 1	Treatment 2	Control Group
eeds	63.74	49.26	71.84
Power	8.08	8.08	8.08
Vitamins/Medical	16.20	16.02	28.02
Watering	5.31	5.31	5.31
Labor	14.32	10.88	14.34
Chicken	30.02	30.02	30.02
Other Cost	12.46	11.97	15.77
Total cost per broiler chicken	150.13	131.54	173.38
Average cost per treatment	21.40	18.77	24.75

Using ANOVA (Table 4.1) to determine if the cost per treatment differs with each other, the result revealed that there is no significant difference among the three groups since the computed p–value of 0.8527 was less than 0.05. This means that the Ho is accepted implying that there is no significant difference in the cost of feed per consumption of each treatment.

Table 4.2. Comparison of the Cost Analysis of the Three Treatments

Source of Variation	*SS*	*df*	*MS*	*F*	*P–value*	*F crit*
Between Groups	125.56	2	62.78	0.1608	0.8527	Ho Accepted
Within Groups	7028.81	18	390.49			
Total	7154.367981	20				

IV. CONCLUSIONS AND RECOMMENDATIONS

Golden apple snail shell can be mixed with feeds for broiler production without negative effect on its carcass recovery. It is one of the easy and efficient ways [11] of developing surrogate feeds. They can replace commercial feeds as food for broilers especially if the farm area or area near it were infested by golden apple snail. Although the consumption cost among the treatments and the commercial feed do not differ, it is practical to use T_2 as a substitute for commercial feeds since it is less than P3.00 to P5.00 per consumption cost.

Based on the findings and conclusions, the researchers offered the following: Golden apple snail shell with the range 0 to 50% can be mixed to the feeds for broiler for a better carcass recovery and consumption cost. Also, it can be a source of income of farmers for the reason that it can be converted into nutritious feeds for broilers. Likewise, it can lessen the expenses of the poultry raisers because the price of commercial feeds is slightly higher than the alternative feeds that are made from the shell of golden apple snail.Lastly, the project feasibility study is recommended to identify the potential market of the proposed alternative feeds and for further verification of the result of this study.

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Main Socioenvironmental Impacts of Mining in the Caatinga Landscape in Northern Bahia/Brazil

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Abstract ***— Mining is one of the basic sectors of Brazilian economy and it has contributed to the development of cities and small villages, since it is operated with social and environmental responsibility, based on the precepts of sustainable development. Much has been questioned about the social and environmental responsibility of mineral activity, raising questions about the impacts caused by the implementation of these projects. The present article aims to present the social and environmental reality of Quixaba village, in the county of Sento Sé, in the north of Bahia, with the voluntary exploration of an amethyst deposit, as well as to point out the main environmental impacts based on Environmental Law. It should be noted that this deposit has not been identified until then within the Brazilian mineralogical heritage, having been recently (re) discovered by people from the region. However, it is in the heart of semi-arid region, in a protected area called Boqueirão da Onça National Park, dominated by massifs and mountain ranges (more than 1,200 meters) in the center and north, and by plains to the south. This research is essentially based on the Geosystemic Theory, the Ecodynamic Method and the GTP Theory. Therefore, it is fundamental to develop conservation measures and sustainable management of protected environments, since landscapes are products and records of the geological evolution of the planet. Therefore, it is essential that local and environmental authorities understand the urgency of proper and responsible management of the areas explored by mining, mainly because the region has enormous geological, paleontological, archaeological, faunal, floristic and paleoenvironmental heritage.***

Keywords— Mining; Environmental patrimony; APA; Paleoambientes; Impacts.

I. INTRODUCTION

Due to its continental dimensions and geological diversity, Brazil is a country with a huge mineral vocation and a major producer of basic inputs from mining. The Brazilian mineral production has grown in the last decades, because of significant investments made by mineral exploration companies, together with the efforts made by the federal and state governments in the execution of extensive programs of systematic geological surveys.

Mineral assets are one of the great non-renewable assets of geodiversity, being an important factor in sustainable development and improvement of the of Brazilians quality of life. However, the distribution of mineral resources is a metallogenic function tendency of tcrustal elements that formed the geological provinces of Brazil, being responsible for the great mineral diversity of these resources and their wide geographic distribution.

Based on these initial premises, it is pointed out that the present article aims to discuss the socio-environmental reality at Quixaba village, in a city callede Sento Sé, in the north of Bahia, with the voluntary exploration of an amethyst deposit, pointing out the main environmental impacts based on legislation. It is worth noting that this deposit has not been identified until the present time, in the Brazilian mineralogical map, having been (re) discovered (because it always existed) recently, by gold miners of the region. In order to discuss the highlighted points the law 7.805, from july18, 1989 will be used as basis. This law refers to the mining permit system, in the Code of Mining and Correlated Legislation, and CONAMA Resolution Nº. 237/97 in addition, we will base the discussions of the results on the Geosystemic Theory, on the Ecodynamic Method and on the GTP Theory.

Therefore, regarding to environmental impacts of mining, it should be considered that the practice of organized mining may cause less impacts and can be monitored and supervised by the public authority and environmental agencies. However, if mineral extraction occurs informally, without planning, control and inspection, it will become a source of large environmental liabilities.

This work aims to reflect on clandestine mineral extraction practices, their main socio-environmental impacts and, above all, on the need to legalize land use

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and occupation, in order to minimize the previously irreversible impacts caused by mineral extraction in the thesis area.

II. HISTORIOGRAPHY OF MINERAL RESOURCES IN SENTO SÉ

The county of Sento Sé (figure 1A), founded in 1832, is located in the north of Bahia, on the shores of Sobradinho Lake, in São Francisco river region, with an area of 12,699 km² and a population of 41,102 (2014), away from the capital (Salvador) 689 km. It has a semi-arid climate and is in the semi-arid region of the Brazilian Northeast, sheltering enormous geological, paleontological, archaeological, faunistic, floristic and paleoenvironmental patrimony (Figure 1B).

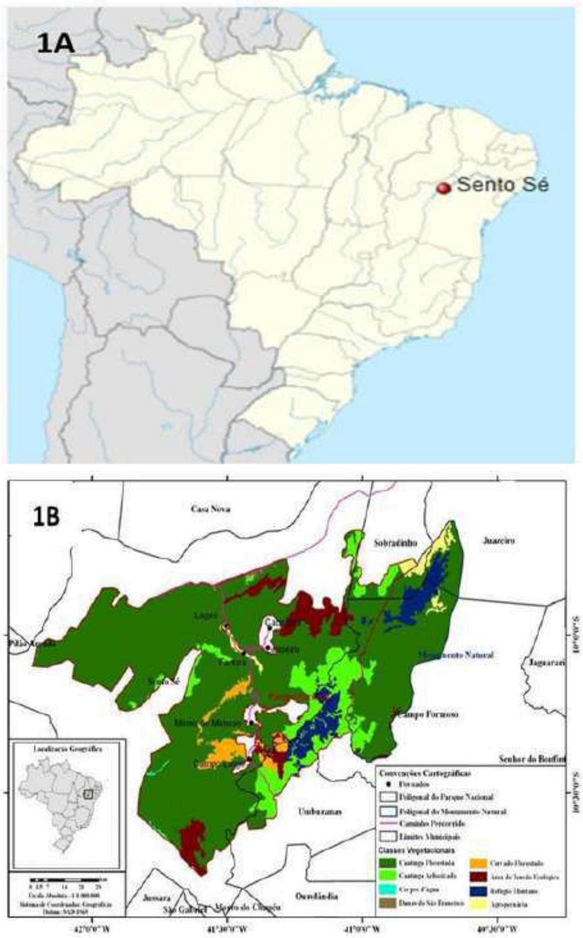

Fig.1A and B - Map of Senso Sé / BA and Natural Heritage Area

Source: KESTERING; ALVES; LIMA FILHO, (2013)

The historiography of Sento Sé reveals a legend, passed down from generation to generation, that a "bohemian" slave used to disappear from the slave quarters in the backward hours of servile labor. He never knew for sure where he was going, but when he returned he always brought nuggets of gold to present his master. It is said that at his death he took with him the secret of the place he had visited and had obtained gold. Apparently, the slave's secret had already been revealed, or at least deciphered, since the region now houses migrants from various parts of Brazil in search of precious stones.

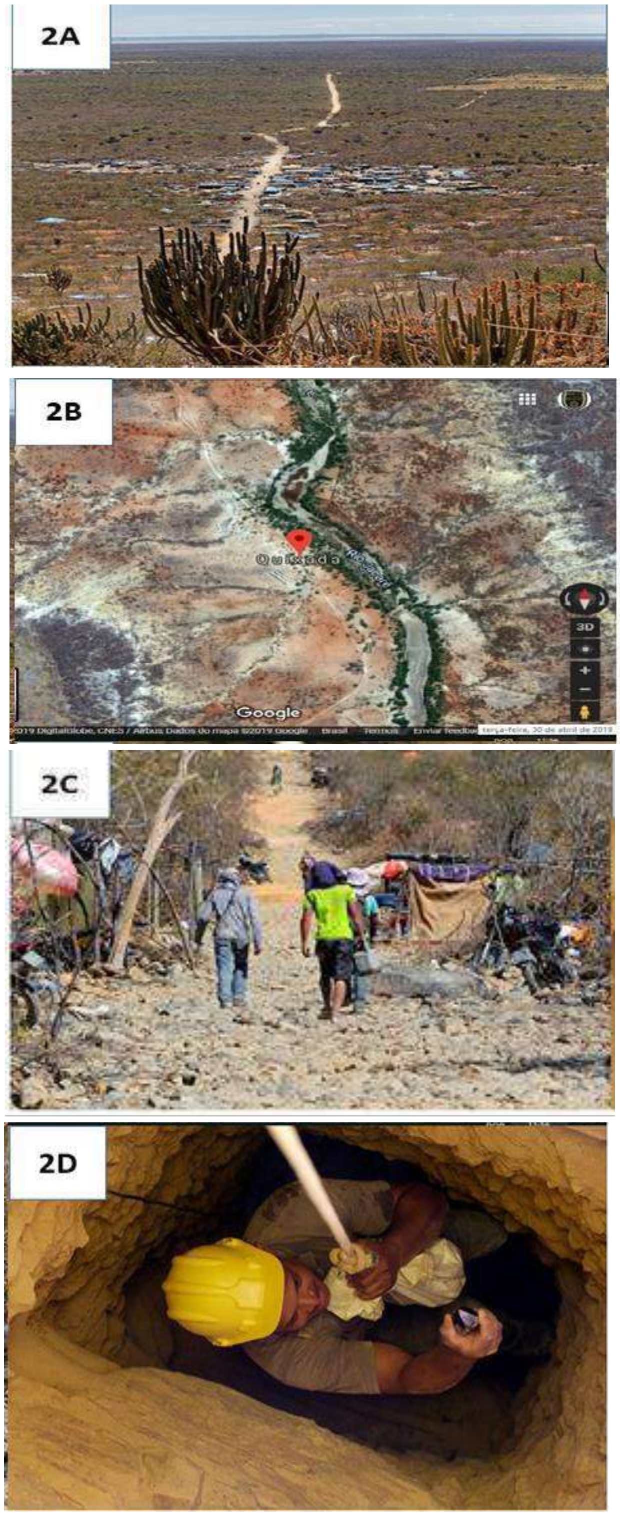

Fig.2 A, B, C and D - Overview of the Garimpo

Source: *Website* A tarde Uol (2018)

The locality of Quixaba, which is 50 km away from the headquarters (Sento Sé), has a modified daily routine and the trade is overpowering with the arrival of the miners.

At the top of Serra da Quixaba (Fig. 2A and B), there is a precious stone mining that was discovered by chance, and already has more than 8,000 people exploring it (Figure 2C). Since gems are in a difficult location (Figure 2D), it contributes even more to the risk of accidents, because miners do not use any Personal Protective Equipment (PPE) and there is not any on-site supervision.

The National Department of Mineral Production (DNPM) has already visited the gold digging and has made the necessary arrangements for its legalization of the mining that is still clandestine today. According to Law n. 7.805/89, Art. 3 "The granting of the mining permit depends on prior environmental licensing granted by the competent environmental agency". Thus, once the Public Power of Sento Sé meets all DNPM requests, the gold miner will need to organize themselves in a cooperative, duly registered and paying the mandatory taxes to Federal Government, since all the wealth that is in the subsoil is a domain of the Union, since "the mining permit shall be granted by the Director General of the National Department of Mineral Production (DNPM), who shall regulate, by means of an ordinance, the respective qualification procedure" (Article 4).

III. MINING IN THE LIGHT OF BRAZILIAN ENVIRONMENTAL LAW

According to Article 14 from Mining Code of 1969, mineral exploration is understood as the execution of the work required to define the mine, its evaluation and determination of feasibility of its economic exploitation (Mining Code and Correlated Legislation in Brazil, mineral resources have legal status, are part of the subsoil, and have a legal regime that is totally different from private land ownership. that mineral goods are of the Union and therefore can only be researched and exploited by companies that means an individualized and specific legal act for each one, obtaining a Mining Title, a Union concession granted by the National Department of Mineral Production (DNPM), on behalf of federal government.

Thus, the Mining Code (1967) removes the landowner's preference in the mineral exploration. In addition, the Brazilian Constitution requires an Environmental License (LA), forcing the company to carry out an Environmental Impact Study (EIA) to be able to operate later. Article 225, item IV, corroborates, reaffirming that it is necessary to require, in the form of the law, the EIA, for the installation of a work or activity potentially causing significant degradation of the environment.

Although there is still no systematic evidence for the entire Brazilian territory, perception of mining in the text on Agenda 21 of the Mineral Sector is not positive:

> Mining is often cited as an aggressive activity of the environment, which cannot contribute to the sustainable development of the regions where it is located. This view portrays the reaction to the destructive use of environmental resources by modern society that degraded and polluted in the name of progress (p. 28).

Unfortunately, natural resources are finite, and after the closure of the mining activity, environmental impacts and soil degradation remain, exposed to open air, without any accountability in the process of remediation and restoration of the degraded area. According to Law 7,805/89 in its Article 11, the DNPM will establish the areas of mining, taking into account the occurrence of mineral good, the interest of the mineral sector and social and environmental reasons. It also establishes that in areas of mining, the work should preferably be carried out in an associative way, through cooperatives (Art. 12), with the areas of garment being conditioned to the Prior License of the competent environmental agency (Art. 13).

IV. MATERIAL AND METHODS

4. 1 Location of the research area

The gold-digging of Serra da Quixaba, in the north of Bahia, is located in Boqueirão da Onça National Park (figure 3), a place that would give full protection to some 900,000 hectares in the heart of the Caatinga. The park in Boqueirão da Onça did not leave the paper, but it is in a region of difficult access, with little valued land, few roads, without paved asphalt and sparsely populated, with three inhabitants per thousand square kilometers.

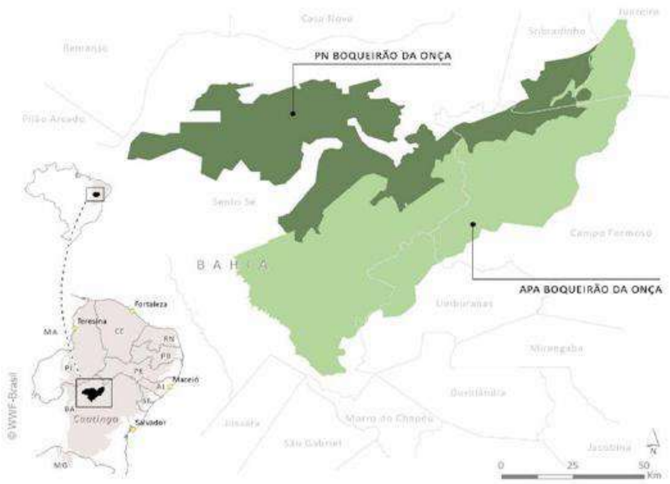

Fig.3: National Park
Source: *World Wide Fund for Nature* (2018)

This region of northeastern sertão does not lack water and it is possible to become a large environmental reserve.

Boqueirão da Onça is a refuge for several animals and preserves species of rare plants, besides having numerous rock formations and an immensity of 800 thousand hectares of native forest. The rare villages that exist are rustic, with pavement and stonewalls, narrow streets and small houses. The residents live on the exploitation of amethysts, which are found in artisanal mining. Many uninhabited lands being an ideal environment to protect the fauna/flora of the region.

The climate is semi-arid, but totally pleasant, due to the altitude. At more than one thousand meters of altitude, it is cold, in a region predominantly hot and dry. Boqueirão da Onça has one of the largest conserved caatinga areas on the planet. It is possible to find endemic species, originated in the region, besides gorges, despite the beauty, inspire solitude. An open-air laboratory where you can study/research. There are isolated points, far from everything, without tourism activities and preferred place of Ibama to release the animals seized in captivity.

4.2 Methods

For the study in thesis, it was sought to base essentially on the following theories/theoreticians:

a) Geosystemic Theory, which, according to conception, aims, a priori, at the integration of natural and anthropic variables, together with the second which merge the resources, uses and problems, which are configured in the synthesis step in homogeneous units, which thus leads to the conclusive stage of application, in which the real quality of the environment is clarified, resulting in a time analysis - integrated space of the interrelationships society and environment in the construction of the landscape;

b) Ecodynamic Method, which states, "an ecodynamic unit is characterized by a certain dynamic of the environment that has more or less imperative repercussions on biocenoses" (p.32). Within the study of the ecodynamicism proposed by Tricart, this one categorizes the ecodynamic units in three, namely: stable means, means intergrades and strongly unstable means;

c) The GTP theory, aiming to re-approximate these three concepts to analyze how a given geographic space works in its entirety. Thus, essentially, it is a matter of understanding the interactions between different constituent elements to analyze the dialectic existing between the landscape, the territory and the geosystem. The visualization of the relationships between landscape elements should lead the researcher to understand the dynamics of the studied area and how to dialogue with the surrounding areas.

In addition to the methodological assumptions described, the work is based on Law No. 7,805, which deals with the mining permit system, in the Mining and Correlated Legislation Code, and in CONAMA Resolutions.

V. RESULTS AND DISCUSSIONS

5.1 The impacts caused by the extraction of informal mining

5.1.1 Socioeconomic impacts

Growth boosts the creation of new ideas, arising from the need to create new products and improve existing ones, emphasizing the standard of living of the population, as well as the economic, and for that to happen, human capital is essential for innovative thinking and for manage the technology with knowledge. However, we should not confuse growth with development. Not always when there is growth, there is development.

The mining of Serra da Quixaba has provoked uncontrolled population growth (figure 4A and 4B) in that district, so that it has made it possible for many to make large profits, and for others, the dream of becoming a millionaire. However, with the precarious conditions at the site (figure 5A and 5B), with improvised daily life, in the middle of the mountain range, with steep climbs and descents, there are many prospectors and people who have moved there, who are perishing with the thermal amplitudes (a heat that reaches up to 38ºC a day, and a cold that can reach 20ºC at night). Besides the lack of hygiene, security and privacy.

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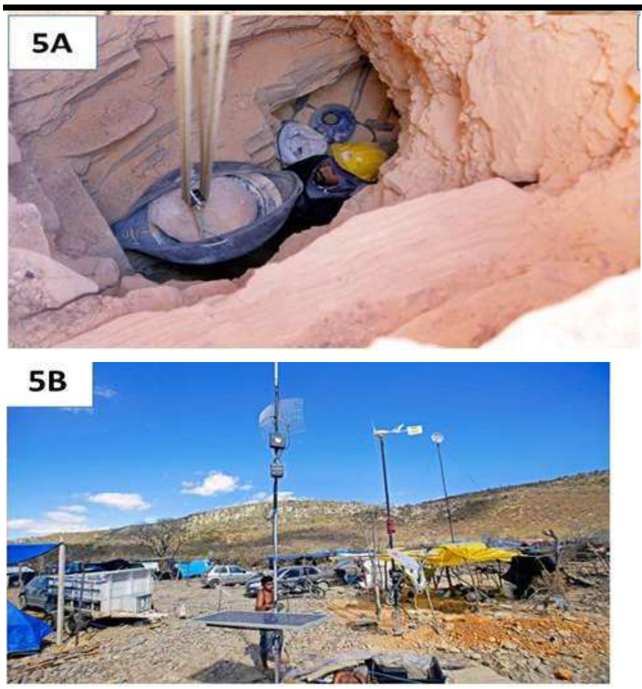

Fig.4 A and 4B; 5A and 5B - Situation in loco
Source: *Website* A tarde Uol (2018)

Those who actually make large profits are the international buyers (Chinese and Indians) who buy the stones and export for greater profits. Beyond them, the crossers, who market the stones with foreigners. In addition, the workers, who act with the manual strength, only acquire timid values with the sales, if compared with the hardness of the handles.

5.1.2 Environmental impacts

The environmental impacts in Serra da Quixaba mining area are uncountable. They range from the devastation of the caatinga biome to deforestation (Figure 6A) and opening of roads to reach the top of the mountain (Figure 6B), to the excavation of the soil in an unregulated way, causing dust, soot, landslides, landslides and even deaths.

Fig.6A and 6B - Situation in loco
Source: *Website* A tarde Uol (2018)

According to Article 1 of Resolution 001 of Conama, environmental impact is constituted:
Any change in physical, chemical and biological properties of the environment caused by any form of matter or energy resulting from human activities that directly or indirectly affect I - the health, safety and well-being of the population; II - social and economic activities; III - the biota; IV - the aesthetic and sanitary conditions of the environment; V - the quality of environmental resources.
Therefore, mining and mineral exploitation are elements generated from incalculable impacts, since fauna and flora of the place suffer serious consequences. In Brazil, the main problems arising from mining can be classified into four categories: water pollution, air pollution, noise pollution, and subsidence. In the studied area, the flora is being degraded to give access to the mine, to be part of the gold-digging (construction of stairs, support grids for entrance in the cut, support for the descent of the workers, forks and supports for the barracks, etc.). Fauna is being unbalanced, by the avoidance of the animals, insects and birds of its habitat, due to the noise and daily movement of cars and people in the place.

5.1.3 Accountability of public authorities and forms of remediation

5.1.3.1 Accountability

According to Decree 97.632, mining enterprises are required to submit the Environmental Impact Assessment (EIA) and the Environmental Impact Report (RIMA), also to submit the Degraded Area Recovery Plan (PRAD) to the approval of the competent state environmental agency. The states and counties have constitutional power to legislate on mining and environment. In addition to these, Federal and State Public Prosecutors also oversee, issue norms and guidelines, most of them conflicting with each other. Following is table (figure 7) of the governmental assignments related to environmental protection and mining planning.

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Distribution of Government Activities in Relation to Environmental Protection and Mineral Exploration

Mining Activity	Municipal Power	State Power	Federal Power
Application for a Grant or License	Laws of Use and Occupation of Soil	Environmental License by Federal Legislation	Deferral or Disallowance
Mineral Search	Laws of Use and Occupation of Soil	Environmental License by Federal Legislation	Follow-up Approval Denial
Mineral Mining	Business license	Analysis of EIA / RIMA and Environmental License by Federal	Legislation Monitoring and Oversight
Mining Recovery	Definition of Future Use of Soil	Environmental License by Federal Legislation	

Fig.7: Synthetic framework of government assignments (Translated)
Source: Adapted from Sintoni (1994)

The impacts caused by mining, combined with competition for land use and occupation, lead to socio-environmental conflicts due to the lack of intervention regulation, which impedes the plurality of interests involved. The elucidation of the conflicts arising from the mining activity, essentially in Permanent Preservation Areas (PPAs) or in Conservation Units (CUs), requires a coordination of the public authorities that act in the locality, in agreement with the civil society and with companies of the branch, so that standards and procedures are implemented with clear and cohesive criteria, valuing the principles of sustainable development.

It is crucial that the areas affected by the implementation of mining be recovered, complying with what is stated in Article 225, § 2 of the Constitution, which imposes on the one who exploits mineral resources the responsibility for recovering the environmental damages caused by the respective activity, obligation to recover the degraded environment, in accordance with the technical solution required by the competent public agency, in the form of a law.

On the basis of this premise, there is the question: in Serra da Quixaba, there is not yet an authorization to operate the mining. Will not the garimpeiros leave the region even before the legalization of operation, since from this, several taxes will be charged? It is a function of the DNPM, and especially of the Municipal Government, to monitor all processes, from EIA/RIMA, PRAD and Prior License, and Operation, in order to avoid abandonment of the mine by garimpeiros, without any environmental responsibility.

5.1.3.2 Remediation

Because of its continental dimensions and diversified geology, Brazil is a country with a huge mineral vocation and a major producer of basic inputs from mining. Currently, it figures in the international scene alongside countries with a traditional mining vocation, such as Canada, Australia, South Africa and the United States. Figure 8A shows the map of mining activity in Brazil, where the distribution of these by almost all geoeconomic regions is observed, and Figure 8B shows the mining areas within protected areas.

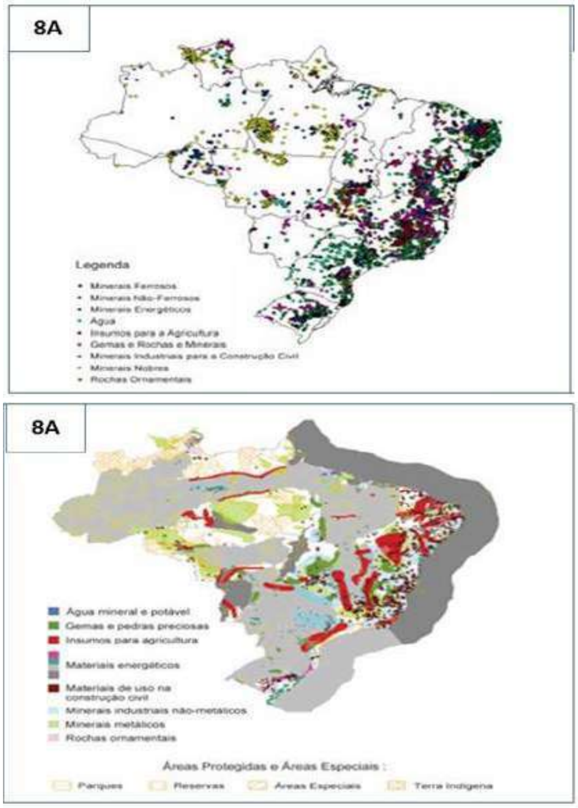

Fig.8 A and B - Mining Area Maps
Source: Farias (2002)

In this sense, the distribution of mineral resources is a function of the metallogenic vocation of crustal elements that form the geological provinces of Brazil, being responsible for the great mineral diversity of these resources and for their wide geographic distribution. However, it points out that organized mining causes less harmful impacts and is much more easily controlled by the public authorities. Moreover, mineral extraction carried out informally, without planning and control, is a source of great environmental liabilities. According to this author, control is very problematic, mainly because it involves parcels of the population that, deprived of this source of sustenance, are marginalized and excluded from any source of income. Thus, categorizes the environments as means: stable (areas that are still conserved), intergrades (areas that are in transition between stable and unstable) and strongly unstable (areas with high environmental degradation), it being possible to draw this categorization in the Serra da Quixaba mining area, according to figures 9A, B and C.

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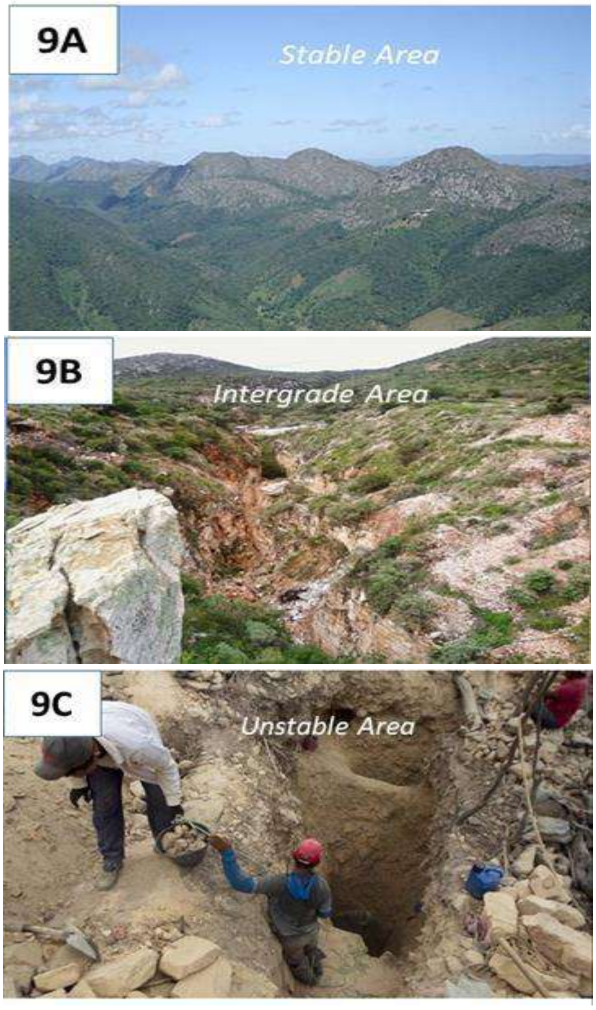

Fig.9A, 9B and 9C - Area Categorization according to the Tricart Theory

Source: Authors (2017)

Finally, it is important to point out conservation proposals in the dune geosystem, based on the characteristics of the ecoregion, since it is located in a protected area, where it would be Boqueirão da Onça National Park. In this conviction, it is suggested the creation of three strategic plans (Figure 10), for the three environments and, according to the GTP Theory.

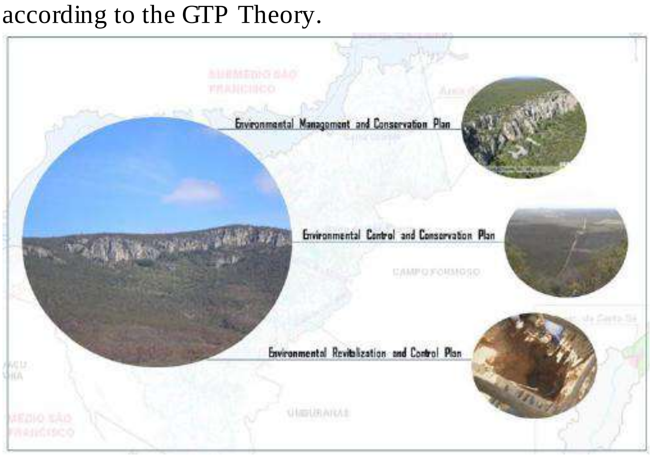

Fig.10: Strategic Plans

Source: Authors (2017)

Each suggested plan has its applicability according to the impaction of the area. The Environmental Management and Conservation Plan is applicable in areas that are still stable, aiming at conservation because it is a fragile and vulnerable environment due to climatic and socioeconomic conditions. The Environmental Control and Conservation Plan should be applied in areas that are in transition from the stable aspect to the middle intergrades. And the Plan of Revitalization and Environmental Conservation is the one that looks for strategies of revitalization/reforestation with native species of the caatinga biome, in the areas considered as strongly unstable and, from the results would draw a conservation control, analyzing the resilience capacity of the respective environments.

It should be emphasized that the proposed proposals should be applied by the Municipal Government of Sento Sé, in partnership with DNPM, with the State Government and with neighboring municipalities and districts affected by the impacts of mining.

According to the Resolution of CONAMA, n. 428 of December, in its Article 1 that the licensing of ventures of significant environmental impact that may affect specific Conservation Unit (UC) or its buffer zone (ZA), thus considered by the environmental licensing body, based on in Environmental Impact Assessment and Environmental Impact Report (EIA/RIMA), may only be granted after authorization from the body responsible for the management of the CU or, in the case of Private Natural Heritage Reserves (RPPN), by the body responsible for its creation . Therefore, any potentially impacting activity in this geosystem must be accompanied by an Environmental Impact Study and its Environmental Impact Report, in order to resolve any and irreversible impacts that may lead to greater imbalances in this territory.

VI. CONCLUSION

The present article sought to discuss mineral exploration and its environmental impacts in Serra da Quixaba, in the north of Bahia, bringing the historiography of Sento Sé natural resources, especially mineral resources. Environmental issues were also discussed from a legislative perspective, since the related project is in an area with a proposal to implement a National Park named Boqueirão da Onça.

It is impossible to discuss environmental impacts in isolation, without linking it to social and economic impacts. In this way, the real profile of the region was traced in the present days with the arrival of the mining, as well as, it was pointed out the need of the public poweraccountability, the necessity of recovery of the degraded areas, suggesting proposals and plans of remediation of the area of mining.

Therefore, it is expected that this article can contribute to the environmental theme, mainly focused on legal issues of protection of the environment in the remediation of degradation by practice of mining.

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Land evaluation, characterization and classification of soil for the proposed oil palm plantation in Ekpri Ibami, Akamkpa Local Government Area, Nigeria.

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Abstract— *Land evaluation, characterization and classification of soil for the proposed oil palm plantation in Ekpri Ibami, Akamkpa Local Government Area, Nigeria was conducted on 50 ha of land using a combination of both conventional and digital survey methods. The objective of the research was to characterize, classify and evaluate the land for the proposed oil palm production. Three soil mapping units were identified (EKP I, EKP II and EKP III) and representative profile pits were dug in each mapping unit. Samples were collected from each pedogenic horizon. Soils morphological, physical and chemical properties were determined using appropriate methods. Results revealed that soils of all of the mapping are characterized by dark greyish brown colour with thin topsoil while yellowish red to strong brown mottles were observed at the subsoil. The soils are coarsed-textured with high sand, low silt, and clay fractions. The soils were strong to moderately acidic (4.8 to 5.3). The soils also have low inherent of natural fertility with low organic carbon content, total nitrogen, and moderately available phosphorus. Low effective cation exchange capacity and high base saturation which may have occurred in available forms in solutions in spite of the low cation reserves in the soil. The individual mapping units were classified as Arenic Eutrudept, Typic Hapludult and Aquic Paleudults for EKP I, EKP II and EKP III respectively. The land was then evaluated and classified based on its suitability for oil palm production. Parametrically, mapping units EKP I and EKP II were marginally suitable and EKP III not suitable for the proposed oil palm production. Non-parametrically, EKPI is moderately suitable, EKP II is marginally suitable and EKP III is not suitable, for growing oil palm. The prevailing limitations on the Ekpri Ibami landscape for oil palm production include fertility, wetness, and topography and soil physical properties. Fertility factor happened to be the most limiting factor in all the mapping units. And thus can be ameliorated through the application of organic manures, NPK fertilizer and liming to improve the nutrient status of the soil.*

Keywords— Land evaluation, characterization, classification, sustainable agriculture.

I. INTRODUCTION

Ekpri Ibami is an agrarian community where dwellers are mainly involved in arable and tree crops production, especially Oil palm with less emphasis on fishing, hunting, and mining of solid minerals. Despite the high agricultural potential of the area, there has been known little or no published land evaluation and soil characterization articles to make sure easy access and transfer of technology to areas with similar soils elsewhere. Due to the little or no information on land evaluation and soil studies in the location, the farmers are often compelled to grow their crops without adequate attention as a result,soils are not used for the purpose that best suits their properties.Land evaluation,however,approaches targeted towards sustainable agriculture and happens to be the starting point towards adequate information on land resources, which land evaluation,provides during soil characterization studies. In this process,soil resources must be studied in detail through processes of soil characterization and land ratings for the land utilization under consideration (Esu, 2004); such information highlights soil characteristics and conditions that are suitable for growing specific crops (Ogunkunle, 2005). It is therefore apt to evaluate soil mapping units obtained from a

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soil survey for Oil palm cultivation. Furthermore, adequate information is often generated during soil surveys to "determine the important characteristics of soils,classify them into mapping units,set up and plot on maps the boundaries between kinds of soils and to correlate and predict the adaptability of soils to various crops".

The earlier reporton the soils of Ekpri Ibami have revealedwell-drained soilswith coarse-textured,strongly acid reaction,moderate organic carbon, and nitrogen but low in available P and basic cations with quartz, kaolinite, and microcline as dominant minerals (Aki *et al.*, 2014). A similar report by Abua and Eyo (2013)of the same study revealed a coarse-textured in the surface horizonwith subsurface accumulation of clay and pH (H_2O) range of 5.4-6.8, but moderate organic carbon and total nitrogen,low available P and exchangeable bases. But, pH value of 3.8 was obtained by Attoe *et al*. (2016) in the Basement complex soils of Akamkpa with moderate organic carbon and total nitrogen but low available P and exchangeable cations as obtained by Aki *et al*., (2014), and Abua and Eyo (2013). The soils,so, have high agricultural potentials but may needadequate soil management strategies.

Owing to the basic principles of conventional soil mapping,digital soil mapping (DSM) techniques, a world trending tool was adopted in this investigation.Digital soil mappingis a computer-assisted production of digital maps of soil types and properties. The method employsthe application of mathematical and statistical models that corroborate information from soil observation with information contained in correlated environmental variables (Dobos *et al.*, 2006) and uses GIS and computer programming to put into a quantitative framework the study of soils (McBratney *et al.*, 2003; Mckenkie and Ryan, 1999).

Oil palm production, which the soils are were investigated for, is one of the most important tree crops that have almost none of its parts as a waste. Its numerous importance has endeared the Federal Government of Nigeria to select it as a value chain crop; also, its cultivation and processing requires little skills and so can be cultivated by rural farmers with little supervision. This is probably the reason why most farmers in the locality grow the crop in either small or large scale. It is proposed to set up an Oil Palm estate at Ekpri Ibami thereby creating jobs for the dwellers.The study was aimed at evaluation, characterization, and classification of soil for the proposed Oil Palm plantation in Ekpri Ibami,Akampa Local Government Area of Cross River State.

II. MATERIALS AND METHODS

2.1 Location of the Study

The study was conducted on Ekpri Ibami landscape on the coordinates (05º18'53''N, 08º13'25''E; 82-111 m ASL) within Akamkpa Local Government Area of southern Cross River State (Fig. 1). The area is within the humid tropical climate characterized by distinct wet and dry seasons. The mean annual rainfall ranges between 1500-3500 mm, relative humidity 80-90 % and mean annual temperature value between 25.4-27.5ºC (NIMET, 2015). These datawere adapted from Calabar weather station of the Nigerian Meteorological Agency being situated within 100 km range of thesynoptic station as proposedby Afangide *et al*.(2010) (Table 1).

The location of the study is on a basement complex geological material of which 40% encapsulates the entire southern Cross Rivers State, Nigeriaspreading up to the African-pan basement complex of Cameroun highlands. The characteristics of the material showthe processes that form the underlying and the influence of the environment where which they occurred.

2.2 Vegetation and land use

The area is covered with secondary forest regrowth with few annual crops identified consist of *Zea mays, Manihot spp, Oryza sativa, Musa spp, Dioscorea spp*, and perennial crops such as *Carica papaya, Elaeis guineensis, Hevea brasiliensis*and *Irvingia gabonensis*. Dominant trees, climbers, and shrubs such as *Daniella oliveri, Ficus spp, Khaya senegalensis,Laxifora spp, Combretum spp,Alcohornea spp, Andropogon spp, and Digitaria spp*, are scattered almost evenly while African bamboo trees grow wildly near the streams and lowland areas.

2.3 Field Study

The research was conducted on a 50-hectare land. A semi-detailed survey was employed at a scale of 1:50 000 which enabled delineation of the boundary through the acquisition of (x), longitude (y) and elevation (z) [xyz]values with the aid of a German Etrex (2000) global positioning system (GPS) device.The xyz values were used to obtain the topographic sheet of the location. Digital terrain model (DTM) of the study area was created from the topographic map; slope map was extracted from the DTM which was classified into different categories.

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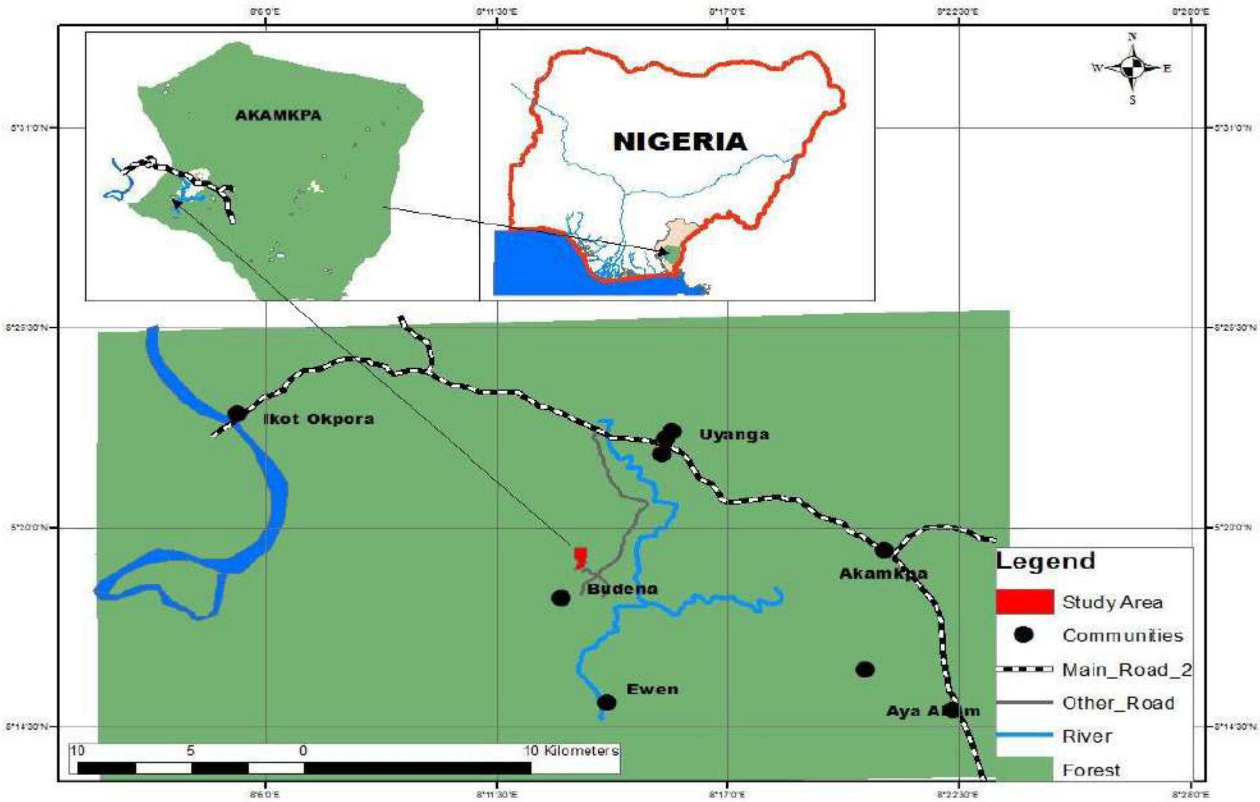

Fig.1: Location Map of the Study Area

Table.1: The Mean annual rainfall, humidity, temperature regime and sunshine duration between 1997 and 2014 in Calabar, Cross River State, Nigeria

Year	Rainfall (mm)	Dry-bulb air temperature (^oC)	Relative Humidity (%)
1997	3282.9	26.9	84.8
1998	2512.6	27.6	83.3
1999	2673.1	27.5	84.7
2000	2678.6	27.2	85.1
2001	3073.6	26.9	82.9
2002	2691.4	26.7	83.3
2003	2113.7	27.5	83.3
2004	1729.1	27.5	85.2
2005	2583.3	27.3	82.7
2006	2951.8	26.9	84.2
2007	2417.5	26.1	84.6
2008	1931.3	26.7	83.7
2009	1401.1	26.7	81.8
2010	2003.7	26.8	82
2011	2969.8	26.1	82.5
2012	4364.1	27.2	86.3
2013	3506.4	26.9	86.4
2014	3433.3	25.4	86.3
Mean	**2684.3**	**26.9**	**84.1**

Source: National Meteorological station (NIMET) Calabar (2015)

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Each slope category became a soil mapping unit.A systematic special purpose grid system detailed soil survey was adopted for ground-truthing of the computer delineated mapping units to establish the actual soil boundaries on the field. Rigid auguring at 50 m x 50 m intervals was made, physical and morphological properties were recorded for use in demarcating the mapping unit's boundaries.

2.4 Soil sampling and preparation

Three (3) mapping units, EKP I, EKP II and EKP III were identified. A pedon was cited on each mapping unit to the depth of 200 cm except interfered by an impenetrable layer or water table. The soils were described according to the guidelines of USDA-NRCS (Soil Survey Staff, 2002) as modified by Ibanga (2003) and Esu (2010). Undisturbed core cylinder samples were collected for bulk density and porosity determinations. Samples were then taken from each pedogenic horizon, bagged, labelled and transported to the laboratory for analysis. Prior to analysis, the samples were air dried, gently crushed and sieved through 2 mm sieve.

2.5.1 Laboratory analysis

The particle size distribution was determined using the Bouyoucos hydrometer method (Gee and Bauder, 1986) and the percent sizes later used to ascertain the soil textural class with the aid of the soil textural triangle provided by USDA. Soil pH was determined potentiometrically in the soil–water ratio of 1:1 (Udo *et al.*, 2009) and organic carbon was determined by the Walkley and Black wet oxidation method. Exchangeable cations were extracted using 1N NH_4OAc (pH 7.0) and, exchangeable Ca^{2+} and Mg^{2+} determined by atomic absorption spectrophotometry (Thomas, 1982) while Na^+ and K^+ were determined by flame photometry. Effective cation exchangeable capacity was determined by summing up the exchangeable bases and exchangeable Al^{3+} as outlined by Udo *et al* (2009). Base saturation was obtained by expressing the sum of exchangeable cations as a percentage of the cations exchange capacity (IITA, 2000) using the formula; **B.S =** $\left[\frac{TEB}{ECEC}\right]$ **X 100 (%).**

2.6 Soil Classification

The pedons were classified based on USDA Soil Taxonomy (Soil Survey Staff, 2010). The Soil Taxonomy was based on the properties of the soil as were found during the study. The physical, chemical and morphological properties and general site information were used as criteria to classify the soil to the subgroup level.

2.7 Land evaluation

The land evaluation was carried out according to the guidelines provided by Sys (1985) (Table 2). This is to make sure the reduction in the risk of production through the matching of the requirements of land use to the land qualities. Three (3) soil units were placed in the suitability classes by comparing the data obtained in the area under study for oil palm production to Oil palm requirement information. The evaluation was both conventional (non-parametric) (FAO, 1976) and parametric methods (Ogunkunle, 1993; Udoh *et al.*, 2006). For the non-parametric evaluation, pedons were first placed in suitability classes by matching their characteristics with the established requirements. The aggregate suitability classes were indicated by the most limiting characterist (c(s) of the soil units. For the parametric method, each characteristic was rated and the index of productivity (IP) for each pedon was calculated using the square root method equation:

$$\text{IP} = Ax\sqrt{B/100\ xC/100\ x \dots xF/100}$$

Where: A is the overall lowest characteristic rating and B, C,…F is the lowest characteristic ratings for each land quality group (Udoh *et al.*, 2006). "Five land quality groups climate (c), topography (t), soil physical properties (s), wetness (w) and fertility (f) were used in this method of evaluation" (Table 2). "Only one member characteristics in each land quality group will be used for calculation purpose because there are usually strong correlations among members of the same group" (Ofem *et al.*, 2016). "Five levels of limitations were used, no limitation (0), slight limitation (1), moderate limitation (2) severelimitation (3) and very severe limitation (4)" (Table 3). One "limitation level was attributed to each land characteristic. The final (aggregate) suitability classes were determined by the number and intensity of the limitation(s)", and the most unfavourable characteristic determined the suitability classification. Suitability classes S1, S2, S3, N2, and N1 were established as reported by Ofem *et al* (2016). For actual (current) productivity index, all the lowest characteristic ratings for each land quality group were substituted into the index of productivity equation above. But, in the case of potential productivity index, it was assumed that the corrective fertility measure would no longer have fertility constraints. So, other qualities except for fertility (f) were used to calculate the potential productivity index. "Suitability classes S1, S2, S3, and N are equivalent to IP values of 100 – 75, 74 – 50, 49 – 25 and 24 – 0, respectively".

III. RESULTS AND DISCUSSION

3.1 Morphological and physical properties:

Threesoil mapping units were identified in the study area denoted by EKP I, EKP II and EKP III.

Soil mapping unit EKP I: The soils are very deep (>100 cm), located at the crest region of the landscape with an elevation value of 108 m above sea level (Table 4).The Ap

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horizon of the soil was observed to be thin (16-17 cm) with an extensive thick B-horizon which may be attributed to eluviation-illuviation processes (FitzPatrick, 1986; Orimoloye *et al*., 2010). The soil were very dark greyish brown (10YR 3/2) with no mottles at surface while the subsurface soils varied from yellowish red (5YR 5/8) ta reddishsh colour (2.5YR 4/6) with mottles occurring within the depths which may be attributed to periods of wetting which had led to inadequate aeration or reduction during the periods of the year, leading to oxidation-reduction reactions. The soils are weakly structured and there is evidence of layers with weak pedogenic processes in the field. The soils are coarse-textured with high sand content = 623.5 g/kg, low silt = 99.0g/kg and low clay = 277.5 g/kg and a sandy loam texture in the surface horizon and sandy clay loam in the subsurface horizon. The high silt/clay ratio indicates a high intensity of weathering (Young, 1976). Bulk density values varied from 1.2 g/cm^3 at the surface horizon to 1.5 g/cm^{3}in the subsurface horizon (Table 5). These values will allow root penetration, and good aeration, water movement for best crop production (Esu, 2010).

Table.2: Modified Land Suitability Requirements for Oil Palm Based on Land Characteristics

Parameters	**Suitability class**				
	S1	**S2**	**S3**	**N1**	**N2**
Land Qualities	100-75	74-50	49-25	24-15	14-0
Climate (c)					
Annual Rainfall (mm)	>1700	1450-1700	1250-1450	-	<1250
MAT(°C)	>22	20-22	18-20	-	<18
Relative humidity (%)	>70	65-70	60-65	-	<60
Topography(t)					
Slope (%)	0-8	8-16	16-30	>30	-
Wetness(w)					
Flooding	Fo	F1	F2	-	F3
Drainage	Perfect, well	Mod. Well	Poor, aeric	Poor, drainable	V. poor, not drainable
Soil Physical properties(s)					
Texture	Cl, Scl, L	Scl	Scl-Lfs	Any	C,Cs
Structure	Blocky	-	-	-	massive, single grain
Depth (cm)	>100	50-100	25-50	-	<25
Fertility(f)					
ECEC (cmol/kg)	> 8.0	5-8	< 5	-	-
Base Sat. (%)	>35	20-35	< 20	-	-
pH(H_2O)	5.5-6.0	5.5-6.0	6.5-7.0	< 4.0,>7.0	< 4.0,>7.0
OC(%)(0-15 cm)	>1.2	1.2-0.5	0.5-0.3	0.3-0.2	< 0.2

Flooding: Fo, No flooding; F1, 1-2 flooding months in>10 yrs; F2, Not more than 2-3 months in 5 yrs out of 10 yrs; F3, 2-4 months almost every year; F4, >4 months in almost every. Texture: Cl, clay loam; Scl, Sandy clay loam; L, Loam; Lfs, Loamy fine sand; c, Clay; Cs, Clayey s and MAT= Mean annual temperature.

Source: Sys *et al*. (1991)

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Table.3: Ratings of limiting characteristics.

Limitation	Rating
Slight to none	100 – 90
Slight	89 – 70
Moderate	69 – 50
Severe	49 – 35
Very Severe	34 – 0
Can be corrected	34 – 20
Cannot be corrected	19 – 0

Soil mapping unit EKP II: It is well deep (>100 cm) with sandy loam texture and they occupy the middle slope of the landscape under study at an elevation of 102 m. The soil colour is characterized with a distinct dark yellowish brown (10 YR 4/4) at the surface horizon coming down to yellowish brown (10 YR 4/6) in the subsurface. Dark red (10 R 3/6) coloured mottles was observed in the subsurface layer and this may be as a result of poor aeration leading to oxidation-reduction reactions. Structural aggregates varied from weak medium granular in the surface and moderate medium coarse subangular blocky in the subsurface. Silt/clay ratio revealed that the soils possess high weathering potentials (>0.15). Bulk density varied from 1.1 g/cm^3 in the surface horizon to 1.5 g/cm^3 in the subsurface horizon. These values obtained have been reported to be suitable for agricultural purposes (Esu, 2010).

Soil mapping unit EKP III: It covered the lower slope of the landscape and situated at the elevation of 82 m above sea level. The soils are poorly drained with depth <50 cm. The hue values were 10 YR and 7.5 YR in the surface and subsurface layers giving a variation of colour, very dark grey-brown and strong brown, this result is similar with the report by Dengiz *et al*. (2012). Also, there was the occurrence of yellowish red (5 YR 4/6) mottle in the subsurface layer which indicates poor drainage conditions. Structural aggregates varied from moderate medium subangular blocky to weak fine granular in the surface and from moderate fine subangular blocky to weak fine medium granular in the subsurface horizon. The soils are predominantly sandy loam texture with particle size distribution (sand= 703.2 g/kg, silt=102.9 g/kg and clay= 203 g). Silt/clay ratio revealed that the soils have high weathering potential (>0.15). Bulk density values ranged from 1.1 g/cm^3 in the surface horizon to 1.5 g/cm^3 in the subsurface horizon.

3.2 Chemical properties of the soil mapping units

The chemical properties of the soil mapping unitsidentified on Ekpri Ibami landscape are presented in Table 6. The soil pH distribution in the soil units followed an irregular increase and decrease with depth. The mean pH (H_2O) were 5.3, 4.8 and 4.9 in soil mapping unit EKP I, EKP II and EKP III, respectively. Negative delta pH (ΔpH) obtained in the soils showed that all the layers of the soils profiles possessed net negative surface charges and such that they can retain basic nutrients for subsequent release into soil solution for plant uptake. Organic carbon and total nitrogen gave an irregular increase ana decreasese in values with depth. Means of organic carbon were 0.56 %, 0.47% and 0.2 % for EKP I, EKP II and EKP III respectively. While the means of total nitrogen content in the proposed land were 0.18 %, 0.04 % and 0.02 % for EKP II and EKP III respectively. Organic carbon content and total nitrogen were rated low as they fall within thin the critical limit of<1.5 % and < 0.2% respectively(Enwezor *et al*.,1989).

Table.4: Morphological properties of EKP I soil mapping unit

Horizon	Depth (cm)	Munsell-colour (moist)	Mottling	Texture	Structure	Consistence	Boundary	Other characteristics
EKP I	**N05˚ 19' 16.84" ; E008˚ 13' 36.1" ; 108 m ASL**							
Ap	0-17	10YR 3/2, vdgb		SL	1mcgr	w ss, f, p	cs	Porous, many fine medium roots, Common medium roots; Iron concretion, animal faecal, termite hill, quartzite inclusion.
Bw1	17-62	5YR 5/8, yr		scl	1msbk	wss, f, p	gs	Many medium pores, many common fine medium roots, weathered rock mica flakes, quartz inclusion, termites, earthworms and ants activities.
Bw2	62-122	5YR 5/8, yr	m (10R 3/6, dr)	scl	2msbk	w ss, f, p	ds	Few medium pores, many common fine medium roots, ants activities.
Crt	122-200	2.5YR 4/6, r	m (10R 3/8, yr)	scl	2msbk	wss,f, p	cs	Many coarse pores, few medium roots, ants activities clear, few thin cutans at ped faces, many iron concretions.
EKP IIN05˚ 19' 11.3" ; E008˚ 13' 36.8" ; 102 m ASL								
Ap	0-8	10 YR 4/4, dyb		SL	1mgr	wss	cs	Fine-coarse medium pores very fine size, few medium roots fine size, ants termites, earthworm and frogs,
Bt1	8-53	10 YR 4/4, dyb		SL	2mcsbk	ws	cd	Many medium pores, many common fine medium roots, weathered rock mica flakes, quartz, termite activities.
Bt2	53-114	10 R 5/6, yb	10 R 3/6, dr	SL	2msbk	wvs	gs	Many fine and medium pores, fine medium coarse roots, fine mica flake and many ants.
Crt	114-200	10 YR 5/6, yb	10 R 3/6, dr	SL	2msbk	wvs	cs	Fine medium coarse roots, few pores; fine mica flakes.
EKP III	**N05º 18' 56.8"; E008˚ 13' 25.1" ; 82 m ASL**							
Ap	0-15	10 YR 3/2, vdgb	nm	Sl	2msbk	wss, sp,f	cs	Many medium pores, many fine roots, many wormholes, termite activities observed.
Bt1	15-25	7.5 YR 5/6,	nm	Scl	2fsbk	wss, p,f	dw	Few thin clay cutan at ped faces, many

		sb					medium pores, many fine roots, termites, ant holes.
Bt2	25-42	10 YR 4/6, db	m(5 YR 4/6, yr)	Sl	2fsbk	wss, p,f	Many medium pores, many fine roots, termites, frogs.

Colour- vdgb: very dark greyish brown, yr: yellowish red, r: red, drb: dark reddish brown, rb: reddish brown, dr: dusky red, **Mottles**-m: mottled **Texture**- SL: sandy loam, scl: sandy clay loam, ls: loamy sand, **Structure**- 1: weak, 2: moderate, 3: strong, m: medium, f: fine, c: coarse, gr: granular, sbk: subangular blocky, pl: plate-like, **Consistence**- wssfp: wet, slightly sticky, friable, plastic, wssp: wet, slightly sticky, ws: wet, sticky, **Boundary**- cs: clear smooth, **ASL**: above sea level.

Table.5: Physical properties of soil unit EKP I

Horizon	Depth (cm)	Particle Size Distribution gkg^{-1}			Textural Class	Silt: Clay	Gravel (% vol)	Bulk density (g/m^3)	Porosity (%)
		Sand	Silt	Clay					
EKP IN05˚ 19’ 16.84” ; E008˚ 13’ 36.1” ;108 m ASL									
Ap	0-17	766.4	83.6	150	SL	0.56	7.7	1.2	55
Bw1	17-62	614.8	65.2	320	SCL	0.20	11.5	1.5	43
Bw2	62-122	616.4	103.6	280	SCL	0.37	64.8	1.5	43
Crt	122-200	496.4	143.6	360	SCL	0.40	63.7	1.4	47
Range		496.4-766.4	65.2-143.6	150-360		0.20-0.56	7.7-64.8	1.2-1.5	43-55
Mean		623.5	99.0	277.5		0.3825	36.9	1.4	47
EKP IIN05˚ 19' 11.3'' ; E008˚ 13' 36.8'' ; 102 m ASL									
Ap	0-8	635.2	134.8	230	SL	0.59	72.0	1.1	58
Bt1	8-53	695.2	142.8	162SL	SL	0.88	0.0	1.4	43
Bt2	53-114	712.2	97.8	190SL	SL	0.51	57.0	1.5	43
Crt	114-200	575.2	44.8	380	SL	0.12	50.0	1.5	47
Range		575.2-712.2	44.8-142.8	162-380		0.12-0.88	0.0-72.0	1.1-1.5	43-58
Mean		654.5	105.1	241		0.54	44.8	1.4	47.8
EKP III	**N05º 18' 56.8''; E008˚ 13' 25.1'' ; 82 m ASL**								
Ap	0-15	754.8	123.6	150	SL	0.82	40.9	1.1	62
Bt1	15-25	619.6	120.4	260	SCL	0.46	67.4	1.4	47
Bt2	25-42	735.2	64.8	200	SL	0.32	0.0	1.5	43
Range		619.6-754.8	64.8-123.6	150-260		0.32-0.82	0.0-67.4	1.1-1.5	43-62
Mean		703.2	102.9	203		0.53	36.1	1.3	51

Table.6: Chemical properties of the soil mapping unit

Horizon	Depth (cm)	pH (H_2O)	pH (KCl)	ΔpH	Org.C (%)	TN (%)	C: N	Avail.P (mgkg^{-1})	Ca	Mg	Exchangeable K	Na	Exchangeable (Al^{3+}+H^{+})	ECEC	BS %
EKP I	**N05° 19' 16.84" ; E008° 13' 36.1" ; 108 m ASL)**														
Ap	0-17	5.0	4.9	-0.1	0.60	0.04	15	20.6	2.2	1.40	0.14	0.48	0.9	5.12	82.0
Bw1	17-62	5.6	4.4	-1.2	0.74	0.06	12	17.3	1.6	1.00	0.10	0.08	1.6	4.34	64.0
Bw2	62-122	5.1	4.8	-0.3	0.50	0.03	17	20.8	2.6	1.50	0.37	0.37	1.7	6.54	74.0
Crt	122-200	5.6	4.7	-0.9	0.40	0.03	13	15.4	1.2	0.80	0.10	0.08	1.6	3.74	58.0
Range		5.0-5.6	4.4-4.8	0.1-0.9	0.40-0.74	0.03-0.06	12-17	15.4-20.6	1.2-2.6	0.50-1.50	0.10-0.37	0.08-0.48	0.9-1.7	3.74-6.54	58.0-74.0
Mean		5.3	4.7	-0.6	0.56	0.18	14	18.5	1.9	1.18	0.18	0.25	1.4	4.94	69.5
EKP II	**N05° 19' 11.3'' ; E008° 13' 36.8'' ; 102 m ASL**														
Ap	0-8	4.8	4.3	-0.5	0.60	0.03	20	24.7	3.0	1.5	0.46	0.47	1.7	7.10	76.0
Bt1	8-53	4.9	4.1	-0.8	0.63	0.05	13	11.4	1.4	1.0	0.09	0.07	1.8	4.40	59.0
Bt2	53-114	4.1	4.0	-0.1	0.30	0.04	15	23.8	3.3	1.5	0.44	0.47	0.8	6.50	44.0
Crt	114-200	5.5	4.5	-1.0	0.36	0.02	18	14.2	1.4	0.8	0.09	0.08	2.3	4.70	51.0
Range		4.8-5.5	4.1-4.5	0.1-1.0	0.30-0.63	0.02-0.05	13-20	11.4-24.7	1.4-3.3	0.8-1.5	0.09-0.44	0.07-0.47	0.8-1.8	4.40-7.10	44.0-76.0
Mean		4.8	4.2	-0.6	0.47	0.04	17	18.5	2.3	1.2	0.27	0.27	1.7	5.70	58.0
EKP III	**N05º 18' 56.8''; E008° 13' 25.1'' ; 82 m ASL**														
Ap	0-15	4.9	4.1	-0.8	0.2	0.01	20	16.5	1.0	0.8	0.09	0.07	2.2	4.20	47.0
Bt1	15-25	4.4	4.2	-0.2	0.4	0.03	13	13.2	2.3	1.7	0.35	0.34	0.4	5.10	92.1
Bt2	25-42	5.5	4.6	-0.9	0.1	0.01	10	12.9	2.6	1.0	0.07	0.06	1.5	5.30	71.0
Range		4.4-5.5	4.1-4.6	0.2-0.9	0.1-0.4	0.1-0.03	10-20	12.9-16.5	1.0-2.6	0.8-1.7	0.07-0.35	0.06-0.34	0.4-2.2	4.20-5.30	47.0-71.0
Mean		4.9	4.3	-0.6	0.2	0.02	14	14.2	2.0	1.2	0.17	0.16	1.4	4.90	70.0

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The mean values of the carbon/nitrogen ratio for the soils were 14, 17 and 14 for EKP I, EKP II and EKP III respectively. The small or narrow C: N (<25) (Paul andClark, 1989) will positively influence microbial activities to ensure rapid mineralization of organic matter with the consequent release of nutrient elements into the soil solution for crop plant assimilation (Akpan-Idiok *et al*.,2012) thus the carbon-nitrogen ratio of the soils falls within the given range. The available phosphorus content followed an irregular increase and decrease with depth in all the mapping units with the mean values of 18.5 mg/kg, 18.5 mg/kg and 14.2 mg/kg for EKP I, EKP II and EKP III respectively. Phosphorus content in soils was rated medium for mapping unit EKP I and EKP II and low for EKP III when compared with the critical value of 15 mg/kg (Enwezor *et al*., 1990 & Adepetu, 2000).

The exchangeable bases of the all soil mapping units were low. Exchangeable Ca dominated the soil exchange site. A similar result was reported elsewhere (Fasina *et al*., 2006 & Noma *et al*., 2004). Means of exchangeable acidity were 1.4 cmol/kg, 1.7 cmol/kg and 1.4 cmol/kg for EKP I, EKP II and EKP III respectively. This showed that preponderance of exchangeable (Al^{3+} + H^{+}) played a major role in soil acidity in the humid tropical soils of Ekpri Ibami. Effective cation exchange capacity values of the sois of Ekpri Ibami landscape were 4.94 cmol/kg, 5.70 cmol/kg and 4.90 cmo/kg for EKP I, EKP II and EKP III respectively. The ECEC were generally low as they fall within the critical value of (<8 cmol/kg) as provided by FAO, (1976) for soils of the ecological zone. This shows that the soils at their natural pH remain low in cation exchange and has low ability to retain nutrients (Yakubu, 2006). The means base saturation of the different mapping units were 69.5 %, 58.0 % and 70.0 % for EKP I, EKP II, EKP III respectively. A similar result was obtained by Abua and Eyo (2013) and Bulktrade (1989)in soils of Akamkpa. Meanwhile, according to FAO (1999) base saturation > 50% is regarded as fertile and rated high while<50% are regarded as not fertile soil and rated low. With the high base saturation, the basic nutrients must have occurred in available forms in soil solution in spite of the low cation reserves in the soil.

3.3 Soil classification

The soil morphological, physical and chemical properties were used to classify the soil unit according to USDA (2010). The mapping units identified at Ekpri Ibami landscape proposed for Oil palm production and denoted by EKP I, EKP II and EKP III and discussed as follows:

EKP I: This group of soil showed a moderate level of weathering and lacks the extensive amount of clay accumulation. They were identified with a weak horizon and with the presence of a cambic diagnostic horizon along with an ochric epipedon and moist colour value of 3 or more. Soils are developed on colluvial deposits. The soil is characterized with a weak coarse granular structure at the surface, improperly drained down the soil depth during the rains and are placed in the order of **Inceptisols**. With udic moisture regime occurring within the study location, the soil fits into the **Udepts** suborder and with a base saturation of 50 % in one or more horizons at a depth between 25 and 75 cm, they are further placed into **Eutrudepts** great group. The soils were further classified as **Arenic Eutrudept** at subgroup category due to the irregular decrease in organic-carbon content between a depth of 25 cm and at a depth of 125 cm.

EKP II: With the presence of argillic horizon and low base saturation less than 50% (with NH_4OAc) in with the depths 20 and 100 cm from the surface mineral soils. Thus qualified to be placed in order **Ultisol** and great group **Hapludult** and subgroup **Typic Hapludult**, according to USDA Soil Taxonomy classification.

EKP III:with increasing depth, do not have a clay decrease of 20 percent or more (relative) from the maximum clay content and so fit into **Paleudults** at the great group level and at subgroup level classified as **Aquic Paleudults.**

3.4 Land evaluation for the proposed oil palm production

3.4.1 Land qualities and land use requirement for oil palm production

3.4.1.1 Climate (c)

Climate parameters considered in the study were annual rainfall, mean annual temperature, and relative humidity. In Akamkpa LGA of Cross River State rainfall is a sufficient factor for oil palm production and has a mean annual rainfall of 2000mm (NIMET, 2015). The mean annual temperature and relative humidity in this region are greater than 25 º C and 80% respectively as shown in Table (7). The climatic characteristics were rated 100 % since they exceed 2000 mm, 25ºC and 75% provided by Sys (1985) for S1.

3.4.1.2 Topography (t)

The topography of the study area was on strongly undulating landscapeswith EKP I mapping unit occurring on relatively flat terrain (0-8 %), crest region. EKP II and EKP III mapping units were located at a convexly shaped angle with gradient range of 8-16 %. According to the criteria set by Sys (1985) for oil palm production EKP I is

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rated highly suitable (S1) while EKP II and EKP III are moderately suitable (S2) (Table). But, soils of EKP III may pose harvest and transportation challenges. Contour farming and strip cropping should be adopted along the slope gradient with reduced grazing and removal of vegetation. This practice will check erosion in EKP II mapping unit.

Table.7: Suitability class scores of the pedons Oil palm cultivation

Parameters	**EKP I**	**EKP II**	**EKP III**
Climate (c)			
Annual Rainfall (mm)	S1(100)	S1(100)	S1(100)
MAT(°C)	S1(100)	S1(100)	S1(100)
Relative humidity (%)	S1(100)	S1(100)	S1(100)
Topography(t)			
Slope (%)	S1(100)	S2(65)	S2(70)
Wetness(w)			
Flooding	S1(100)	S1(100)	S3(49)
Drainage	S1(85)	S1(85)	N1(20)
Soil Physical properties(s)			
Texture	S1(90)	S2(50)	S2(60)
Structure	S1(75)	S1(75)	S1(75)
Depth(cm)	S1(100)	S1(100)	S3(44)
Fertility(f)			
ECEC(cmol/kg)	S3(47)	S2(60)	S3(44)
Base Saturation (%)	S1(80)	S1(70)	S1(90)
pH(H_2O)	S2(55)	S2(50)	S3(40)
OC(%)(0-15 cm)	S3(48)	S3(45)	S3(49)
Aggregate Suitability			
Potential	S3(38.1)	S3(26.3)	N2(10.9)
Actual (current)	S3(37.3)	N1(23.6)	N2(9.9)

3.4.1.3 Wetness (w)

In terms of soil wetness, the characteristics considered under this land quality group were flooding and drainage. They were no flooding problems in EKP I and EKP II mapping units as they were very well drained and had no characteristics of limiting drainage probably due to the sandy and gravelling properties of the soilsand were rated 100 % (S1) for both flooding and drainage. But EKP III mapping unit is influenced by the inflow of water from the streams and rated between 25-49% (S3) for flooding and drainage.

3.4.1.4 Soil physical properties (s)

Soil physical properties considered were texture, structure and soil depth. Comparing the land qualities (Table 2). All the mapping units were rated between 40 – 90 % for both soil texture and structure. But, soil texture is generally optimum for oil palm production with moderate to high suitability. EKP I = S1, EKP II = S2 and EKP III= S3.

3.4.1.5 Soil fertility (f)

The soils effective cation exchange capacity by summation, base saturation (BS) and organic carbon were evaluated as potential fertility characteristics they are not easily altered. The matching scores as shown in Table (6) showed that the ECEC values of the three mapping units were marginally suitable (S3) the criteria required for oil palm production as suggested by Sys (1985) with an average score of 49 % while the base saturation is highly suitable (S1) with suitability scores between 80-100 %. The pH of the different mapping units was moderately suitable (S2). The organic carbon scores showed that the EKP I, EKP II and EKP III are moderately suitable (S2) and pose greater fertility challenge in the production of oil palm, so, requires the increase in organic matter content.

3.5 Oil palm Suitability

The aggregate scores, S3(38.1), S3 (26.3) and N2 (10.9) are potentially suitable scores for EKP I and EKP II and EKP III respectively. While S3 (37.3), N1 (23.6), N2 (9.9) are actual (current) suitable scores. Table (8) presents the non-parametric and parametric rating of the different soil mapping units.

Potential Suitability: EKP I and EKP II are marginally suitable for oil palm production and must, need continuous conservation and crop management practices such as appropriate and adequate fertilizer application, contouring, minimum tillage etc to ensure increased yield while conserving the soil nutrient level while EKP III is not suitable.

Actual (Current) Suitability: the aggregate scores showed that EKP I is marginally suitable while EKP II and EKP III are not suitable.

Table.8: Suitability classification of the mapping units

Mapping units	Potential		Current	
	Nonparametric	Parametric	Non parametric	parametric
EKP I	S2f	S3(38.1)	S2f	S3(37.3)
EKP II	S3fs	S3(26.3)	N2tfs	N1(23.6)
EKP III	N2wf	N2(10.9)	N2wf	N2(9.9)

f=fertility limitation; w=wetness limitation; s=soil physical characteristic limitation; t= topography

3.6 Soil management practices for Oil palm production at Ekpri Ibami

The soils of Ekpri Ibami in Akamkpa Local Government Area of Cross River represented with mapping units EKP I, EKP II and EKP III were generally good for the agricultural purpose. But, their specific limitations have placed them into different suitability classes. The general management practices are as follows;

3.6.1 Maintenance of soil fertility

The basic cations such as Ca, Mg and K appear to be very low in the soils of the study area due to high rainfall resulting into leaching of these cations out of soil solum (Esu, 2005). So, the proper soil management strategist to conserve or improve the fertility may include spreading of crop residues on the soils after harvesting, the inclusion of grasses and legumes during fallow.

3.6.2 Adoption of conservation practices

For best production; it is recommended that the practice of annual burning of bushes and plant residues at the start of farming, extensive grazing of the bushes by livestock and the felling of trees be discouraged while contour farming and strip cropping along slopes be encouraged to reduce the speed of runoff and subsequently reduce erosion and leaching in the area.

3.6.3 Organic manures and chemical fertilizer application

Oil palm requires balanced and the sufficient amount of micro and micronutrients for production. The study shows that the soil mapping units are generally low in soil basic nutrient yielding marginally to moderate suitability. However, to ameliorate the nutrient status of the soil adequate application of fertilizer in a split dosage at 3 months interval should be adopted (FDDD, 1989). Farmyard manure (FYM) of 75 to 100 kg or 90 to 100 kg of green manure and 5 kg neem cake should be added per each oil palm tree along the second dose of fertilizer. The practice will also reduce soil water erosion in the study area and will return the much-needed plant nutrient element to the soil (Esu, 2005; Onyekwere *etal.*, 2001; Akpan-idiok, 2012).

IV. CONCLUSION

Three mapping units (EKP I, EKP II and EKP III) derived from basement complex parent material of Akamkpa Local Government Area were mapped, characterized, classified and evaluated for its suitability for oil palm cultivation. The soils were strong to moderately acidic (4.8 to 5.3). The soils also have low inherent of natural fertility with low organic carbon content, total nitrogen, and moderately available phosphorus. Low effective cation exchange capacity and high base saturation which may have occurred in available forms in solutions in spite of the low cation reserves in the soil.

Parametrically, mapping units EKP I and EKP II were marginally suitable and EKP III not suitable for the proposed oil palm production. Non-parametrically, EKPI is moderately suitable, EKP II is marginally suitable and EKP III is not suitable, for growing oil palm. The prevailing limitations on the Ekpri Ibami landscape for oil palm production include fertility, wetness, and topography and soil physical properties. Fertility factor happens to be the most limiting factor in all the mapping units. And thus can be ameliorated through the application of organic manures, NPK fertilizer and liming may also improve the soil fertility status. At EKP III which is seen to be limited by wetness and drainage, proper drainage channels should be constructed since the proposed tree crop requires well-drained soil to encourage microbial biomass. Also, waterlogging crops can be grown in the area.

There is no declaration of conflict of Interest.

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Bioremediation of Textile dyes by Fungal-Bacterial Biofilms

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***Abstract*— *Textile waste pollutants are the most polluting waste water and their treatment is greatly challenging for their safe discard. Microbial communities have potential ability to decolorize synthetic commercial dyes used for textile dyeing. Therefore, this study was aimed to develop potential dye degrading microbial biofilms from endophytic fungi and soil bacteria. Endophytic fungi were isolated from the leaves of Eleusine indica (Linn) and bacteria were isolated from soil samples obtained near textile effluent dumping site in Biyagama Industrial zone, Sri Lanka. Biofilms were developed after screening the fungal and bacterial isolates with Malachite green and Nigrosin disodium dyes separately and the decolorization assay was performed for biofilms along with monocultures to evaluate their ability for dye decolorization. The highest significant ($P< 0.05$) decolorization percentages were observed by Tricoderma harzianum (F2) and Bacillus subtilis (B1) for both dyes. All the biofilm combinations showed higher decolorization percentage than that of the monocultures. Thus, it can be concluded that the biofilms can be used as an efficient biological tool for textile effluent treatment.*

***Keywords*— Biofilms, Textile dyes, Bioremediation, Decolorization.**

I. INTRODUCTION

Environmental pollution has been recognized as one of the major hazards of the modern world. Due to the rapid industrialization and urbanization, various types of chemicals manufactured and applied in day to day life (Moorthi *et al*.,2007). Among industrial effluents, wastewater from textile industries is one of the most difficult to be treated since the dyes used are usually synthetic and contain complex aromatic molecular structures (Padmesh *et al*., 2005). During manufacturing and processing, approximately 10- 15% of the dye is lost and released directly as wastewater that accumulates in the environment (Elisangela *et al*., 2009). Unfortunately, most of these dyes persist in the environment due to their high stability to physical factors like light and temperature (Drumond Chequer *et al*., 2013). The disposal of such reactive dyes into the environment even at very low concentrations causes considerable damage as they intensely affect the photosynthetic activity of aquatic organisms by limiting the light penetration and their breakdown products may be toxic to them (Wang *et al*., 2009). Nigrosin disodium and Malachite green are good examples which are used in many fields and also have a wide variety of toxicological effects (Zhang *et al*., 1995). Both dyes have been reported for their negative impact on living cells and organisms (Culp and Beland, 1996). Because of the toxicity to major microorganisms, its presence in wastewaters makes difficult the biodegradation.

Commonly applied treatment methods for the removal of colored effluents consist of integrated processes involving various combinations of biological, physical and chemical decolorization methods (Galindo and Kalt, 1999; Azbar *et al*., 2004). However, chemical and physical methods for the treatment of dye wastewater are not widely applied to textile industries because of the costs and disposal problems (Yang *et al*., 2009). The available conventional waste water treatment systems are unable to completely remove the recalcitrant dyes and other organic residues from such effluents. Therefore, there is an immense effort to develop a cost-effective and ecofriendly alternative to conventional waste treatment methods.

Among all the technologies bioremediation of textile dye containing effluents using microorganisms which are capable of degrading dye, is still seen as an attractive alternative solution (Shahid *et al*., 2013). Although, bacterial, fungal and algal species have the ability to adsorb and/or degrade dyes (Stolz, 2001; Don Santoz, 2007), the moderate decolorization rate and complexity of textile effluents limit the performance of microorganisms in bioremediation (Banat *et al*., 1996; Elisangela *et al*., 2009; Wang *et al*., 2009). Further, it has been observed that the biological treatment along with the current conventional microbiological treatment processes have not been not proved satisfactory for color elimination (Robison *et al*., 2001). Therefore, bioremediation through microbial biofilms is emerging as one of the promising approaches due to its power of degrading various environmental pollutants, high tolerance towards harsh environment, low-cost and environmentally friendliness (Mitra and Mukhopadhyay, 2015). Thus, present study focused on investigating the potential of fungal bacterial biofilms to decolorize the selected textile dye effluent.

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II. METHOD

2.1 Isolation of endophytic fungi and soil bacteria

Endophytic fungi were isolated from the leaves of *Eleusine indica (Linn)*, a plant grown near a dumping site of textile effluents in Biyagama Industrial zone, Sri Lanka. The leaves of the *Eleusine indica (Linn)* were cut into smaller pieces and surface sterilized with 70% ethanol, 1% clorox, and rinsed with sterilized water. The PDA plates inoculated with the surface sterilized leaf parts were incubated at 25°C and observed for growth. The pure fungal cultures were transferred periodically onto fresh Potato Dextrose Agar (PDA) plates. Bacteria were isolated from soil samples obtained from the same location near the textile effluent dumping site. The pure bacterial cultures were transferred periodically onto fresh Nutrient Agar (NA) plates.

2.2 Screening of microbial isolates on dye decolorization

Malachite green (4-[(4-dimethylaminophenyl) phenyl-methyl]-N, N-dimethylaniline) Molecular formula: C23H25ClN2 and Nigrosin disodium; 4-amino-3-[(4-nitrophenyl) diazenyl] - 5-oxo-6- (phenylhydrazinylidene) naphthalene-2, 7-disulfonate, Molecular formula: (C22H14N6Na2O9S2), dyes in textile industries were used to screen the fungal and bacterial isolates for their decolorization ability. Fungal and bacterial isolates were inoculated into Erlenmeyer flasks containing 100 ml of nutrient broth and potato dextrose broth supplemented with filter sterilized 0.5g/L of Malachite green and Nigrosine disodium dyes separately. Dyes containing uninoculated medium served as control and all assays were performed in triplicate. Inoculated medium and control were incubated at room temperature (25^0C) for five days on rotary shaker at 150 rpm. Growth and dye decolorization were noted every day. After incubation, samples were withdrawn, centrifuged at 5000 rpm at 4°C for 10 minutes using cooling centrifuge (REMI C-24BL). Absorbance reading of the cell free supernatant was measured using Spectrophotometer at 620 nm for Malachite green and at 600 nm for Nigrosin disodium dyes. The decolorizing activity was expressed in terms of percent decolorization which was determined by using the following formula;

Percentage of decolorization = {Initial Absorbance - Final absorbance/Initial Absorbance} X100

2.3 Formation of the biofilms

The screened fungal (F1 and F2) and bacterial (B2, B3 and B6) isolates having the dye decolorization ability were inoculated into Potato dextrose and Nutrient broths separately and incubated at 29±1 °C for 10 days and at 25± 1°C for 3 days respectively. Fungal- bacterial biofilms were developed by combining the fungal and bacterial isolates grown in potato dextrose and nutrient broths and incubated at room temperature with continuous shaking for five days. The strength of the attachment of the biofilm was continuously observed under optical microscope model BX43F by staining with Methylene blue. Biofilms which had the best attachments (F2B3, F2B2B3, F2B2) and their mono cultures were selected to analyze their efficiency of heavy metal absorption in the liquid media.

2.4 Dye decolorization assay

Ten milliliters of five-day old biofilms and mono cultures were inoculated into series of 250mL Erlenmeyer's flask contained 100ml of Potato Dextrose Broth (PDB) and Nutrient Broth (NB) mixture added with filter sterilized 0.5g/L of Malachite green and Nigrosine disodium dyes separately. The inoculated media were incubated on rotary shaker in 150 rpm speed at room temperature 27°C for 10 days with control contained medium having dye without biofilm and monoculture inoculation and all assays were performed in triplicate. During the incubation, 5 ml of samples were withdrawn at two days' time intervals and centrifuged at 5000 rpm at 4°C for 10 minutes using cooling centrifuge (REMI C-24BL). Absorbance reading of the cell free supernatant was measured using Spectrophotometer at 620 nm for Malachite green and at 600 nm for Nigrosin disodium dyes and the decolorizing activity was expressed in terms of percent decolorization using the formula mentioned above.

2.5 Statistical analysis

Values reported in this paper are the means ± Standard Deviation. The decolorization capacity of each isolate was studied by one-way ANOVA followed by post-Hoc multiple comparisons by Duncan's method using Minitab16 software package.

2.6 Molecular identification of microbial components in the responsive biofilm for the dye decolorization

Genomic DNA of the fungal component of the biofilm was extracted from 5-day old fungi cultures grown on plates using thermolysis method (Zhang *et al*., 2010). Fungal DNA was amplified using universal primers of fungal DNA ITS1 (5'–TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'–TCC TCC GCT TAT TGA TAT GC-3') (White *et al*., 1990). PCR products were purified and were sent for sequencing and obtained sequences were compared with the other related sequences using BLAST search in GenBank (NCBI) (Liu *et al*., 2000). Identification of the bacterial component in the responsive biofilm was done through 16S rDNA sequence analysis. The genomic DNA of each biofilm-forming isolate grown in TSB for 24 to 48 hours was extracted using ZR Fungal/Bacterial DNA Kit™ (Zymo Research California USA) according to the manufacturer's protocol. Polymerase Chain Reaction (PCR) procedure was done using primers 11F (5'GTTTGATCMTGGCTCAG 3') and 1492R (5'TACGGCTACCTTGTTACGACTT3') with 1 µL of undiluted genomic DNA extract as template. Agarose gel electrophoresis was employed to detect amplification of the 16S rDNA of the biofilm-forming isolates. The amplified products were sequenced at the Macrogen Sequencing facility in Korea and sequences were compared with those stored in the Genbank databases of the National Center for Biotechnology Information available on-line using pairwise alignment or BLAST algorithm.

III. RESULTS

3.1 Screening of fungal and bacterial isolates on dye decolorization

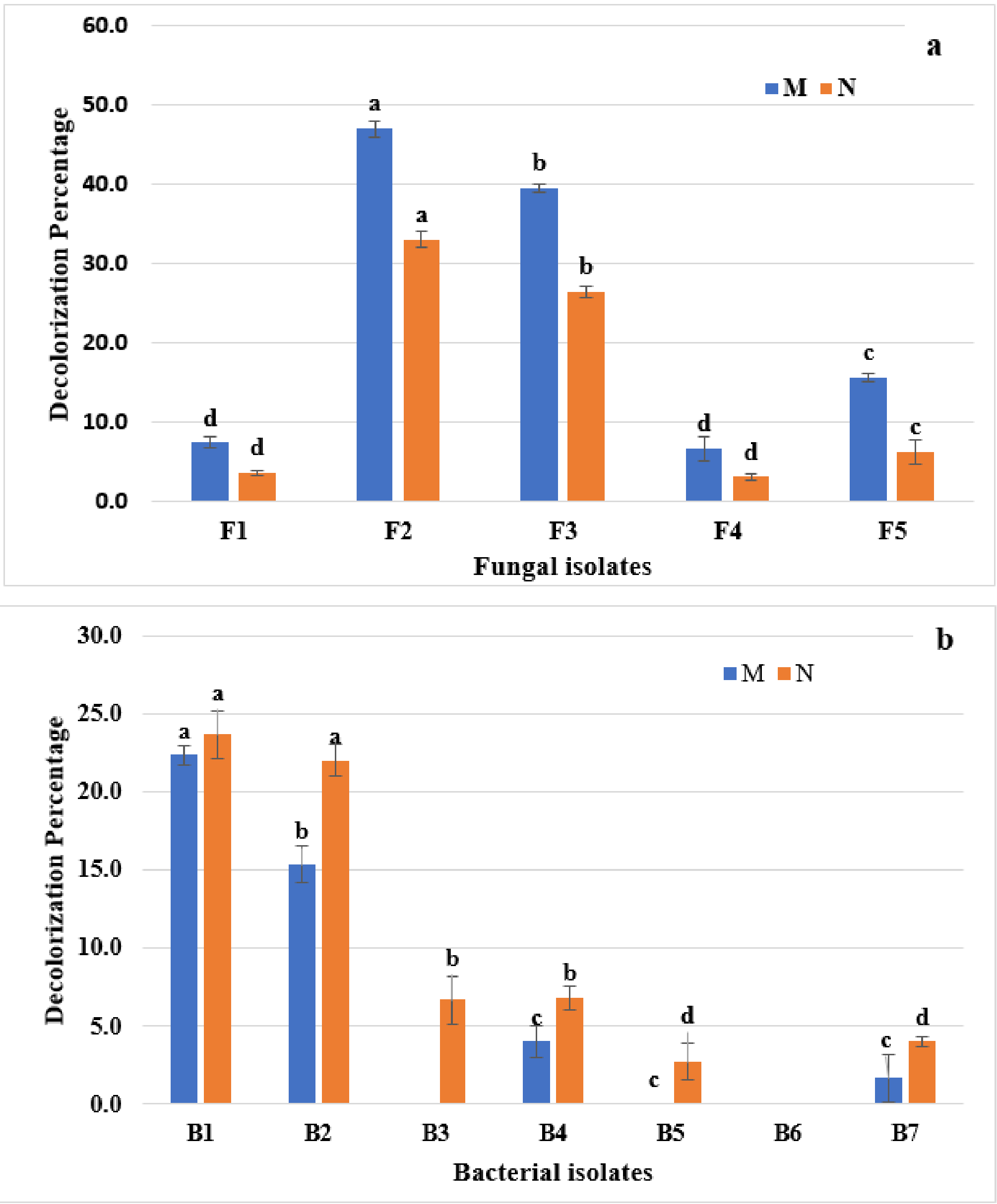

Fig. 1- a decolorization ability of different fungal isolates on malachite green and nigrosin disodium dyes. b. decolorization ability of different bacterial isolates on malachite green and nigrosin disodium dyes. M- malachite green and N- nigrosin disodium. columns with the same letter are not significantly different at 5% probability level. vertical bars show standard deviations.

All the fungal isolates showed decolorization of both Malachite green and Nigrosin disodium dyes (Fig.1a). The results revealed that fungal isolate F2 and F3 showed higher decolorization percentage for both dyes than that of the other fungal isolates. Out of all fungal isolates, the highest significant ($P< 0.05$) decolorization percentage for both Malachite green (47± 0.762) and Nigrosin disodium (33 ± 0.845) was observed from F2 fungal isolate. All the bacterial isolates showed decolorization of both dyes except B5 and B6 (Fig.1b). Further, bacterial

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isolates B1 and B2 showed higher decolorization percentage for both dyes than that of the other bacterial isolates. The highest significant (P< 0.05) decolorization percentage for Malachite green (22.3± 0.435) and the highest decolorization percentage for Nigrosin disodium (23.7 ± 0.612) was observed from B1 bacterial isolate. Decolorization was not recorded from B6 isolate for both dyes. It was observed that all the fungal isolates decolorized more Malachite green dye than Nigrosine disodium dye whereas all the bacterial isolates decolorized more Nigrosine disodium dye than Malachite green dye.

3.2 Biofilm formation

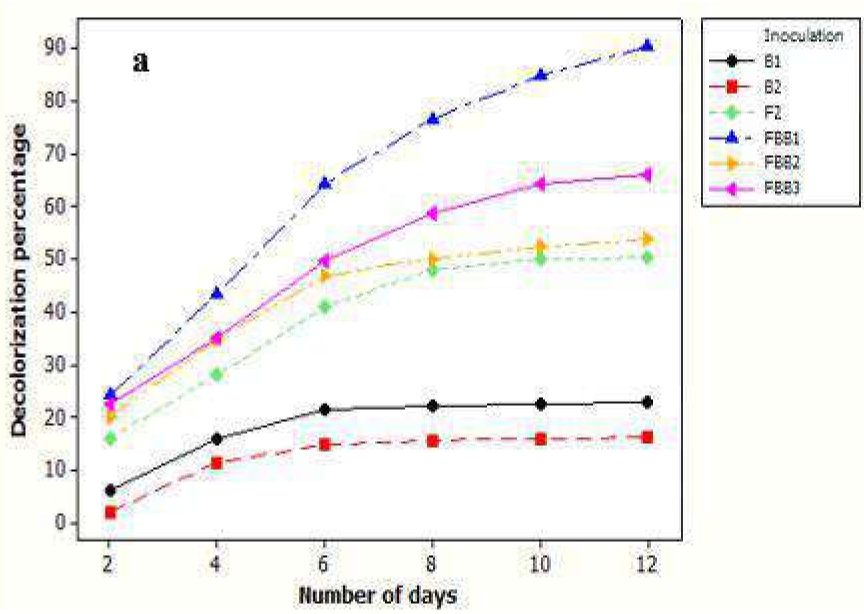
Microscopical observation revealed that except the microbial combinations FBB6, all other combinations showed a biofilm formation and the strength of the attachment varied depending on the microbial composition. Out of all combinations, the highest strength of attachment was observed in FBB1. Biofilm combinations FBB2 and FBB3 also showed higher attachment compared to other biofilm combinations. Therefore, the biofilm combinations (FBB1-FBB3) showed the higher attachments under microscope, were considered for the decolorization assay.

Table 1- Strength of attachment of different monocultures during the formation biofilms

Microbial combination	Strength of the attachment
F2B1 (FBB1)	********
F2B2 (FBB2)	*****
F2B2B1 (FBB3)	****
F3B1 (FBB4)	*
F3B2 (FBB5)	**
F3B1B2 (FBB6)	-

3.3 Dye decolorization assay

All the biofilm combinations showed higher decolorization percentage than that of the monocultures at every time intervals from day 2 to day 12 for both dyes (Fig.2). Although, a sharp increment was observed in the dye decolorization percentage up to day 6 by the fungal isolate F2 and biofilm combinations FBB2 and FBB3 for both dyes, the rate of increment was gradually decreased with the time until day 12. After day 6, it was clear that the dye decolorization rate by the bacterial isolates became constant. However, this pattern was not observed from the biofilm combination FBB1 where the rate showed continuous increment even after day 6 until day 12 and the increment rate showed high compared to other biofilms. Out of all the biofilms, FBB1 showed the highest decolorization percentage values for both dyes at every time intervals until 12. Further, all the bacterial isolates (B1 and B2) showed the least dye decolorization percentage values at every time intervals from day 2 to day 12.



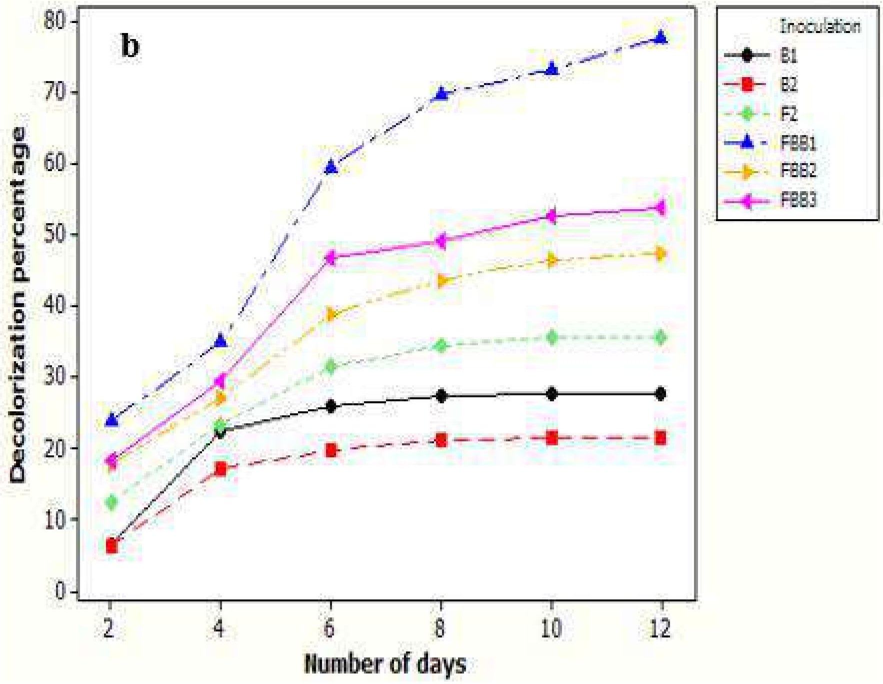


Fig. 2- a Decolorization ability of different biofilm combinations and their monocultures on malachite green dye with the time. b. Decolorization ability of different biofilm combinations and their monocultures on malachite green dye with the time

It was observed that the biofilm combinations showed higher dye decolorization percentage than that of the monocultures for both dyes (Fig. 3). Out of the two dyes all the biofilm combinations and the fungal isolate, F2 decolorized more Malachite green dye than Nigrosin disodium whereas bacterial isolates decolorized more Nigrosine disodium dye than Malachite green dye. Out of all the microbial treatments, the highest significant ($P< 0.05$) dye decolorization percentage was observed by the biofilm combination FBB1 for both dyes. Further, molecular identification confirmed the composition of the highest responsive biofilm FBB1 as *Tricoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens*.

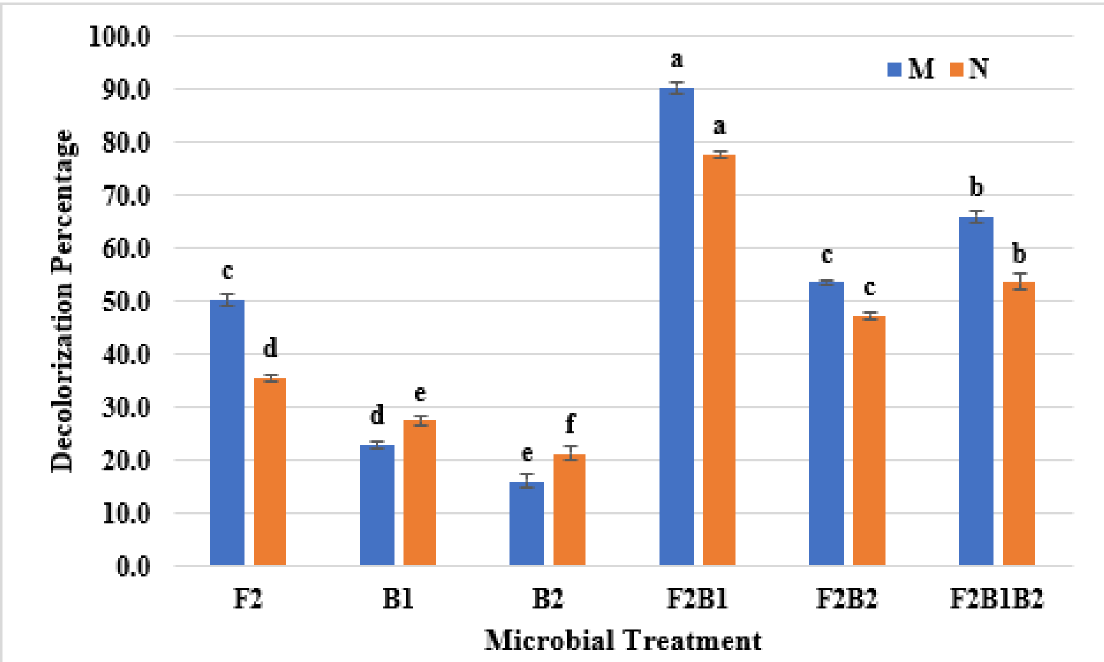


Fig.3- Decolorization ability of different biofilm combinations and their monocultures on malachite green and nigrosin disodium dyes. Columns with the same letter are not significantly different at 5% probability level. Vertical bars show standard deviations.

IV. DISCUSSION

On the basis of the observations of the current study, it was found that the endophytic fungal isolates performed better in decolorizing targeted dyes than bacterial isolates. A previous study reported that fungi have more tolerant to high concentrations of polluting chemicals than bacteria (Casieri *et al*., 2008). Fungi produce various extracellular enzymes, resulting in enhanced bioremediation rates for most of the pollutes (Kaushik and Malik, 2009). Further, fungi have a greater physical contact with the environment due to the presence of increased cell-to-surface ratio. Therefore, fungal systems appear to be most appropriate in the treatment of textile dyes (Ezeronye and Okerentugba, 1999) and also, they had shown better dye reduction potential over the bacteria (Fu and Viraraghavan, 2002). It has been reported that the white rot fungus *Phanerochaete chrysosporium*, grown under ligninolytic conditions, had shown to metabolize crystal violet by sequential N demethylation of the parent compound, which had catalyzed by lignin peroxidase (Cha *et al*., 2001). In a previous report it was demonstrated that supernatants from *Fomes sclerodermeus* with laccase activity were able to degrade malachite green dye (Papinutti *et al*., 2006). Decolorization studies with *Trichoderma virens* showed high decolorization ability (99.6%) for brilliant blue dye (Sweety *et al*., 2017). The current study also showed the ability of the reduction of both dyes by the bacterial isolates. The reason for effective and faster decolorization of the textile dye by bacteria might be associated with the metabolic activities and interactions of the strains (McMullan *et al*., 2001; Phugare *et al*., 2011). It has been reported in a previous study that the significant ability of dye reduction by bacterial species, *Bacillus* sp. and *Pseudomonas* sp. isolated from textile dye effluent contaminated soil (Sriram *et al*., 2013). Further, the efficacy of *Bacillus subtilis* in decolorization of azo dyes have been previously reported (Cheria *et al*., 2012; Ali *et al*., 2014).

The current study clearly showed that biofilms has high decolorization ability than their monocultures. It has been reported that different microbial species present in consortia of biofilms each with different metabolic degradation pathway are capable of degrading several pollutants including dyes and heavy metals either individually or collectively (Gieg *et al*., 2014: Mitra and Mukhopadhyay, 2015). Decolorization of synthetic dyes using consortia offers advantages over the use of single microbial strains (Sudha *et al*., 2014) due to higher degrees of biodegradation resulted from synergistic metabolic activities of the microbial community (Allam, 2017). It has been reported that mixed bacterial cultures from different habitats showed high decolorization of dye molecules in 15 days (Knapp & Newby, 1995). Similar results have been reported by another study that immobilized bacterial consortium of the three bacterial species (*Sphingomonas paucimobilis, Rhizobium radiobacter, and Bacillus subtilis*) had the ability to decolorize azo dyes more efficient than free bacterial cells of single culture (Allam, 2017). Mahmood *et al*. (2015) found that, the consortium of 6 bacterial isolates was able to decolorize 84% of 200 ppm of red, green, black, yellow, and mixed dyes within 24 hours while individual strain required 72 hours.

V. CONCLUSION

Malachite green and Nigrosin disodium dyes are highly degradable with fungal bacterial biofilms in comparison with their mono cultures. Biofilm combination *Tricoderma harzianum, Bacillus subtilis* and *Pseudomonas fluorescens* showed the highest dye reduction ability for both Malachite green and Nigrosin disodium dyes. Therefore, it can be concluded that the biofilms can be used as an efficient biological tool for textile effluent treatment.

VI. ACKNOWLEDGEMENT

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Response of Common Bean Genotypes (*Phaseolus vulgaris* L.) to Drought for Growth and Yield Characteristics in the Southern Highlands of Tanzania

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***Abstract*— *Common bean cultivation is affected by drought up to 60% worldwide and makes the second for yield loss contribution after diseases. Despite the loss, it is estimated that over 75% of rural households in Tanzania depend on common bean for daily sustenance. The objective of the study was to evaluate the response of common bean genotypes in growth and yield characteristics under induced moisture stress in the field at Inyala Agriculture Training Institute in the Southern Highlands of Tanzania. In this study, eighteen common bean genotypes investigated included SER125, MR13905-6, and 41-EX- VAM, BFS20, RCB233, CZ109-22, CZ104-61, KG25-21, SER82, PASS, SER83, KG104-72, SER16, KG4-30, SER45, SER124, BFS60 and RCB266. The experiment was designated in a 3 x 18 split plot arranged in a complete randomized block design (CRBD) with three replications. The main plots were the three moisture treatments such as non moisture stress, stress at flowering and stress at mid pod filling and the sub plots were the common bean genotypes. The plants' variables recorded were number of days to 50% flowering, number of days to 85% maturity, number of pods per plant, weight of pods per plant, weight of seeds per plant, number of seeds per pod, 100 seed weight and yield per hectare. The collected data were subjected to analysis of variance (ANOVA) using GenStat computer software 14th edition. The results showed significant ($p < 0.05$) differences between moisture treatments and bean genotypes. Genotypes SER16, BFS60, KG104-72 and CZ109-22 were significantly superior in grain yields Also, BFS60 was recorded with highest number of pods per plant, weight of pods per plant and weight of seeds per plant, while KG104-72 was recorded as the earliest in 50% flowering and 85% maturity. Genotype SER16 also excelled in weight of seeds per plant. These genotypes therefore can be considered as drought tolerant common bean genotypes and also can be used as parental materials for breeding programmes.*

***Keywords*— Common bean, drought, stress, yield components and yield**

I. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the widely cultivated crops. It is considered to be one of the most important legumes for human consumption (CIAT, 2001; Emam *et al*., 2010) and is an important source of dietary protein, calories, dietary fibers, and minerals, especially iron and zinc. In Africa, it is a primary staple in parts of the Great Lake Regions (Singh *et al*., 2000; Tryphone and Nchimbi-Msolla, 2010). Bean consumption also has medicinal benefits to human health; eating beans may provide protection from cardiovascular disease by a small depression in blood cholesterol (Kabagambe *et al*., 2005). In epidemiological studies of colon cancer, low incidence was observed in many Latin American countries where the consumption of common bean is high (Hengen and Bennink, 2002). Clinical studies consistently showed that when consumed exclusively as a carbohydrate-rich foods, beans reduced postprandial glucose elevations in both diabetic and non-diabetic participants (Tomson *et al*., 2012).

In Tanzania, it is estimated that over 75% of rural households depend on it for daily dietary requirements (CIAT, 2008). Despite the importance of common beans in Tanzania and other developing countries, its production mostly relies on local cultivars (Miklas *et al*., 2006; Chataika *et al*., 2010). Like other plants, the development and productivity of beans is adversely affected by biotic and abiotic factors (Jaleel *et al*., 2009). Among the abiotic factors, drought is the major factor limiting crop production worldwide (Jones and Corlett, 1992). Moisture stress is ranked second after insect pests and diseases that cause grain yield losses with about 60% of world bean production area. With the evolving phenomena of climate change, it is anticipated that drought will exert increasing impacts on crop productivity (Man *et al*., 2011). Drought causes reduction in yield, yield components and biomass accumulation of common beans (Munoz-Perea *et al*., 2006). In the Southern Highlands of Tanzania, the bulk of bean production is done by small scale farmers who depend entirely on rainfall. In these areas intermittent and or terminal droughts are experienced in some years, whilst supplementing the crops with irrigation during drought periods is not common and unaffordable for small scale farmers. Therefore, variety evaluation for drought tolerance in common beans is the appropriate approach for plant breeders to identify superior genotypes for varieties development. The objective of this study was to determine the effect of drought on common bean production in the Southern Highlands of Tanzania.

II. MATERIALS AND METHODS

The experiment was conducted at Inyala Agricultural Training Institute which is located at latitude 84°7'S, longitude 36° 51 E' and altitude of 1100 meters above sea level (m. a. s. l). This location experiences a unimodal rainfall pattern that occurs between November and May every year. The overall average temperature is 17.5 °C. The heaviest rainfall occurs from December to March. The soil characteristics of this area are loam, slightly acidic with a pH of 5.54. Before planting, the land was cleared, ploughed and harrowed using oxen-pulled equipment. Soil sample composite was collected using a hand hoe at a depth of 15-20cm and analyses for physical and chemical characteristics at Uyole soil laboratory as presented in Table1. Weather data including rainfall, minimum and maximum temperatures, relative humidity and solar radiation were recorded at Uyole weather station (Table 2). During planting, fertilizers used were:-triple super phosphate (TSP) (45% P_2O_5) and Urea (4 6 % N). The experiment was laid out in a 3 x 18 split plot arranged in a randomized complete block design (RCBD) with three replications. Subplot, plot size was 2 x 2 m and the spacing used was 0.50 x 0.10 m making a plant population of 84 plants per plot. The main plot (factor A) was moisture treatment with three different stresses periods and sub plot (factor B) was 18 common bean genotypes. Planting was done during the offseason in June 2014 cropping season by putting two seeds per hole at 5cm depth in each row. Fertilizers were then applied uniformly at a rate of 25.3 kg P/ha and 22.5 kg N/ha. Seven days after planting seedlings were thinned to one seedling per hill. Spraying with Amecron 50 EC insecticide at the rate of 2mls/l was carried out to control bean stem maggot, termites and other insects by using a knap sack sprayer. Weeding was done three times using a hand hoe to make sure that there are no weeds in the plots. Moisture stress was induced to the main plots assigned for stress at flowering and mid pod filling when the plants had already attained 50% flowering and mid pod filling stages, respectively. Moisture supply was done through flooding. The duration for moisture stresses applied at both flowering and mid pod filling stages were 20 days. When the bean attained harvestable maturity, they were harvested, sun dried and weighed. The obtained weights of genotypes grown under non-moisture stress and moisture stress at flowering and mid pod filling were subjected to analysis of variance using GenStat computer software and the means were separated using Turkey test.

III. RESULTS

3.1 Soil Analysis

Physical and chemical properties of soil are presented in Table 1. The soil was sandy loam, with a slightly acidic condition (pH 5.3), and cation exchange capacity (CEC) of 15.41 cmol (+)/kg which was high. The quantity of exchangeable bases for potassium and calcium were low (0.12 cmol (+)/kg) and medium (4.49 cmol (+)/kg) respectively, while magnesium was 2.14 cmol (+)/kg, which was also medium. Total nitrogen of the soil was medium (0.13%), while phosphorus was 15.30 mg/kg which was also medium and percent organic carbon was low (0.82%).

Table 1: Physical and chemical characteristics of soil collected from the experimental site

Parameter	Unit	Quantity	Remarks*
Physical characteristics			
Clay	%	28.43	
Silt	%	33.01	Sandy loam
Sand	%	48.59	
Chemical characteristics			
Soil pH(1:25) H2O	pH	5.30	Slightly acidic
CEC	cmol(+)/kg	15.41	High
K	cmol(+)/kg	0.12	Low
Ca	cmol(+)/kg	4.49	Medium

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Mg	cmol(+)/kg	2.14	Medium
TN	%	0.13	Low
OC	%	0.82	Low
P	mg/kg	15.30	Medium

*According to Landon (1991)

3.2 Weather condition

Weather data were collected in 2015, however there were no precipitations data since the experiments were conducted during off season. Non experimental data collected were mean monthly maximum temperatures, which ranged from 17.06°C in September to 27.23°C in October and minimum temperatures, ranged from 5.20°C in May to 14.05°C in November. The mean relative humidity was lowest in August (57.93%) and highest in July (72.7%). Solar radiation was lowest in June (17.72MJm^{-2}day^{-1}), and highest in September (18.73MJm^{-2}day^{-1}).

Table 2: Summarized mean monthly weather data collected during the experiment

Month	Rainfall (mm)	Temperature (^{0}C) Maximum temperature	Minimum temperature	Relative humidity (%)	Radiation (MJm^{-2}d^{-1})
May	0	23.92	5.2	72.1	18.68
June	0	23.52	8.69	70.73	17.72
July	0	20.5	8.6	72.7	18.21
August	0	23.75	7.37	57.93	18.49
September	0	17.06	11.3	60.17	18.73
October	0	27.33	10.3	62.97	18.17
November	0	23.08	14.05	69.6	18.62

Source: Uyole Meteorological Station (2015)

3.3 Effects of moisture tress treatments and common bean genotypes on growth characters

3.3.1 Days to 50% flowering

There were highly significant ($p \leq 0.001$) differences between genotypes on number of days to reach 50% flowering among common bean genotypes (Table 3). The earliest genotype was KG104-72 (34.33) followed by SER82 (36.0), SER125 (36.67) and RCB266 (36.89). These were however, statistically similar. The latest genotypes were 41-EX-VAM (48.56), SER45 (47.56), CZ109-22 (43.56) and MR13905-6 (43.22). In this study, there were no significant differences between moisture treatments on 50% flowering (Table 3).

3.3.2 Days to 85% physiological maturity

There were highly significant ($P \leq 0.001$) differences between genotypes on number of days to reach 85% physiological maturity (Table 3). The earliest genotype to reach 85% maturity was SER82 (83) followed by KG104-72 (84.22), RCB266 (85.44) DAP and SER125 (85.67). However, the earliest genotype (SER82) was statistically similar to KG104-72 and RCB266. The latest genotype was PASS (94.1 days) followed by RCB233 (93.67 days). Genotypes with moderate days to reach 85% was KG25-21 (88.78) followed by CZ109-22 (88.67) and SER83 (88.56) and these were statistically similar. On the other hand longest days for maturity was recorded from PASS (94.11) DAP and RCB233 (93.67), however these two genotypes were statistically similar.

Table 3: Effects of moisture stress treatments and common bean genotypes on growth characteristics

Moisture treatments (a)	50% flowering	85% maturity
SP0	40.37a	94c
SPI	41.3a	84.43a
SPII	41.5a	88.15b
Mean	**41.06**	**88.86**
F.test0.05	**Ns**	**
CV%	**4.0**	**1.0**
Genotype (b)		
KG104-72	34.33a	84.22ab

SER82	36.00ab	83.00a
SER125	36.67abc	85.67bc
RCB266	36.89a-d	85.44abc
SER16	37.78a-e	86.00bc
KG25-21	39.11b-f	88.78ef
PASS	40.89c-f	94.11i
SER124	40.89c-f	86.11bcd
BFS20	41.56def	92.11ghi
BFS60	41.89ef	89.44ef
CZ104-61	42.33ef	92.78hi
KG4-30	42.33ef	87.22cde
SER83	42.44ef	88.56def
RCB233	43.00fg	93.67i
MR13905-6	43.22fg	90.11fg
CZ109-22	43.56fg	88.67ef
SER45	47.56gh	92.78hi
41-EX-VAM	48.56h	90.78fgh
Mean	**41.6**	**88.86**
F.test$_{0.05}$	***	***
CV%	**2.0**	**1.5**

Means in the same column followed by the same letter(s) are not statistically different (P<0.05) by Duncan's New Multiple Range Test. *** = Significant at 0.001.SP0 = without moisture stress, SPI=Stress at flowering and SPII=Stress at mid-pod filling

There were statistical (P≤0.01) differences between moisture regimes in reaching 85% maturity (Table 3). The earliest to reach maturity was stressed at 50% flowering followed by stress at mid pod filling and the latest was unstressed condition.

3.4 Effects of moisture stress and common bean genotypes on yields and yield components

3.4.1 Yield

There were highly significant (P≤0.001) differences between genotypes on yields (Table 4). Genotype with highest yield was SER16 (1419 kg/ha) followed by KG104-72 (1375 kg/ha) and KG4-30 (1374 kg/ha), however KG104-72 and KG4-30 were statistically similar (Table 4). Genotype with lowest seed yield was 41-EX-VAM (763 kg/ha) followed by PASS (824 kg/ha), BFS20 (935 kg/ha), SER45 (1062 kg/ha) and MR 13905-6 (1123 kg/ha). The remaining genotypes had moderate seed yields and were statistically similar. There were highly significant (P≤0.001) differences between moisture regimes on yield. The unstressed treatment produced highest yield (1759 kg/ha) followed by stress at mid pod filling (977 kg/ha) and lowest yield was from stress at 50% flowering (824 kg/ha); however, yields under stress at mid pod filling and 50% flowering were statistically the same.

Table 4: Effects of moisture treatments and common bean genotypes on yield and yield components

Moisture treatments (a)	**Yield (kg/ha)**	**Number of pods per plant**	**Weight of pods per plant (g)**	**Number of seeds per pod**	**weight of seed per plant (g)**	**100 seed weight (g)**
SP0	1759b	17.48c	21.0c	5.12b	14.9c	23.72b
SPI	824a	9.9a	10.23a	4.71a	6.49a	23.43b
SPII	977a	11.44b	16.3b	5.01b	10.99b	20.00a
Mean	**1187**	**12.94**	**15.75**	**5.011**	**10.79**	**22.38**
F.test$_{0.05}$	**	***	***	ns	***	**
CV%	**6.8**	**1.9**	**10**	**1.4**	**10.5**	**1.3**
Genotype (b)						
41-EX-VAM	763a	12.42abc	11.90a	5.156bc	7.90a	17.49a
PASS	824ab	9.49a	14.40a-d	3.933a	9.29abc	30.21g
BFS20	935abc	12.71abc	16.55c-f	4.689b	10.97b-f	25.00f
SER45	1062bcd	19.16d	19.89f	5.244bc	13.68f	20.31b

MR13905-6	1123cde	12.31abc	14.71a-d	4.711bc	10.03a-e	24.29ef
CZ104-61	1152c-f	12.58abc	14.75a-d	4.978bc	9.82a-e	23.50ef
SER82	1184c-f	13.02bc	14.07abc	4.822bc	9.10ab	20.56bc
SER83	1211def	12.82abc	15.88b-e	5.178bc	11.01b-f	21.33bcd
KG25-21	1232def	13.07bc	14.51a-d	4.889bc	10.65a-e	21.32bcd
SER125	1233def	12.75abc	13.96abc	5.328bc	9.74a-d	23.33ef
SER124	1254def	14.49bc	17.76def	5.200bc	12.09c-f	21.15bcd
RCB233	1258def	14.58c	18.40ef	5.156bc	12.69ef	20.64bc
BFS60	1300def	12.44abc	17.80def	4.867bc	12.35def	23.30ef
RCB266	1322def	11.40abc	12.83ab	5.356c	8.96ab	21.07bcd
CZ109-22	1335def	12.64abc	15.87b-e	5.244bc	10.80b-e	22.44cde
KG4-30	1374ef	11.09ab	17.77def	5.178bc	12.74ef	22.85de
KG104-72	1375ef	11.29abc	15.00a-e	5.200bc	10.18a-e	23.34ef
SER16	1419f	14.67c	17.47c-f	5.078bc	12.30def	20.77bc
Mean	**1187**	**12.94**	**15.75**	**5.011**	**10.79**	**22.38**
F.test0.05	***	***	***	***	***	***
CV%	**9.7**	**6**	**4.4**	**4.5**	**7.6**	**3**

Means in the same column followed by the same letter(s) are not statistically different (P<0.05) by Duncan's New Multiple Range Test. ns = Non significant, ** = Significant at 0.01, *** = Significant at 0.001. SP0 = Without moisture stress, SPI=Stress at flowering and SPII=Stress at mid-pod filling

3.4.2 Number of pods per plant

There were highly significant (P≤0.001) differences between genotypes on number of pods per plant (Table 4). Genotype with significantly highest number of pods was SER45 (19.16). This was followed by SER16 (14.67) and RCB233 (14.58); however, these were statistically similar. Lowest number of pods per plant was recorded from PASS (9.49) followed by KG4-30 (11.09), KG104-72 (11.29) and RCB266 (11.40); however, KG4-30 and RCB266 were statistically not different. There were also statistical (P<0.01) differences between moisture regimes on number of pods per plant. The largest number of pods was from unstressed treatment (17.48) followed by stress at mid-pod filling (11.44) and the lowest was from stress at 50% flowering (9.9). All these regimes differed significantly from each other. Although, these two treatments (SPI and SPII) were statistically similar (Table 4).

3.4.3 Weight of pods per plants

There were highly significant (P≤0.001) differences between genotypes on weight of pods per plant (Table 4). Genotype with highest weight of pods per plant was SERF45 (19.89 g) followed by RCB233 (18.40 g), BFS60 (17.80 g), KG4-30 (17.77 g), SER124 (17.76 g), SER16 (17.47 g) and BFS20 (16.55g). However, BFS60, KG4-30 and SER124 were statistically not different. Genotypes with lowest weights of pods was 41-EX-VAM (11.9 g) followed by RCB266 (12.83 g), SER125 (13.96 g) and SER82 (14.07 g), although genotypes SER125 and SER82 were statistically the same. The rest of genotypes had moderate weights of pods per plant. Results across moisture treatments were significant (P≤0.001). Significantly highest weight (21 g) of pods per plant was under unstressed treatment (21 g) followed by those stressed at mid pod fill (16.3 g) and the least weight (10.23 g) was found in stress at flowering.

3.4.4 Number of seeds per pod

There were highly significant (P≤0.001) difference between bean genotypes on number of seeds per pod (Table 4). Genotype with significantly highest number of seeds per pod was RCB266 (5.36). This was followed by SER125 (5.33), while genotype with lowest number (3.93) of seeds per pod was PASS. The remaining genotypes had moderate number of seeds per pod and they were statistically similar. The effect of moisture treatments on number of seeds per pod was significant (Table 4). The highest number of seeds per pod was recorded from those with unstressed treatment (5.12) followed by those stressed at mid pod filling (5.01). The lowest was from those stressed at flowering (4.71)

3.4.5 Weight of seeds per plant

Common bean genotypes showed significant (P≤0.001) differences between genotypes on seeds weight per plant (Table 4). Genotype with significantly highest weight (13.68 g) of seeds per plant was SER45 and this was followed by KG 4-30 (12.74 g) and RCB233 (12.69 g); however, these two genotypes were statistically similar. Genotype with lowest weight of seeds per plant was 41-EX-VAM (7.90g) followed by RCB266 (8.969), SER82 (9.10g) and PASS (9.29). There were statistical (p≤0.001) differences between moisture treatments on weight of seeds per plant. The weight of seeds per plant under unstressed treatment was highest (14.9 g) followed by those stressed at mid pod filling (10.96 g) and least weight (6.52 g) of seeds per plant was from those stressed at 50% flowering.

3.4.6 Weight of 100 seeds

Common bean genotypes differed significantly (P≤0.001) on 100 seed weight (Table 4). Genotype with statistically highest 100 seed weight (30.21 g) was PASS. The latter was followed by BFS20 (25.09 g). Genotypes that followed BFS20 were MR.13905-6 (24.29 g), CZ104-61 (23.50 g), KG104-72 (23.34g), SER125 (23.33g) and BFS60 (23.30 g) and these were not statistically different. Genotype with statistically lowest 100 seed weight was 41-EX-VAM (17.49 g). There were significant (P≤0.01) differences between moisture regimes on 100 seed weight. The highest 100 seed weight was from unstressed treatment (23.72 g) followed by stress at flowering (23.4 g) and these were statistically similar. The least 100 seed weight was from stress at mid-pod filling (20.62 g).

IV. DISCUSSION

4.1 Response of Bean Genotypes to Different moisture Stress Periods on Growth Characteristics

Days to attain 50% flowering among the common bean genotypes differed significantly (P≤0.05) as summarized in Table 3. These results indicate that, there is genetic variability among the common bean genotypes tested on number of days to reach 50% flowering and these results are in agreement with the findings of Yoshinda (1981). The latter revealed genetic variation among genotypes of common bean on days to attain 50% flowering. Similarly, Das (2005) reported considerable variability of traits including number of days to 50% flowering of snap beans. Among the 18 common bean genotypes, there were significant differences among tested genotypes on days to 85% maturity. The variation among genotypes is influenced by the genetic constitution of the individuals. The stress treatments influenced the time taken to attain flowering and maturity in such a way that, beans flowered and matured earlier in a stressed than in non-stressed environments. This situation could be enhanced by a harsh condition (moisture deficit) that faced plants, as a result plants accelerate senescence. Further, in that plants tend to attain reproduction hence propagation before they die giving it a life survival strategy under stressful conditions, the C/N ratio is reached faster where incipient to flowering occurs hence earlier maturity and hence the life circle of the plant is shortened. This act enables the reserved food in leaves to be partitioned to harvestable parts in order to maintain its generation. This type of adjustment mechanism to moisture stress condition was reported by Sabaghpour *et al*. (2003) who found that early phenology, such as early maturity was the most important mechanism for genotypes to escape terminal drought stress as associated with high initial growth vigour. Guar *et al*. (2008) associated the early maturing of the chickpea in the dry areas of India to their drought mechanism. The genotype with early maturity would be less vulnerable to terminal drought and hence suited as drought tolerant genotype. The genotypes with such traits could be selected for better performance and as source of genes for improvement. Similar result was found by Beaver and Rosas (1998) in their study for drought tolerance of common beans, where selection for earlier flowering in red beans permitted the identification of the bean genotypes with a shorter life cycle without affecting its yield potential and with greater rate of partitioning assimilates.

4.2 Effects of moisture stress on yield and yield components

Subjecting genotypes to moisture stress during the reproductive stage reduced grain yields in this study. Moisture regimes affected significantly seed yields. SER16 yielded the highest (2183 kg/ha) under unstressed condition (Table 4). Other genotypes that yielded better were RCB266, BFS 60, kg104-72 with average of 2071 kg/ha, 1999 kg/ha and 1939 kg/ha respectively. The overall difference of yields per hectare across moisture treatments was 44.5% from non-stressed treatment to stress at mid pod filling and 53.2% from unstressed treatment to stress at flowering. Emam et al. (2010) reported that drought stress is one of the limiting factor in crop growth and yield which reduces dry matter production, grain yield, and yield components through decreasing leaf area and accelerating leaf senescence and plant death. Albert, drought stress during all developmental stages significantly reduce the number of pods per plant, seeds per pods, 100 seed weight and consequently yield (Beshir et al., 2016). However, Emam, (1985) and Emam and Seghatoleslami (2005) stated that common bean grain yield is significantly reduced when moisture stress occurs during the reproductive phase. Reduction in grain yield was caused by reduction in the yield components because the grain yield is the product of several yield components and these components are generally the product of sequential development processes. Any reduction in these yield components directly reduces grain yield (Ardakani et al., 2013). Therefore, a reduction in yield is largely due to reduction in number of pods/plant and seeds/pod. The reduction in grain yield is attributed to lower percentage of pod production when the moisture stress occurs during flowering (Emam, 1985) and from embryos abortion when the moisture stress occurs during pod filling stage (Robins and Domingo, 1956). In this study, higher percentage of yield loss occurred with stress at flowering though it did not differ significantly with stress at pod filling. Thus pod production was affected as shown by lowest number of pods formed at stress during flowering. Ardakani et al. (2013) noted that water stress at flowering reduces yield through increased flower failure or abortion and consequently number of pods is reduced by aborted seeds. Barrios *et al*. (2005) reported that seed yield reduction of up to 60 % observed in common beans under drought stress was attributed to losses of 63.3 % in pods per plant, 28.9 % in seed per pod and 22.3 % in seed weight per plant. In this study, the number of pods per plant was significantly influenced by moisture stress treatments. The introduction of moisture stress lowered the number of pods per plant and seed number per pod. This finding is in agreement with that obtained by Castañeda *et al*. (2009) that high moisture stress during the reproductive stage exposed the plant to floral abortion and resulted in low seed yield. Other authors (Singh 1995; Sponchiado *et al*. 1989) reported that moisture stress imposed during flowering and pod setting caused flower and pod abortion. Generally, the reproductive stage is the most sensitive to drought stress (Nielsen and Nelson, 1998). This phase includes flower formation (Pedroza and Muñoz, 1993), full flowering (Pimentel *et al*., 1999), pod formation (Castañeda *et al*., 200), or grain filling (Nielsen and Nelson, 1998). Moisture deficit caused falling or abortion of reproductive structures in soybeans (Beshir *et al*., 2016) and reduced pollen formation and pollination in common bean (Boutra and Sanders, 2001). The need to maintain high pod number under moisture stress condition in common bean is vital, since it constitutes an important yield component that determines final yield. Confalone *et al*. (1991) reported that the number of pods per plant constitute the main yield component which is mostly affected by moisture deficit during flowering stage and can reduce seed yield up to 70 % depending on the duration and severity of the moisture stress. Lopez *et al*. (1996) reported that total number of flowers in some susceptible varieties may be reduced up to 47 % under drought conditions thereby influencing the number of pods per plant; though pod setting may also vary among different common bean varieties in response to drought.

In this investigation, the heaviest pods per plant were recorded measured from unstressed treatment. The mean difference of pods weight per plant between unstressed treatment and stressed at mid pod filling was 24% and when stressed at flowering, the difference was 52% (Table 4) for the field experiment. Results indicated that moisture stress exerted at flowering and mid pod filling growth stages affected plants by reducing weight of pods, as drought affects formation of pods and development of reproductive parts of the plant. Due to this reason, beans may from empty pods and hence are lighter compared to beans grown under unstressed treatments and not affected by early abortion of seed embryos. In determination of seed weight per plant, results showed that trend of seed weight per plant among genotypes was significant different (Table 4). The highest quantity of seed weight per plant was measured from unstressed treatment followed by stress at mid pod filling and these did not differ significantly from each other. The least was recorded from stress at flowering. The latter differed significantly from the rest. Seed weight per plant under unstressed treatment differed by 32.9% from those stressed at mid pod filling and 56% from those stressed at flowering. This implies that stress at flowering reduce photosynthesis and consequently translocation and portioning of photosynthetic materials decreases. Our results are supported by the findings of Mohammadzadeh et al., (2011).

Number of seeds per pod varied from 4 to 5 among genotypes, however, PASS was observed to contain an average of less than four seeds. Moisture treatments affected number of seeds per pod especially for beans stressed at flowering. This observation corresponds with the findings of Nuñez *et al*. (2005) who also identified number of pods per plant as the principal cause of yield losses of bean subjected to drought stress, followed by the number of seeds per pod and seed weight.

In this study, 100 seed weight obtained under unstressed treatment was statistically the same with those plots stressed at flowering and was significantly different from those plots stressed at mid pod filling (Table 4). 100 seed weight at mid pod filling was the lowest and highly affected by moisture deficit. This observation signifies that, 100 seed weight stressed at flowering was less because when irrigation was resumed after 20 days it was the period of pod filling for most of genotypes. This phenomenon enabled these genotypes to maximize translocation of assimilates to the seeds and finally the seeds size and dry matter weight. However for those stressed at mid pod filling, stress was experienced when the seed size and weight were at critical stage of development. This shortage of soil moisture, lead to under development of seeds and finally, 100 seed weight decrease. Teran and Singh (2002) reported that drought stress, on the average reduced common bean 100 seed weight by 13 %. Singh (1995) observed a decrease in grain yield and mean weight of a hundred seeds along with accelerated maturity among these characteristics.

V. CONCLUSION

Common bean genotypes differed significantly in their growth characteristics, yield and yield components, when evaluated in different moisture stress treatments and across genotypes. Some genotypes yielded better while others were moderate and few yielded poorly. Highest yields among genotypes stressed at flowering were; BFS60, KG104-72, SER16, MR13905-6, CZ109-22, and SER125. Highest yields among genotypes stressed at mid-pod filling were; KG4-30, RCB266, KG25-21, KG104-72 and SER125. Despite the fact that results demonstrated no significant effect of interactions of the drought treatments and common bean genotypes, genotypes that yielded relatively better under both stresses (stress at flowering and mid pod filling) were SER16, KG104-72 and SER125. Genotypes that yielded better under all conditions (non stress, stress at flowering and mid pod filling) were SER16, KG104-72, KG4-30, and CZ109-22. Since the study was based on the observations of morphological response of bean genotypes treated with different moisture stress periods, therefore there is need to investigate the presence of any physiological mechanisms involved in providing tolerance under limited moisture. This knowledge will help to improve selection criteria for drought tolerance of common bean. Genotypes SER16, BFS60 and KG104-72 were observed to be superior in yield under moisture stress conditions; therefore, it is recommended that, those genotypes could be used as a source of breeding materials for drought resistance in areas which are affected by drought at flowering. In areas where droughts occur during mid-pod filling, it is recommend that genotypes KG4-30, RCB266, KG104-72 and SER125 could be used as a source of breeding materials for drought resistance. Since the selection was based on morphological characterization, it is recommended that, marker assisted selection could be deployed to confirm the findings.

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Establishment the Synchronization System for Mass Clonal Propagation of Sugarcane (*Saccharumofficinarum L*) through Shoot tip Culture

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Abstract—*The aim of present investigation was to examine the efficiency of three different sugarcane (Saccharumofficinarum L.) verities viz. NIA 1026-P7, NIA-87 and SG NIA-2476.A total of 20 explants (apical meristems) shoot tip were inoculated on MS Basal media along with various concentrations of 2, 4-D, BAP, Kinetin and IAA. The results of different verities showed that initial growth, number of micro shoots and shoot length was obtained in variety NIA-1026-P7, on MS medium supplemented with low concentration of 2, 4Dichlorophenoxyacetic acid (2, 4-D) along with highest concentration of Benzyl aminopurine (BAP) and Kinetin. The variety SG NIA 2476 expressed modestly and was not sufficient as compared with other varieties. The established plantlets were transferred on the MS half strength rooting medium along with various concentrations of IBA and IAA. The results of different varieties showed that maximum number of micro roots and roots length were established in variety NIA 87, on the half strength MS medium plus 1.50 mg/l IBA, 3.0 mg/l IAA and 25 g/l of sugar.*

Keywords— Auxin, Cytokinin, In-vitro, Plant Growth hormones, Sugarcane.

I. INTRODUCTION

Sugarcane (*Saccharumofficinarum,* L.) is the agro-industrial cash crop of Pakistan belongs to the *poaceae* family and cultivated in tropical and subtropical area of the world. Due to high economic importance in Pakistan mostly contributed share of country; where accounts about 70% of worlds total sugar production [1, 2]. The production of sugarcane described a very promising picture and reached to historical high of 73.6 million tons showing with increase of 12.4 % over the production of 65.5 million tons during 2015-16 and comfortably exceeded target of 67.5 million tons by a considerable margin of 9.0 % [3]. Pakistan occupies 5thposition in respect to area under sugarcane crop and almost 15thposition in cane production but ranks far below in sugar production [4]. Pakistan average yield of sugarcane is 48.9 tonnes per hector as compared with globally which is nearby 65.5 tonnes per hector, however Egypt and India are getting around 60 tonnes and 121 tonnes per hector individually [5]. Inefficient yield and lower sugar recovery cause high production cost that's why Pakistan the minimum aggressive in domestic and global sugar markets [6]. Generally conventional propagation of sugarcane multiplied vegetatively by stem cuttings which suffered from low propagation rates, expensive labour, time consuming and potential transmission of pathogens through seed cane from generation to generation [7]. It is still a problem due to unfavourable climatic conditions. Thus, lack of viable fuzz production makes it difficult to improve sugarcane through conventional breeding in Pakistan [8]. Therefore, now a day's most widely used techniques plant tissue culture like a Micro propagation and *in vitro* propagation is currently the only realistic means of achieving rapid, large-scale production of disease-free quality planting material as seed canes of newly developed varieties in order to speed up the breeding and commercialization process in sugarcane [9].

Plant tissue culture is currently a powerful tool that plays a major role in rapid multiplication of disease free planting material of newly improved varieties through in vitro technique on continuous year rounded basis [10]. Shoot tip culture is relatively simple *in vitro* method for rapid propagation of selected sugarcane materials and the clean or pathogen free plant materials [11]. As a result of the regeneration of plants through tissue culture technique could be a viable option for improving the quality and productivity of sugarcane. So far, a lot of reports have been published in the tissue culture of sugarcane from different countries [12, 13].

The aim of present investigation to determine the efficiency of three different sugarcane varieties were tested their performance under synchronization system for regeneration of shoots and roots.

II. MATERIAL AND METHOD

2.1. Collection of explants/ Growth media preparation

The present experiment was done at Plant tissue Culture Laboratory, Plant Breeding Genetics Division, Nuclear Institute of Agriculture (NIA) Tando Jam. Three sugarcane` varieties Viz. NIA-1026-P7, NIA-87 and SG-NIA-2476 were tested in this experiment. Fresh, healthy and young apical meristems were collected from Experimental Farm, Nuclear Institute of Agriculture (NIA) Tando Jam.The Murashige and Skoog [14]medium containing with different concentration of 2, 4-D, BAP, IAA and Kinetin for shoot induction, for induction of rooting plantlets were transferred half strength MS basal media added various concentrations of IBA and IAA.

2.2. Surface Sterilization

The excised young and mature shoot tip removed leaf sheath on the surface of explants and cut into 1 to 1.5 cm long segments and washed in running tap water for five minutes, surface disinfected using 70 % ethanol for 40 second and then immersed in 10 % sodium hypochlorite solution of commercial laundry bleach (5.25% NaOCl). The pH of medium was adjusted at 5.7-5.8 before autoclaving and media was autoclaved at 121 °C for 20 minutes.

2.3. Tissue culturing

After that plant samples were brought under the laminar airflow cabinet and washed thrice with sterilized distilled water. The soft segment of apical meristem then cut into small disc and placed on MS basal medium supplemented with different concentration of plant growth hormones.

2.4. Culture maintenances

After successfully explants were inoculated in the culture bottles then these culture bottles were transferred in the incubator for one week, after one week it is again transferred in the growth room forUniform culture conditions were maintained as 16-hour photoperiod at 25±2 ^{o}C for growth temperature.

2.5. Obtained data and phenotypic analysis

The experiments were laid out in completely randomized design (CRD) with three replications. The phenotypic data were collected, days taken shoot initiation, number of micro shoots, shoot length (cm), number of micro roots and root length (cm).

2.6. Statistical Analysis

The experimental data were recorded and subjected to factorial design of analysis of variance (ANOVA) under linear models of statistics to observe statistical differences among different traits of wheat using computer program, Student Edition of Statistix (SWX), Version 8.1 (Copyright, Analytical Software-USA). Further least significant difference (LSD) test was also applied to test the level of significance among different combination means 15, 16].

III. RESULTS AND DICUSSION

3.1 Hormonal Concentration Regulates the Micro-Shoot Initiation

The results of different varieties showed that days taken to shoots initiation (40.91) was observed in variety NIA-1026-P7 and later days taken to shoots initiation (50.83) was recorded in variety SG-NIA-2476 (Figure 1).The results of different varieties indicated that maximum number of micro shoots were recorded (40.75) in variety NIA-1026-P7 and minimum number of micro shoots were observed (28.41) in variety SG-NIA-2476 (Figure 2).The results of varieties indicated that highest shoots length were recorded (6.22 cm) in variety NIA-1026-P7 and lowest shoots length were recorded (5.58 cm) in variety SG-NIA-2476 (Figure 3).The results of different varieties indicated maximum number of roots were recorded (14.58, 11.17 and 10.16) in varieties NIA-1026-P7, NIA-87 and SG-NIA-2476 separately (Figure 4).The results of different varieties showed that days taken to shoots initiation concurred with [17] publicised a protocol for stimulation and organogenesis shoots proliferation, found early days to shoots initiation for tip extension were observed 15 and 20 days with the concentration MS media containing 3 % sugar combined with BAP and Kin, 1.50 mg L^{-1} GA_3 3.0 mg L^{-1} resulted about 100 % explants showing shooting. However the result number of shoots which corresponded with [18] developed the method for callus and regeneration was set up under *in vitro* culture developing immature shoot tip as explants, maximum shoot enlistment was observed in MS medium added with 2.50 mg L^{-1} 2, 4-D, 2.0 mg L^{-1}BAP and 0.5 mg L^{-1} NAA.The results equally comparable with [19] publicised that variety B4906 gave the highest (16.88) shoots with 5.94 cm average shoot length in MS medium added with 1.5 mg L^{-1}BAP and 0.4 mg L^{-1} NAA while, Pr1013 delivered highest 11.70 shoots explants^{-1} with 4.48 cm shoot length on MS media sustained by 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA [17].

3.2 Rooting of established shoots inhibited by the interaction of Auxin-Verities

The results of different varieties indicated that highest roots length were observed (5.90 cm) in variety NIA-87 and lowest roots length were recorded (4.66 cm) in variety SG-NIA-2476 (Figure 5). Among the combinations, IBA and NAA indicated best response with professed establishing MS½medium added with 2.50 mgl^{-1} NAA showed best response and the highest number of roots per micro shoots were 13.9 obtained within 8-10days with average root length 4.3 cm for the variety Co-91017. The stronger root growth development was impacted by IBA at the concentration of 1.0 mg $L^{-1}$with the maximum number of 41 roots $plant^{-1}$ and poor quality rooting response was observed at 0.1-0.5 mg L^{-1}IBA combined with 0.5-2.0 mg L^{-1} BAP. The concurred with[19] observed the rooting response at concentration of 2.50 mg L^{-1} IBA and it exhibits 16 numbers of roots at the length of 1.1 cm, whereas 0.5 mg L^{-1} NAA + 2.50 mg L^{-1} IBA indicated rooting response with 11.3 with a number of roots and 3.7 cm root length.

IV. CONCULSION

It was concluded that days to micro shoots initiation, number of micro shoots, can be obtained on MS + 2.0 mg L^{-1} 2, 4-D + 4.0 mg L^{-1} BAP + 20 g L^{-1} sugar in variety NIA-1026-P7 (Figure 6-7). while the shoots length (cm) can be achieved on MS + 1.0 mg L^{-1} 2, 4-D + 4.0 mg L^{-1} IAA + 2.0 mg L^{-1} Kin + 20 g L^{-1} sugar in variety NIA-1026-P7, however the number of micro roots and roots length (cm) can be obtained on MS½ + 3.0 mg L^{-1} IBA + 1.50 mg L^{-1} IAA + 30 g L^{-1} sugar in variety NIA-87.

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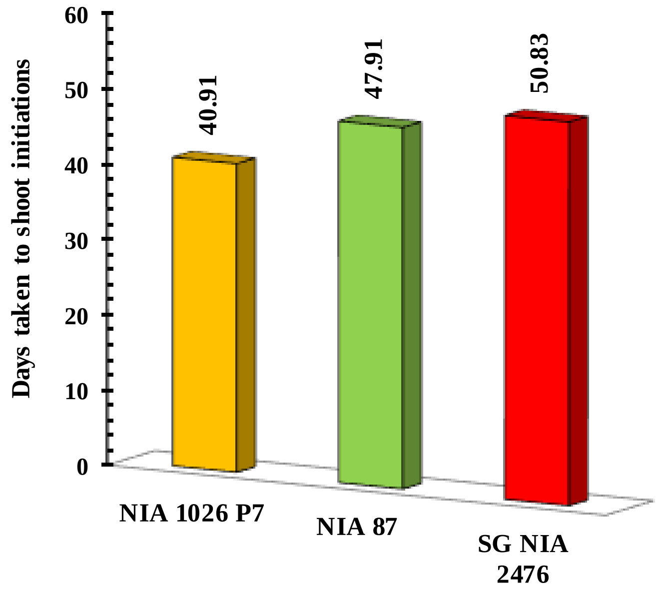

Fig.1: Invitro regeneration of sugarcane through shoot tip culture. *The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.*

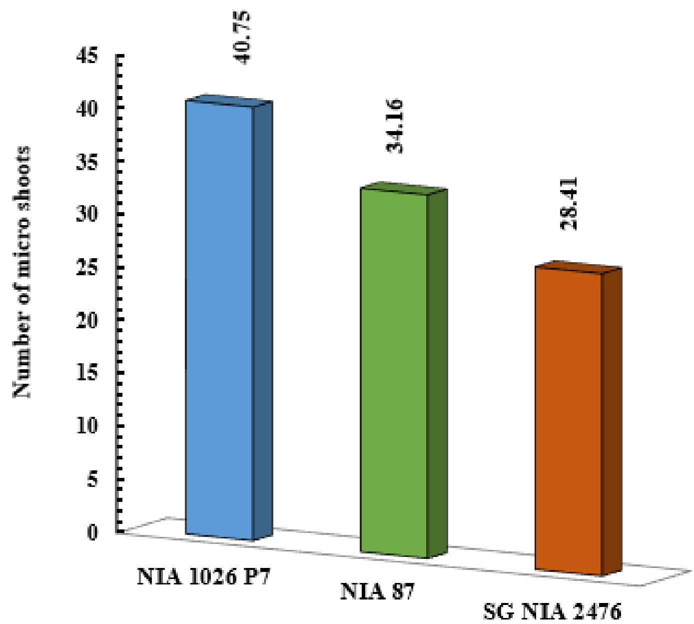

Fig.2: Encountering the number of micro shoots. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.

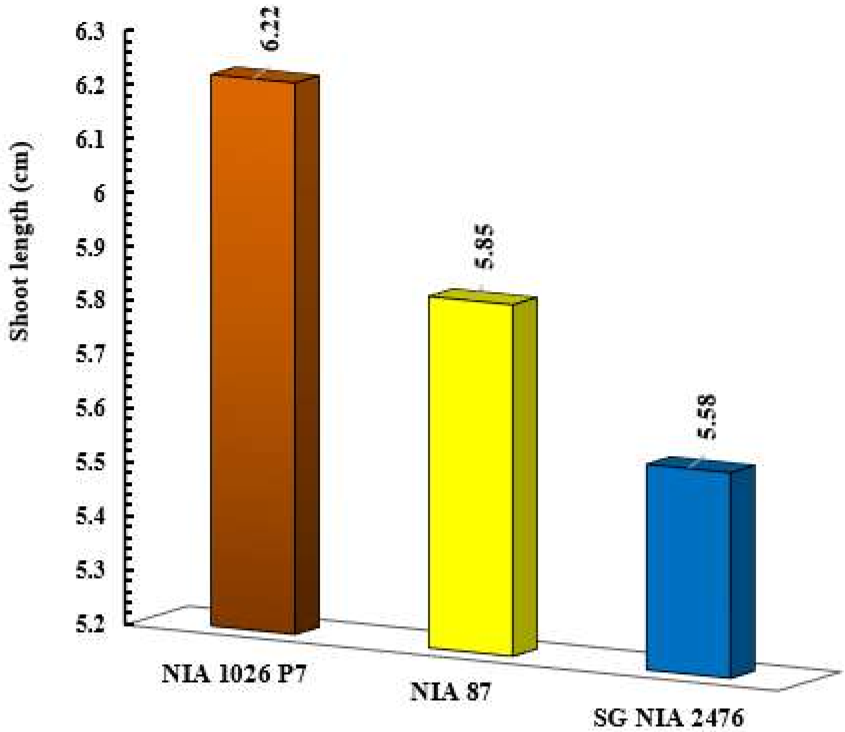

Fig.3: Shoots length modulated by different varieties of sugarcane. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.

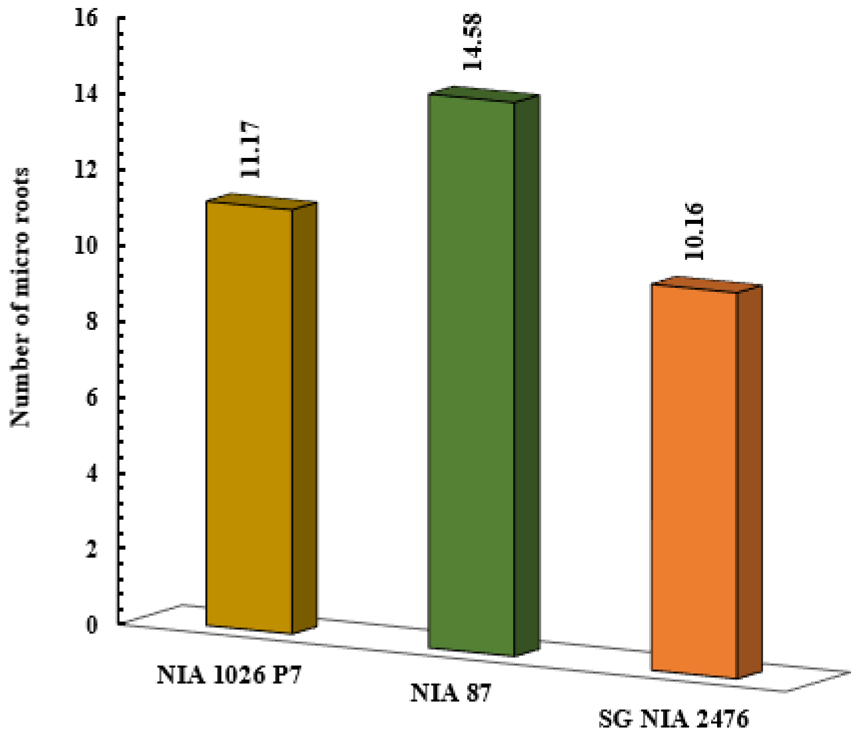

Fig.4: Micro roots maximization in different varieties of sugarcane. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.

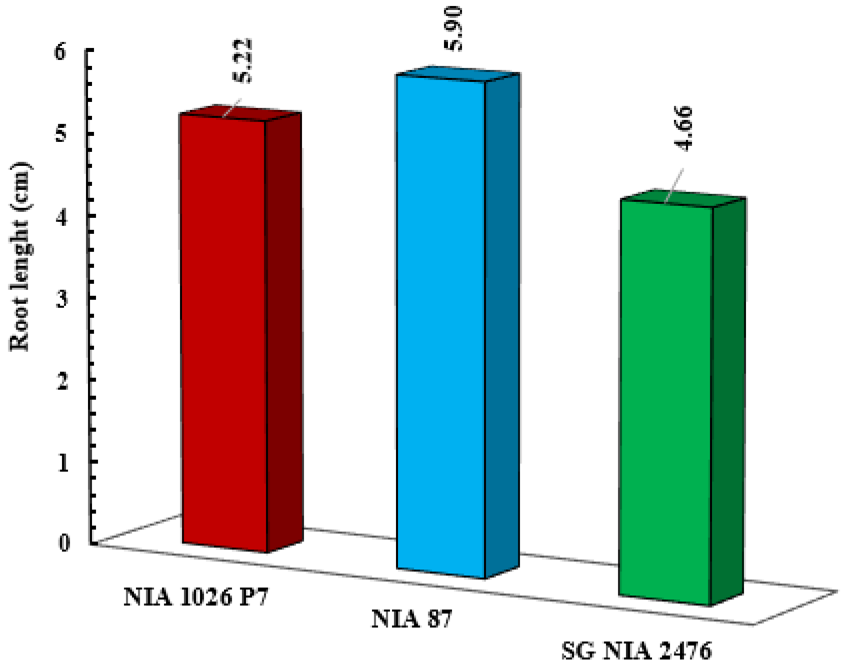

Fig.5: Slight inhibition of root length. The treatment means were compared using Least Significant Difference (LSD) at 5% level of Probability. NIA 1026 P7, NIA87, SG NIA 2476 represents the different verities of sugarcane. Data are the average of three different biological replications.

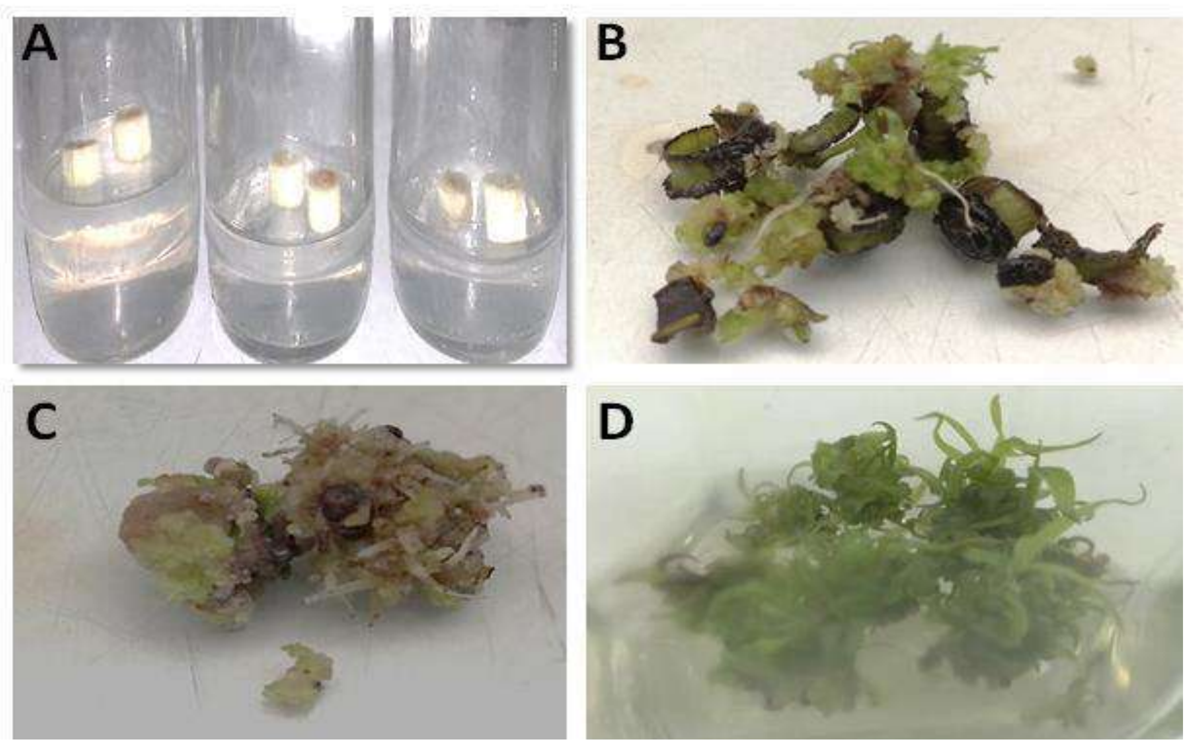

Fig.6: Mass-clonal proliferation of sugarcane on invitro condition. (A)Disc inoculated in the culture bottles(B)Growth starting from small segment of meristic tissue (C) Differentiate the tissue in the micro shoots(D)Establishment of seedling. Experiments performed thrice as different biological replications. Data so obtained at two days interval on each replications. Regenerated shoots were then transferred to rooting media.

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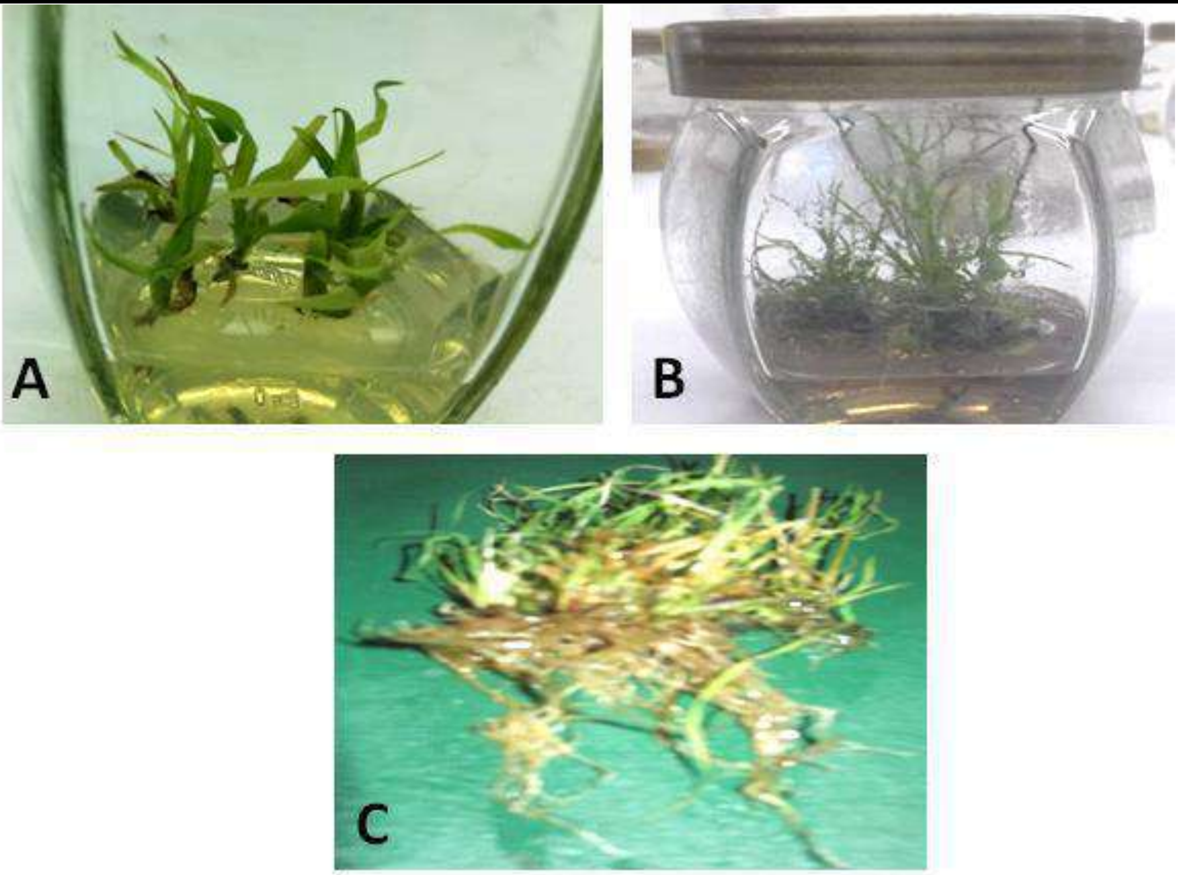

Fig.7: Mass-clonal production and multiplication of sugarcane. (A) Plantlets ready for establishing further height (B) Height of seedling (C) Established maximum micro roots and its length Experiments performed thrice as different biological replications. Data so obtained at two days interval on each replications. Rooted seedlings ready for acclimatization

Supplementary Tables

Table S.1. Regeneration of sugarcane on controlled condition.

	Days taken to shoot initiations		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	40.91 c	47.91 b	50.83 a
	Number of micro shoots		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	40.75 a	34.16 b	28.41 c
	Shoot length		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	6.22 a	5.85 b	5.58 b
	Number of micro roots		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	11.17 b	14.58 a	10.16 b
	Root length		
Varieties	NIA-1026-P7	NIA-87	SG-NIA-2476
Mean	5.22 b	5.90 a	4.66 c

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Table S.2 Composition of MS basal medium (Murashige and Skoog, 1962)

INGREDIENTS OF MS MEDIUM			
Serial No	**Macro Nutrients**		
	Ingredients	**Chemical composition**	**Weight mg l^{-1}**
1	Potassium nitrate	KNO_3	23.5 g l^{-1}
2	Ammonium nitrate	NH_4NO_3	20.5 g l^{-1}
3	Calcium chloride	$CaCL_2$ $2H_2O$	5.5 g l^{-1}
4	Potassium diphosphate	KH_2PO_4	2.5 g l^{-1}
5	Magnesium sulphate	$MgSO_4$ $7H_2O$	4.5 g l^{-1}
6	Potassium nitrate	KNO_3	23.5 g l^{-1}
	Micro Nutrients		
7	Boric Acid	H_3BO_3	0.31
8	Manganesesulphate	$MgSo_4$ $4H_2O$	1.15
9	Zinc sulphate	$ZnSo_4$ $7H_2O$	0.430
10	Ferrous sulphate	Fe SO_4 $7H_2O$	1.390
11	Sodium molybdate	Na_2 M_oO_4 $2H_2O$	0.12
12	Copper sulphate	$CuSO_4$ 5 H_2O	1.25
13	Cobalt chloride	$CoSo_3$ 6 H_2O	1.25
	Iron source		
14	Iron Sulphate	$FeSo_4$ 7 H_2O	27.8
15	Sodium EDTA	Na_2 EDTA	1.865
	Vitamins		
16	Thiamine HCL	$C_{12}H_8N_8$ $OSCL_2$	2.0 mg l^{-1}
17	Myo-inosital		200 mg l^{-1}
	Other		
18	Sugar	$C_{12}H_{22}O_{11}$	20 g l^{-1}
19	Agar		7.5 g l^{-1}
20	pH		5.8

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Use of Various concentrations of plant growth regulator (PGR) of Sweet Corn on the Growth and Production of sweet potato (Ipomoea batatas L.)

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Abstract -- *The use of Plant Growth Regulating can be sourced from synthetic products and products made from natural or organic ingredients. This study aims to determine the effect of the use of various plant growth regulating (PGR) concentrations of sweet corn on the growth and production of Sweet Potatoes. The study was conducted in Wiringpalennae Sub-District, Tempe Subdistrict, Wajo Regency, South Sulawesi Province, Indonesia 2018. The method used was Randomized Block Design (RBD), consisting of four (4) treatments, namely without PGR (z_0), PGR 3 ml / l water (z_1), PGR 5 ml / l water (z_2), and PGR 7 ml / l water (z_3). Each treatment was repeated three times so that the number of treatment combinations was 12 combinations. The results of the study indicate that the administration of 7 ml / l of water in sweet potato produced an average production of fresh tuber 60.51 t ha^{-1}.*

Keywords— Growth, PGR Sweet Corn, Production, Sweet Potatoes.

I. INTRODUCTION

Sweet potato (Ipomoea batatas L) is a crop of potatoes group, originally from Latin America (Widowati et al., 2010). Sweet potato plants in Indonesia are one of the most important plants, both as an alternative staple food in the dry season and as an additional food in order to diversify food ingredients. Indonesian sweet potato production can be said to be still low. The results of the wet bulb on average at a rate of as much as 15.20 Notional 2014 tons per hectare harvested area of 156 758 ha with a total production of 2,382,658 million tons, while in 2015 increased by 16.10 tons per hectare, while harvested area decreased namely 143,125 ha with a total production of 2,297,634 million tons (BPS, 2016). According to Astawan and Widowati (2007) that with advanced technology some superior varieties of sweet potatoes can produce more than 30 tons of wet bulbs / ha.

Efforts to increase sweet potato production are still being carried out, so that efforts can be taken with various kinds of technology packages, one of the ways that can be done to achieve high production goals is through the provision of Plant Growth Regulating (PGR). Along with the current development of agriculture towards sustainable agriculture, it is directed to the actors of farming to use organic matter as a source of nutrients and Plant Growth Regulating (PGR) to increase plant growth and yield. The use of Plant Growth Regulating can be sourced from synthetic products and products made from natural or organic ingredients. Organic farming in a broad sense is an agricultural production system that relies on natural materials and avoids or limits the use of synthetic chemicals, especially plant growth regulators, with the aim of providing agricultural products (especially food) that are safe for the health of producers and consumers and maintain environmental balance by maintaining its natural cycle.

Plant growth regulator is an organic compound instead of nutrients in low concentrations can encourage, inhibit or qualitatively alter plant growth and development (Widyastuti and Tjokrokusumo 2001 in Asrijal et al., 2018a). One source of PGR can be used to stimulate plant growth can be obtained from the seeds of sweet corn crop is processed and then fermented in liquid form so it can function as a plant growth regulator. According to Asrijal at al. (2018b), Results of research on the use of plant growth regulator (PGR) sweet corn with a concentration of 1 ml / l of water on onion yield an average production of 12:08 t ha^{-1}.

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II. MATERIALS AND METHODS

The study was conducted in Leweng Village, TakkalallaSubdistrict, Wajo District. The method used was Randomized Block Design (RBD), consisting of four (4) treatments, namely without PGR (z_0), PGR 3 ml / l water (z_1), PGR 5 ml / l water (z_2), and PGR 7 ml / l water (z_3). Each treatment was repeated three times so that the number of treatment combinations was 12 combinations. Data were analyzed according to Gaspersz (1991).

III. RESULTS AND DISCUSSION

3.1. Research result

3.1.1. Number of Bulbs

The results of the average observation of the number of sweet potato tubers and their variance showed that the treatment of various concentrations of sweet potato PGR on the number of sweet potato tubers showed no significant effect (Fig. 1).

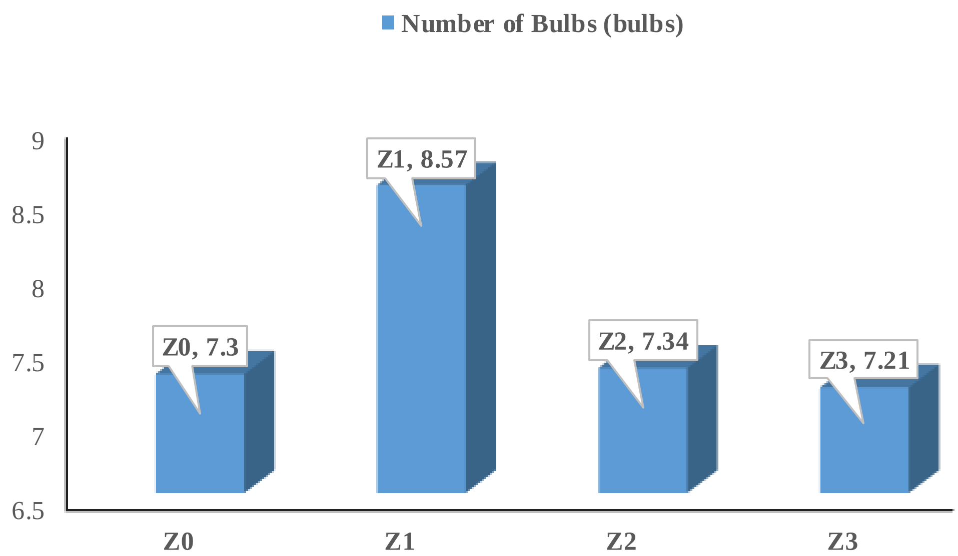

Fig. 1. Average diagram of observations of the bulbs number of Sweet Potatoes.

3.1.2. Bulb Diameter

The results of the average observation of sweet potato tuber diameter and variance showed that the treatment of various concentrations of PGR sweet corn on the tuber diameter of sweet potatoes showed no significant effect (Fig. 2).

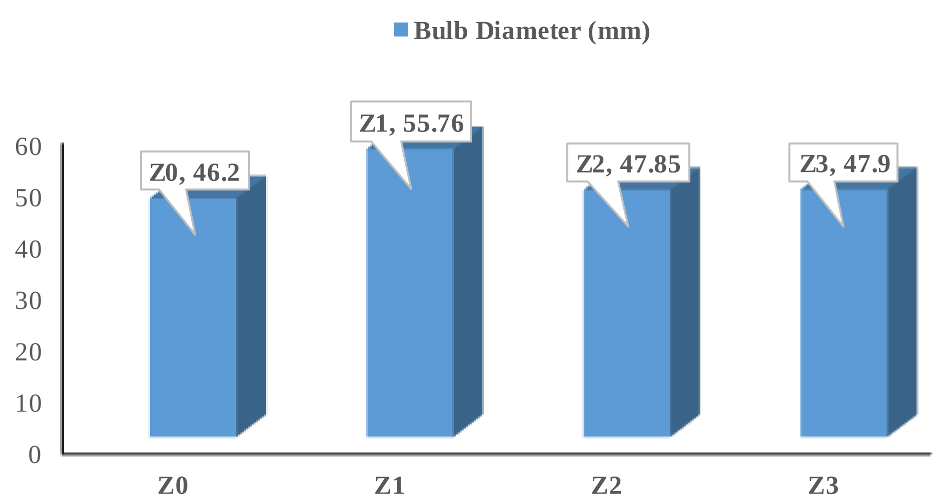

Fig. 2. Average diagram of observed of the bulb diameter of Sweet Potatoes

3.1.3. Production per hectare

The results of the observation of the average production per hectare of sweet potato and its variance showed that the treatment of various concentrations of PGR sweet corn to the production per hectare of sweet potato showed a non-significant effect (Fig. 3).

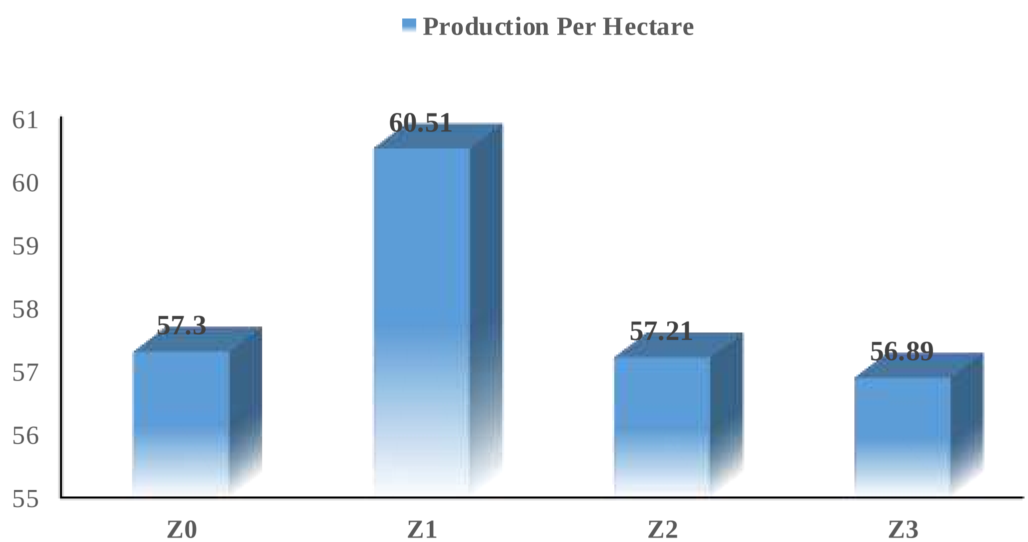

Fig. 3 Average chart of production observations per hectare of Sweet Potatoes (tons)

3.2. Discussion

Based on the results of research and analysis of data on the use of various concentrations of plant growth regulator (PGR) sweet corn on the growth and production of sweet potato shows that the parameter observation bulb number, diameter bulb and bulb production per hectare shows the results of the highest average in the treatment of PGR concentration of 3 ml / l water, respectively 8.57 bulbs per plant (Fig. 1), 55.76 mm bulb diameter (Fig. 2), and 60.51 t ha^{-1} (Fig. 3). This is caused because the content of plant growth regulator, auxin and gibberellin primarily of sweet corn extract is able to stimulate the growth and development of sweet potato bulbs.

According to Manik (2011). Auxin serves to help in the process of accelerating growth, both root growth and stem growth, accelerating germination, helping in the process of cell division, accelerating fruit ripening, reducing the number of seeds in the fruit. High levels of giberelin will stimulate cell division and elongation at the apex of the shoot, especially in the meristematic cells, thus spurring vegetative growth (Komariyah, 2012).

According to Soelaiman (2012). Two groups of growth regulators that are very important in tissue culture are auxin and cytokinin. Auxin is a growth regulator that plays a role in root formation, callus, inhibitory shoot formation inhibitors and axillary buds, and cell differentiation. Low concentration of gibberellin does not stimulate root formation, but at high concentrations stimulates root formation (Jauhar et al., 2013). (Karadeniz et al., 2006); (Pandingan and Nainggolan, 2006) Gibberellins function in the process of seed formation, which stimulates the formation of pollen (pollen), enlarges fruit size, stimulates flower formation, and ends the period of seed dormancy.

IV. CONCLUSIONS

The treatment plant growth regulator at a concentration of 3 ml / l of water gives an average yield higher with fresh bulb production 60.51 t ha^{-1},

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Identifying Popular Indigenous Leafy Vegetables for Sustainable Interest in Vegetable Production in the Tamale Metropolis in the Northern Region of Ghana

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Abstract— *One way of achieving a sustained interest in vegetable production in a catchment area is to identify the most popular vegetable with high demand. The present study determined the Popular Indigenous leafy vegetables in the Tamale Metropolis in the Northern region of Ghana. A structured questionnaire was used to access the requisite data. The items were divided into sections. Most of the items were close-ended questions with few open-ended ones to make room for more different opinions. The questionnaire covered the bio data of leafy vegetable marketers and consumers, buying and selling volumes of leafy vegetables, Leafy vegetable marketers and prospective leafy vegetable consumers were the target population. The population of 60 marketers of leafy vegetables and 274 consumers in the Tamale Metropolis were considered. Respondents (consumers) were chosen as they came to buy leafy vegetables at the market. The data obtained was subjected to one way analysis of variance (ANOVA), using Statistical Package for Social Sciences (SPSS Version 16.0). Amaranthus dubius, Corchorus olitorius, and Hibiscus sabdariffa were rated the most popular leafy vegetables.*

Keywords— Vegetable Production, Northern Region of Ghana.

I. INTRODUCTION

Ghana has varying climatic conditions across the country which coupled with inadequate food distribution channels has led to existence of different food crops in the different regions. Leafy vegetables serve as the main source of nutrients, especially in poor-resource households in the country. This is the case of the residents of northern Ghana, which comprises the Northern, Upper East and Upper West regions. Residents of Northern Ghana, consume significant quantities of greens gathered from their surroundings/farms (Amaglo and Nyarko, 2012).

Vegetables are the succulent edible plant parts that may be eaten as supplementary food or side dishes in raw state or in the cooked form and various preparations. They may be sweet, aromatic, bitter, hot and tasteless and sometimes require salting and considerable seasoning to render them more tasty and acceptable. Products of vegetables play an importantrole in the diet of West Africans. Some crops grown for one purpose may also be put to dual use, thus cowpea and cassava grown for their protein-rich seeds and carbohydrate-rich roots respectively, also have their leaves harvested for vegetables (Seidu *et al*., 2012). Traditional leafy vegetables play a very important role in income generation and subsistence. They provide employment for peri-urban dwellers because of their generally short, labour intensive production systems, low levels of investment and high yield (Schippers, 2000).

Several attempts have been made by successive governments and other development partners to improve the productivity of vegetable farmers in an effort to reduce poverty in the region through small scale irrigation schemes, dug-outs and others. These interventions have contributed to the improvement in the production and supply of vegetables in the area. (Fuseini, 2018).

Vegetables are also a preferred cash crop because of their potential for lifting poor farmers out of poverty. It is reported that urban agriculture can meet large parts of the urban demand for certain kinds of food such as fresh vegetables, poultry, potatoes, milk, fish and eggs. The most commonly grown urban vegetables are the most perishable (leafy) ones such as *Amaranthus dubius, Corchorus olitorius,* and *Hibiscus sabdariffa*, which have to be produced in market proximity. Sub-Saharan Africa regions are reported to have the world's lower intake of micronutrient-rich fruits and vegetables with the mean consumption being less than half the World Health Organization (2003) recommendations on daily intake of 400g per capita per day in most countries. Low consumption of fruits and vegetables is the main contribution to micronutrients deficiencies, especially in populations with a low intake of nutrient-dense animal sources and dietary products (Seidu *et al.,* 2012). It is an indisputable fact that leafy vegetables are vital sources of minerals and vitamins for human health and development. Climatic conditions such as high temperatures, low humidity short periods of rainfall in the northern part of the country does not favour the cultivation of crops withlong maturation periods. The main objective of the study was to determine the commonly grown leafy vegetables in the Tamale Metropolis in the Northern region of Ghana.

A. Importance and benefits of Vegetables

Vegetables are very vital to human, both economically and nutritionally. Economically, they are relatively cheap to grow and act as a quick source of income to many rural women (Ijeomah *et al.,* 2012). Nutritionally, they are a good sources of vitamins, minerals and dietary fibre and water to aid digestion. Vegetables are rich in minerals such as potassium, sodium, calcium, iron, zinc and phosphorus. They also have high contents of thiamine, ascorbic acid, riboflavin and β-carotene. The water content is about 70% or more. They also contain many phyto-chemicals which are needed for health-promotion and disease prevention. Vegetables are consumed in very small quantities, and are used in almost every meal or used alone as salad or as a side dish with main meal (Ijeomah *et al.,* 2012).

Many authors have documented the importance of indigenous leafy vegetables of Africa as valuable sources of food, income and traditional medicine. Traditional African vegetables are extremely important for nutrition and farm income throughout Africa. They often supply most of the daily requirements of proteins, minerals and vitamins of poor rural people (Adebooye, 2005).

Quite a large number of African indigenous leafy vegetables have long been known and reported to have health protecting properties and uses. Several of these indigenous leafy vegetables continue to be used for prophylactic and therapeutic purposes by rural communities. This indigenous knowledge of the health promoting and protecting attributes of African indigenous Leafy Vegetables (ALVs) is clearly linked to their nutritional and non-nutrient bioactive properties. ALVs have long been, and continue to be reported to significantly contribute to the dietary vitamin and mineral intakes of local populations (Smith, Francisca I. *et al.,* (2007).

Indigenous vegetables are important sources of vitamins, minerals and other nutrients for rural families since ancient times. These substances protect us from illness and ensure proper metabolism. Since we cannot synthesize vitamins and minerals, they must also be supplied in the food we eat. Vitamins are often destroyed or lost during food processing. Othersources of vitamins and minerals are green leafy vegetables such as spinach and *Kontomire* (cocoyam leaves); both are found in southern Ghana. A larger variety of wild and cultivated leafy vegetables such as bitter leaf, cassava leaves, baobab leaves and Guinea sorrel are found in Northern Ghana (Kwapata, 1991).

Vegetables are a good source of roughages, which by providing an indigestible matrix stimulates intestinal muscles and keep them in working order and also prevent constipation through their laxative effect. The fibre content of vegetables generally adds to bulk of the food which prevents us from frequent hunger (Norman, 1992). Vegetables play an important role in the diet of West Africans. Some crops grown for one purpose may also be put to dual use, thus cowpea and cassava grown for their protein-rich seeds and carbohydrate-rich respectively also have their leaves harvested for vegetables (Okigbo, 1983).

Vegetables are highly beneficial for maintaining health and preventing diseases. Dark green leafy vegetables provide high amounts of micro-minerals which play vital roles in nutrient metabolism and retard degenerative diseases (Darkwa and Darkwa, 2013). Vegetables are important dietary sources of nutrients, vitamins and fibre for humans' vital health and wellbeing. Well balanced diets rich in fruits and vegetables are especially valuable for their ability to prevent vitamin A and C deficiency (Sowley *et al.,* 2011). Leafy vegetables contain many typical plant nutrients, but since they are photosynthetic tissues, their Vitamin K levels in relation to those of other fruits and vegetables, as well as other types of foods, are particularly notable. Leafy vegetables are typically low in calories, low in fat, high in protein per calorie, high in dietary fibre, high in iron and calcium, and very high in vitamin C, carotenoids, lutein folate, magnesium as well as vitamin K (Schippers, 2000).

Some indigenous leafy vegetables such as okra, vegetable jute, impart a glutinous constituency to stew and soup and thus facilitate swallowing of food such as "Banku" "Fufu" and "Gari". It has been also discovered that vegetables are rich source of vitamins K, A, and C as well as minerals such as calcium, iron, phosphorus, some appreciable amount of thiamine, Niacin and riboflavin, carbohydrate and crude proteinas compared to exotic leafy vegetables (Schippers, 2000).

Green leafy vegetables are rich sources of carotene and vitamin B and C. Carotene often called pre-vitamin A is changed to vitamin A indigestion. Food rich in vitamin C play an important supportive role in preventing Iron deficiency. Also, leafy vegetables are high in calcium, iron, and phosphorus. Furthermore, leafy vegetables are high in water content and fibre. Because of their succulence, they aid digestion (Schippers, 2000). *Amaranthus dubius* for instance is consumed as cooked leafy vegetable. Its leaves is known to be good food with medicinal properties for young children, lactating mothers and for patients with fever, haemorrhage, anaemia, constipation or kidney complaints (Schippers, 2002). It is rich in vitamin C and it is used to support the treatment of patients suffering from HIV/AIDS (Babalola *et al.,* 2010).

Fibre is responsible for maintaining a healthy digestive tract and supporting a wide array of other bodily systems in less crucial ways as well. Eating green fruits and vegetables will help to ensure that you do not run into any problems with your digestive system. While each different green fruit and vegetable contains a slightly different mixture of vitamins, it is likely that any one that your choice will have a good source of at least one important nutrient of this type. Vitamin A and vitamin C are two of the most commonly occurring vitamins in these types of vegetables and fruits. Vitamin A helps your body to process a number of different nutrients, like calcium and potassium, while vitamin C is essential for the building up of your immune system and to prevent you from getting sick (WHO/FAO, 2003).

II. MATERIALS AND METHODS

Tamale Metropolis was the study area for this research work. Tamale Metropolis lies between latitudes 9°16’ and 9°34’ North and longitudes 0°36’ and 0°57’ West. The Metropolis occupies approximately 750 km^2 which is about 13% of the total land area of the Northern Region of Ghana (Sowley *et al*., 2011). Tamale has a tropical wet and dry/savannah climate with a pronounced dry season in the low-sun months, no cold season, and wet seasonis in the high-sun months. According to the Hold ridge life zones system of bioclimatic classification Tamale is situated in or near the tropical dry forest biome. The mean temperature is 27.9 degrees Celsius (82.3 degrees Fahrenheit). Average monthly temperatures vary by 5.5 °C (9.9°F). The annual precipitation averages 1090 mm (42.9 inches) which is equivalent to 1090litres/m^2 (26.74gallons/ft^2) or 90.8 mm (3.6 in) per month. On average there are 97 days per year with more than 0.1 mm (0.004 in) of rainfall. The driest weather is in December when an average of 3 mm (0.1 in) of rainfall occurs. The wettest weather is in September when an average of 231 mm (9.1 in) of rainfall occurs. On the average there are 2723 hours of sunshine per year. (Meteorological Service Department-Tamale, 2015). The study was conducted on some popular indigenous leafy vegetables in the Tamale Metropolis. A pilot survey was conducted to gather information on the popular indigenous leafy vegetables consumed in Tamale Metropolis. A structured questionnaire was used to access the requisite data. The items were divided into sections. Most of the items were close-ended questions with few open-ended ones to make room for more different opinions. The questionnaire covered the following broad areas; the bio data of leafy vegetable marketers and consumers, buying and selling volumes of leafy vegetables, and sources of the vegetables.

Leafy vegetable marketers and prospective leafy vegetable consumers were the target population. The population of 60 marketers of leafy vegetables and 274 consumers in the Tamale Metropolis were considered. Respondents (consumers) were chosen as they came to buy leafy vegetables at the market. The data obtained was subjected to one way analysis of variance (ANOVA), using Statistical Package for Social Sciences (SPSS Version 16.0).

III. RESULTS

BIO DATA OF RESPONDENTS

The age ranges of the marketers of leafy vegetables were 21-30, 31-40 and more than 41. Out of a total number of 60 vegetable marketers, 14 (23.3%) of them were within the age range of 21-31 years, 22 (36.7%) of the total were within the age range of 31-40 years, majority (40%) of the leafy vegetable marketers were in the age range of 41 years and above.

The level of education of leafy vegetable marketers were categorized into No formal education, Middle School Leaving Certificate (MSLC), Junior High School (JHS), Senior High School (SHS), and higher than SHS. Twenty five (41.7%) of the marketers had no formal education while ten (16.7%) were MSLC graduates. Junior High School (JHS) graduates were 15 (29.7%) of the total number. Seven (11.7%) of the total number of marketers

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were Senior High School SHS graduates and the remaining two (3.3%) of marketers had education higher than SHS. Majority of the vegetable marketers had no formal education. Education higher than SHS category of marketers recorded the least.

Out of 274 answers, 65 (23.7%) were within the age range of 21-30 years, 121 (44.2%) of the total number of consumers were within the age range of 31- 40 years, while the remaining 88 (32.1%) were 41 years and above.

Table.1: Bio-data of respondents

		Marketers		Consumers	
		Frequency	%	Frequency	%
Gender	Male			135	49.3
	Female			139	50.7
Age	21-30	14	23.3	65	23.7
	31-40	22	36.7	121	44.2
	41+	24	40	88	32.1
Educational level	No formal	25	41.7	127	46.4
	MSLC	10	16.7	05	1.8
	JHS	15	29.7	52	19.0
	SHS	07	11.6	63	23.0
	Tertiary	02	3.3	27	9.9

Leafy vegetable marketers rated the vegetables as follows; 23 representing 38.3% of the total number of marketers rated *Corchorus olitorius* as the most popular leafy vegetable. Seventeen (28.3%) of the marketers rated *Hibiscus sabdariffa* as the most popular leafy vegetable. *Amaranthus dubius* was rated next most popular leafy vegetable to *Hibiscus sabdariffa* with 13 (21.7%) of the total number of marketers. Only 5.3% of the total number of marketers, rated cocoyam leaves, and 6.4%rated bitter leaf as the most popular leafy vegetable in the Tamale Metropolis.

From Table 4.2, out of the total number of sixty marketers, 14, (23.3%) were in the age range of 21-30 years; 22 marketers, representing 36.7% of the total number of the marketers were in the age range of 31-40 years. The remaining 24 marketers, representing 40%, were in the age range of 41 years and above. With this statistics, the youngsters (21-30 year range), six marketers, representing 42.9% of this group, sold *Corchorus olitorius* most, none sold cocoyam leaves and four marketers, representing 28.6% of the population sold *Hibiscus sabdariffa*. Others were three marketers, representing 21.4%, sold *Amaranthus dubius* and only one marketer,representing 7.1%, sold bitter leaf. On the other hand, in the 31-40 year group who were interviewed, seven marketers, representing 31.8%, sold *Corchorus olitorius;* three marketers, representing 13.6%, sold cocoyam leaves, whilst seven representing 31.8%, sold *Hibiscus sabdariffa*. *Amaranthus dubius* was sold by five marketers representing 22.7% of the total number of this year group (31-40), while none of them sold bitter leaf. Finally, ten marketers, representing 41.7% of the total number were in the 41 years and above group sold *Corchorus olitorius*. Three marketers representing 12.5%, sold cocoyam leaves and five marketers, representing 20.8%, sold *Amaranthus dubius* and none of them sold bitter leaf. In this case *Corchorus olitorius* was rated the most popular leafy vegetable with a high percentage of 41 and above year group of marketers.

Corchorus olitorius was rated the most popular indigenous leafy vegetable with 107 (39.09%). This was followed by *Hibiscus sabdariffa* with 90 (32.84%) of the consumers rating it as the most popular leafy vegetable used at home. *Amaranthus dubius* was rated next by 51 (18.6%) of total number of consumers, as the most popular leafy vegetable used at home. 22 representing 8.02% rated bitter leaf as the most popular leafy vegetable used at home. The least rated leafy vegetable was cocoyam leaves where only four (1.45%) of the total number of consumers rated it as the most popular leafy vegetable used at home

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Table.2: Popularity of leafy vegetable sold by age

Indigenous leafy vegetable	**Description**	**Age**			
		21-30	**31-40**	**41+**	**Total**
Corchorus olitorius	Count	6	7	10	23
	% within most popular leafy vegetable used at home	26.1%	30.4%	43.5%	100.0%
	% within age of marketers	42.9%	31.8%	41.7%	38.3%
Cocoyam leaves	Count	0	3	3	6
	% within most popular leafy vegetable used at home	0.0%	50.0%	50.0%	100.0%
	% within age of marketers	0.0%	13.6%	12.5%	10.0%
Hibiscus sabdariffa	Count	4	7	6	17
	% within most popular leafy vegetable used at home	23.5%	41.2%	35.3%	100.0%
	% within age of marketers	28.6%	31.8%	25.0%	28.3%
Amaranthus dubius	Count	3	5	5	13
	% within most popular leafy vegetable used at home	23.1%	38.5%	38.5%	100.0%
	% within age of marketers	21.4%	22.7%	20.8%	21.7%
Bitter Leaf	Count	1	0	0	1
	% within most popular leafy vegetable used at home	100.0%	0.0%	0.0%	100.0%
	% within age of marketers	7.1%	0.0%	0.0%	1.7%
Total	Count	14	22	24	60
	% within most popular leafy vegetable used at home	23.3%	36.7%	40.0%	100.0%
	% within age of marketers	100.0%	100.0%	100.0%	100.0%

From Table 4.3 total number of 107 consumers representing 39.1% of the total number of 274 chose *Corchorus olitorius* as the most popular leafy vegetable used at home. Out of this, 50.5% of them were males and 49.5% were females. Thereby, indicated 40.0% of the total male consumers rated *Corchorus olitorius* as the most popular leafy vegetable used at home and 38.1% of the total female consumers rated *Corchorus olitorius*. *Hibiscus sabdariffa* was rated the second most popular leafy vegetable used at home as 90 consumers, representing 32.8% of the consumers. Out of this population, 50.0% of them were males and 50.0% were females, thereby, indicating 49.0% of the total male consumers rated *Hibiscus sabdariffa* as the most popular leafyvegetable used at home while 51.0% of the total female consumers rated *Corchorus olitorius* as most popular leafy vegetable used at home. Fifty one consumers, representing 18.6% of the total number of consumers, consisting of 18.5% males and 18.7% females indicated 49.0% of male consumers and 51.0% of female consumers respectively rated *Amaranthus dubius* as the most popular leafy vegetable used at home, placing it third on the list. Cocoyam leaves was rated the least with 1.5% and 8.0% by male and female consumers respectively of the total population of 4 consumers.

Table.3: Choice of popular leafy vegetable used by gender

Indigenous leafy vegetable	**Description**	**Gender**		
		Male	**Female**	**Total**
Corchorus olitorius	Count	54	53	107
	% within most popular leafy vegetable used at home	50.5%	49.5%	100.0%
	% within gender of consumers	40.0%	38.1%	39.1%
Cocoyam leaves	Count	2	2	4
	% within most popular leafy vegetable used at home	50.0%	50.0%	100.0%

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	% within gender of consumers	1.5%	1.4%	1.5%
Hibiscus sabdariffa	Count	45	45	90
	% within most popular leafy vegetable used at home	50.0%	50.0%	100.0%
	% within gender of consumers	33.3%	32.4%	32.8%
Amaranthus dubius	Count	25	26	51
	% within most popular leafy vegetable used at home	49.0%	51.0%	100.0%
	% within gender of consumers	18.5%	18.7%	18.6%
Bitter Leaf	Count	9	13	22
	% within most popular leafy vegetable used at home	40.9%	59.1%	100.0%
	% within gender of consumers	6.7%	9.4%	8.0%
Total	Count	135	139	274
	% within most popular leafy vegetable used at home	49.3%	50.7%	100.0%
	% within gender of consumers	100.0%	100.0%	100.0%

Table 4.4 shows that, out of the total number of 274 consumers, 65 representing 23.7%, were in the age range of 21-30 years, 121, consumers, representing 44.2% of the total number of the consumers were in the age range of 31-40 years, and 88 consumers, representing 32.1%, were in the age range of 41 years and above. With this, 21 consumers, representing 32.3% of 21-30 year group, rated *Corchorus olitorius* was mostly used at home, none rated cocoyam leaves and 22 consumers, representing 33.8%, rated *Hibiscus sabdariffa*. Seventeen consumers, representing 26.2% of this group (21-30 years), rated *Amaranthus dubius* was mostly used leafy vegetable at home and only five people, representing 7.7%, rated bitter leaf. On the other hand, 31-40 year group who were interviewed, 55 consumers, representing 45.5%, rated *Corchorus olitorius*; two consumers, representing 1.7%, rated cocoyam leaves as mostly used leafy vegetable at home, whilst 39 consumers (32.2%), rated *Hibiscus sabdariffa*. *Amaranthus dubius* was rated by16 consumers, representing 13.2% of the total number of this year group (31-40) of consumers, while nine consumers, representing 7.4% of them rated bitter leaf. Finally, 31 consumers, representing 35.2% of the total number of the 41 years and above group of consumers, rated *Corchorus olitorius*. Only two consumers, representing 2.3%, rated cocoyam leaves and 29 (33%) consumers rated *Amaranthus dubius*. Finally, eight consumers, representing 9.1%, rated bitter leaf as mostly leafy vegetable used at home. It is clear that *Corchorus olitorius* was rated the highest with 39.1% with the concentration of 31-40 year range of 45.5%.

Table.4: Consumer rating of leafy vegetables used by age

Indigenous leafy vegetable	**Description**	**Age**			
		21-30	31-40	41+	Total
Corchorus	Count	21	55	31	107
olitorius	% within most popular leafy vegetable used at home	19.6%	51.4%	29.0%	100.0%
	% within age of consumers	32.3%	45.5%	35.2%	39.1%
Cocoyam leaves	Count	0	2	2	4
	% within most popular leafy vegetable used at home	0.0%	50.0%	50.0%	100.0%
	% within age of consumers	0.0%	1.7%	2.3%	1.5%
Hibiscus	Count	22	39	29	90
sabdariffa	% within most popular leafy vegetable used at home	24.4%	43.3%	32.2%	100.0%
	% within age of consumers	33.8%	32.2%	33.0%	32.8%
Amaranthus	Count	17	16	18	51
dubius	% within most popular leafy vegetable used at home	33.3%	31.4%	35.3%	100.0%

	% within age of consumers	26.2%	13.2%	20.5%	18.6%
Bitter Leaf	Count	5	9	8	22
	% within most popular leafy vegetable used at home	22.7%	40.9%	36.4%	100.0%
	% within age of consumers	7.7%	7.4%	9.1%	8.0%
Total	Count	65	121	88	274
	% within most popular leafy vegetable used at home	23.7%	44.2%	32.1%	100.0%
	% within age of consumers	100.0%	100.0%	100.0%	100.0%

IV. CONCLUSION

The survey conducted to identify the most popularly used indigenous leafy vegetables in the Tamale Metropolis, leafy vegetables were ranked in order of popularity by both leafy vegetable marketers and consumers. *Amaranthus dubius, Corchorus olitorius,* and *Hibiscus sabdariffa* were rated the most popular leafy vegetables, meanwhile *Corchorus olitorius* was rated the most popularly used indigenous leafy vegetable. Farmers of leafy vegetables should be encouraged to cultivate *Amaranthus dubius, Corchorus olitorius* and *Hibiscus sabdariffa*. This could help in the efforts to reduce unemployment situation in northern region of Ghana.

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Effects of pre-sowing treatments on seed germination and seedling growth of *Glycine max(L.) Merrill*

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Abstract— *The study assessed the effects of some pre-sowing seed treatments on seed germination and seedling growth rate of three varieties of Glycine max– TGX 1935-3F (variety 1), TGX 1448-2E (variety 2) and TGX 1951-3F (variety 3). The treatments included four different concentrations of sulphuric acid (0.1, 0.2, 0.3, 0.6mole/dm^3) termed T1, T2, T3 and T4 respectively, for five minutes and three hot water temperature variations (40, 60, and 80^{0}C) termed T5, T6 and T7 respectively, for twenty minutes. Germination was monitored as emergence of radicle and plant growth parameters were also measured. Treatments T4 (93.33%, 86.67%, 96.67%) and T7 (90.00%, 83.33%, 93.33%) had the highest germination percentages in the three varieties. Plant height in T2, T3 and T4was significantly different ($p< 0.05$) from the control in all acid pre-treatments for all varieties; for hot water pre-treatment, plant height in varieties 1 and 3 were significantly ($p< 0.05$)different from the control in all treatments. Furthermore, T6 and T7 (variety 2) were also significantly different from the control. Number of leaves showed significant difference from the control in T3, T4 (variety 1), T4 (variety 2), and T2, T3, and T4 respectively. Additionally, T5, T6 and T7 were significantly different from the control in varieties 1 and 2. Leaf area was significantly different ($p< 0.05$)from the control in T1, T2, T3, T4 (varieties 1 and 3), and in T4 (variety 2). Moreover, T5, T6 and T7 were significantly different from the control in varieties 1 and 3, and in T7 in variety 2. Number of buds was significantly different ($p< 0.05$) in T2, T4 (variety 1), and in T3 and T4 in variety 3. There was also significant difference in the number of buds in T5, T6 and T7 in variety 3. The results suggested treatments T4 and T7 had the best overall effect on germination and overall growth, and hence hereby recommended.*

***Keywords—* Glycine max, Dormancy, Acid treatment, Hot water treatment, Cellular Phase Transition.**

I. INTRODUCTION

Glycine max (L.) Merrill commonly known as soybean, is a species of legume native to East Asia and grown for its numerous uses (Felker and Bandurski, 1979; Burkhill, 1994). Almost all types of soil support its growth except deep sand with poor water retention (Iwe, 2003). Soybean is used commercially as human food, and livestock feed and for the extraction of oil. It has the adaptability to fix nitrogen. It is composed of about 40% protein and 20% oil. This composition ranked it highest in protein content and second highest in terms of oil content food crops(Iwe, 2003). Because of its importance in providing many uses and services, it has attracted some attention.

However, one of the problems with this species is that some varieties produce some hard seed coats that do notreadily imbibe water even for prolong period of soaking (Shao*etal*., 2007). The cuticle of such seeds contain disproportionately high amount of hydroxylated fatty acid, which makes germination difficult. The seeds therefore require treatment before sowing to enhance germination and improved seedling growth (Shao *et al*., 2007).Germination is a multifaceted event, involving up regulation of genes promoting germination and down regulation of genes promoting dormancy (Matilla and Matilla-Vazquez, 2008). Germination has been shown to be affected by the endogenous seed concentration and soil levels of plant growth hormones (Graeber *et al*., 2012; Kucera *et al*., 2005). Furthermore, activation of proteinase triggers the mobilization of storage proteins (Tiedemann *et al*., 2001). Other enzymes such as carboxyl peptidase and amino peptidase have also been implicated in the emergence of radicle and plumule (Finch-Savage and Leubner-Metzger, 2006). In addition, environmental factors such as acidity, temperature, salinity have been elucidated to affect hormonal balance. Gibberellic acid (GA) enhance seed germination by promoting enzymes that inhibit Abscisic acid (ABA) pathway and subsequently, ABA concentration (Atia*et* *al*., 2009). Ethylene and GA antagonize ABA during dormancy initiation, termination and germination, but the crosstalk has not been fully elucidated (Rodríguez-Gacio*et al*., 2009; Miransari and Smith, 2014; Matilla and Matilla-Vazquez, 2008). Although dormancy is controlled at the molecular level (Graeber *et al*., 2012), the physical characteristics of the testa, pericarp and endosperm have profound effects on germination (Kucera *et al*., 2005).

Various studies have employed different methods of breaking or reducing dormancy and improving germination rate including cold water (Adeola and Dada, 1983; Eze and Orele, 1987), hot water (Awodola, 1994; Adewusi and Ladipo, 2000; Otegbeye and Momodu, 2002) and acid treatment (Ibrahim and Kalu, 2006). According to Umar(2005), these treatments made the seed coat permeable and enhanced germination. The purpose of this study was to investigate the effects of pre-sowing seed treatment on germination and seedling growth of *Glycine max*.

II. MATERIALS AND METHODS

The study was conducted at the nursery section of the Department of Biological Sciences, University of Agriculture Makurdi, Nigeria.

Plant Materials

Seeds of three varieties of *G. max*were obtained from the Seed Technology Centre, University of Agriculture Makurdi, Nigeria. The three varieties of *G. max* used were– TGX 1935-3F, TGX 1448-2E and TGX 1951-3F; termed variety 1, 2 and 3 respectively. The varieties were hand-picked to ensure uniformly large-size, and seeds were checked to ensure they were physically undamaged.Physical identification of the varieties was done – with TGX 1935-3F, TGX 1448-2E, and TGX 1951-3F having brown, yellow and black seed coats respectively.

Experimental Design and Treatments

A 3x3x8 Factorial experiment (3 varieties, 3 replicates and 8 treatments) laid out in a Completely Randomized Design was adopted for the study. There were seventreatments (and a control), with three replicates for each treatment per variety, and ten seeds were assessed for each replicate.The treatments included acid scarification by immersing the seeds in sulphuric acid (H_2SO_4) at different concentrations (0.1, 0.2, 0.3 and 0.6 mol/dm^3), termed Treatments T1, T2, T3 and T4 respectively, for 5 minutes. Also, seeds were immersed in hot water of varying temperatures (40,60, and 80^{o}C) for 20 minutes, termed Treatments T5, T6 and T7 respectively. Acid treated seeds were washed with distilled water to ensure complete removal of trace acid before planting in poly pots. Control seeds were sown without any pretreatment.All seeds were planted at 2cm depth in poly pots. The study lastedfor five weeks. Data on germination were collected in week one, and data on growth parameters for four weeks. Growth parameters such as plant height, number of leaves, leaf area, and number of buds were evaluated. Germination percentage was calculated using the equation described by ISTA (1999).

Statistical Analyses

Statistical analyses were done using Minitab 16 Statistical Software. One-way analysis of variance (ANOVA) was used to compare multiple means at 95% confidence interval. Mean separation was done using Least Significant Difference (LSD) and$p<0.05$ was considered statistically significant.

III. RESULTS

Germination Percentage:

Table 1 shows the germination percentage of the three varieties of *G. max* seeds subjected to pre-sowing acid and hot water treatments. The results indicated increases in germination percentage with increases in acid concentrations and water temperatures respectively in the three varieties, while T4 and T7 yielded the highest germination percentage.

Table.1: Germination percentage of three varieties of G. max. seedstreated with sulphuric acid and hot water pre-sowing treatments

Treatments	Germination %		
	Variety 1	Variety 2	Variety 3
Control	60.00	53.33	66.67
T1	63.33	70.00	80.00
T2	66.67	80.00	83.33
T3	70.00	83.33	86.67
T4	93.33	86.67	96.67
T5	73.33	66.67	60.00
T6	76.67	76.67	80.00
T7	90.00	83.33	93.33

Variety 1 (TGX 1935-3F);Variety 2 (TGX 1951-3F);Variety 3 (TGX 1448-2E); T1 (0.1 mol/dm^3); T2 (0.2 mol/dm^3); T3 (0.3 mol/dm^3); and T4 (0.6 mol/dm^3). T5 (40 ^{o}C); T6 (60 ^{o}C); and T7 (80 ^{o}C).

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Growth Parameters of Acid Treatments:

Variety 1 - TGX 1935-3F

Significant differences ($p<0.05$) from the control were observed in plant height in treatments T1, T2, T3 and T4 (Figure 1). The tallest plantswere seen in treatment T4. The number of leaves in treatments T3 and T4 were significantly different (Figure 1) from the control. Also, the leaf area in treatments T1, T2, T3 and T4 was significantly different from the control (Figure 1). Furthermore, significant differences from the control were observed in the number of buds of treatments T2 and T4.

Variety 2 - TGX 1951-3F:

Significant differences ($p<0.05$) from the control were observed in plant height of treatments T2, T3 and T4 (Figure 1). The number of leaves and leaf area of treatment T4 were also significantly different from the control (Figure 1).

Variety 3 - TGX 1448-2E:

Significant differences ($p<0.05$) from the control were observed in the plant height and number of leaves (Figure 1) of treatments T2, T3 and T4. The leaf area of all treatments (T1 to T4) was significantly different (table 4) from the control. Additionally, the number of buds (Figure 1) of treatments T3 and T4 were significantly different from the control.

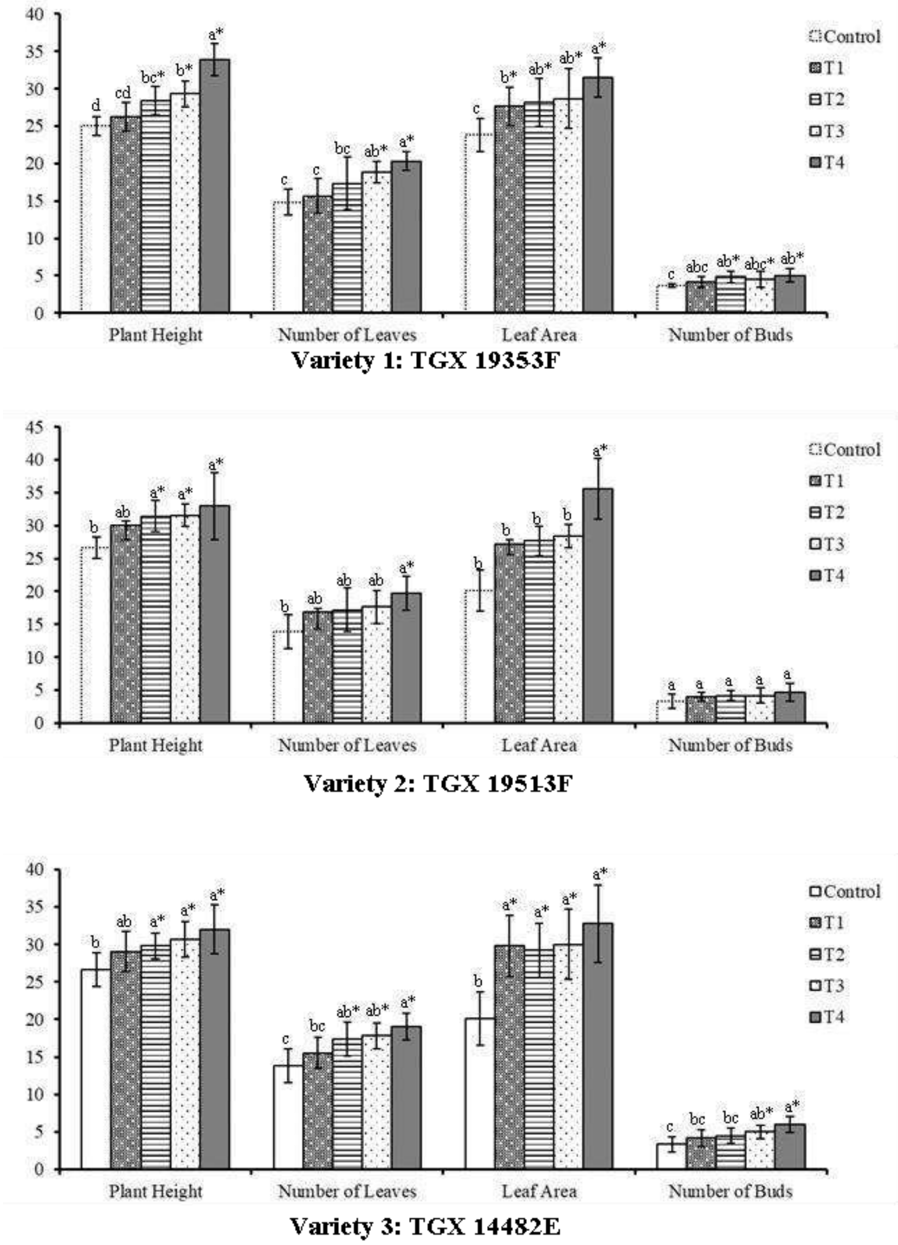

*Fig.1: Effects of sulphuric acid pre-sowing seed treatments on growth parameters of three varieties of G. max.Vertical bars represent means; error bars represent ± standard deviation; n = 10; * = significantly different from the control ($p<0.05$). Means with the same superscript are not significantly different. Plant Height (cm); Leaf Area (cm^2);T1 (0.1 mol/dm^3); T2 (0.2 mol/dm^3); T3 (0.3 mol/dm^3); and T4 (0.6 mol/dm^3) acid pre sowing treatments for 5 minutes.*

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Growth Parameters of Hot Water Treatments:

Variety 1 - TGX 1935-3F

Plant height, number of leaves and leaf area in all hot water treatments, T5, T6 and T7, were significantly different($p<0.05$) from the control (Figure 2).

Variety 2 - TGX 1951-3F:

Plant heights of treatments T6 and T7 were significantly different from the control (Figure 2). Furthermore, there was significant difference from the control in plant height and leaf area of Treatment T7. Number of leaves and buds were not significantly different from the control in all treatments.

Variety 3 - TGX 1448-2E

All treatments showed significant differences from the control ($p<0.05$) in the plant height, number of leaves, leaf area and number of buds (Figure 2).

IV. DISCUSSION

Increased germination percentage in pre-sowing treated seeds in this study is in line with similar findings reported by (Amira and Mohammed, 2013) whose work showed increased germination percentage of *Cassia fistula* seeds treated with sulphuric acid and hot water and (Aliero, 2004) on the positive effect of sulphuric acid on germination of *Parkiabiglobosa*. Additionally, Agbogidi*et al*. (2007) demonstrated that acid treatment of *Dacryodes edulis* significantly improved germination, plant height, number of leaves and leaf size with increasing time of acid pre-sowing exposure. Similaraly, Moosavi*et al*., (2014) observed improved germination percentage, vegetative and reproductive traits of 8-hour seed water pre-treatment of soybeans. These studies have highlighted the role of softening the seed coat in seed germination.

The pre-sowing treatments appear to soften the seed coat, thereby triggering a cascade of molecular events that led to the breaking of dormancy; hence, higher germination percentages and head start for seedlings whose seeds were subjected to such pre-sowing treatments. Furthermore, the germination and growth parameters measured in the higher acid and hot water pre-sowing treatments (T4 and T7) showed better overall performance suggesting the efficiency of the treatments in breaking dormancy and improving seedling growth.

Advances in molecular biology – metabolomics, transcriptomic, genomics, proteomics, bioinformatics – allow biologists to create genome-wide network models revealing in-depth insights into seed germination and regulation of plant cellular phase transitions (Bassel*et al*., 2011; Linkies*et al*., 2010). Such studies have shed light on genes involved in germination and hence, deciphered the genetic process of germination. Bassel*et al* (2011) elucidated the possible co-evolution of germination and dormancy, and the potential interplay with cellular-phase transition genes, suggesting that dormancy arose as an evolutionary adaptive stage by linking genetic pathways involved in cellular phase transition and abiotic stress.

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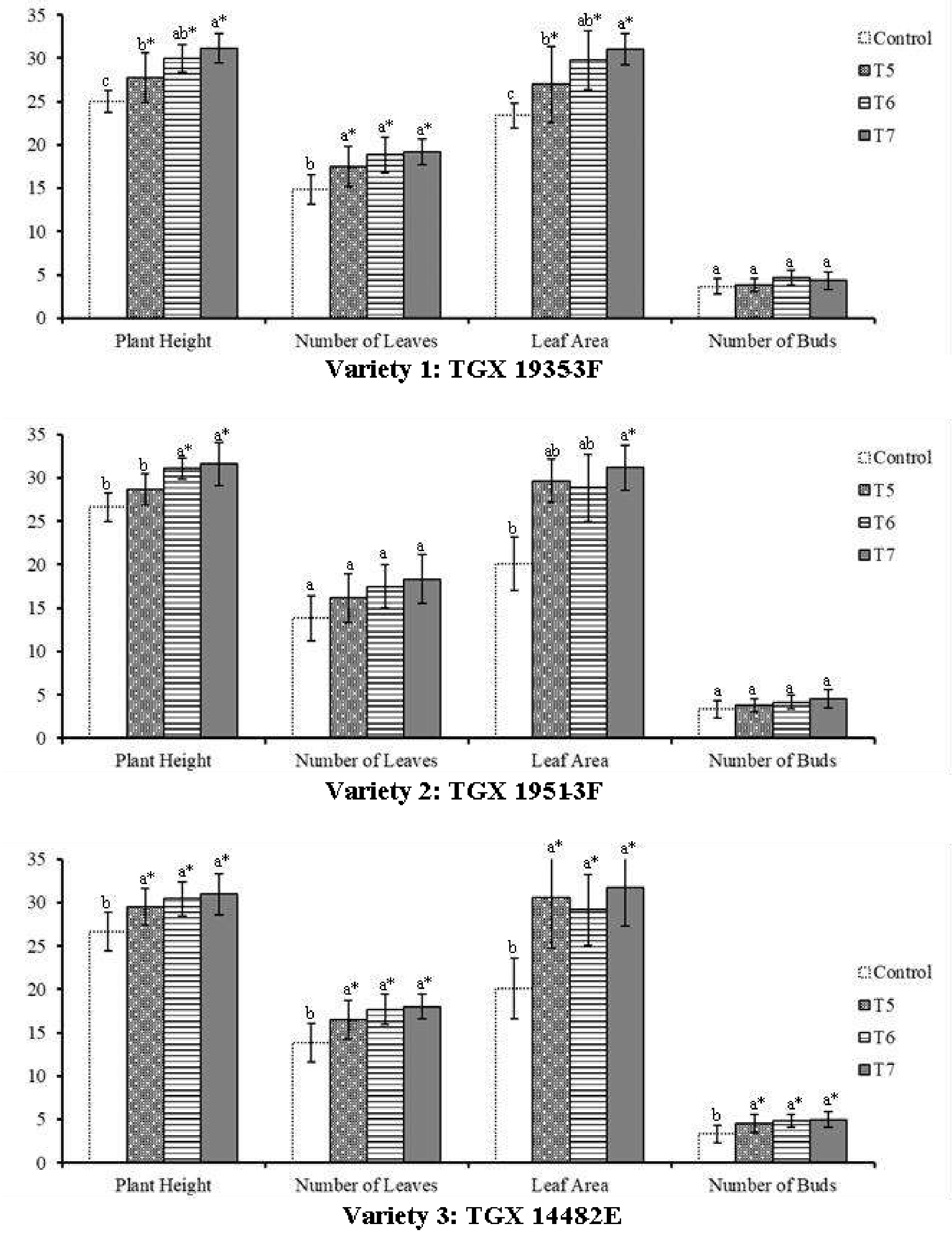

*Fig.2: Effects of hot water pre-sowing seed treatments on growth parameters of three varieties of G. max. Vertical bars represent means; error bars represent ± standard error. n = 10; * significantly different from the control ($p<0.05$). Means with the same superscript are not significantly different. Plant Height (cm); Leaf Area (cm^2); T5 (40 °C); T6 (60 °C); and T7 (80 °C) hot water pre-sowing treatments for 20 minutes.*

V. CONCLUSION

The study suggests pre-sowing treatments have profound effect on germination and seedling development of soybean by softening the seed coat and triggering molecular cascades involved in germination, breaking dormancy and cellular phase transition, leading to higher germination percentages and better seedling growth.

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Phytochemical and Vitamin Contents of *Mangifera indica* (Mango) Fruits Subjected to Ripening by Artificial Methods

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Abstract -- *Phytochemical and vitamin contents of ripe, unripe as well as unripe mango (Mangifera indica) fruits subjected to different ripening methods (use of dark polybag, calcium carbide and hot water) were investigated. The Phytochemicals namely carotenoids, phenols, terpenes, alkaloids, tannins, saponins, phytosterols, flavonoids and glycosides were quantified. Natural ripening (control) increased carotenoids (6.53±0.02mg/100g to 11.46±0.04mg/100g), phenols (15.13±0.02 mg/100g to 25.27±0.02 mg/100g), terpenes (0.08±0.02 mg/100g to 1.77±0.02 mg/100g) but decreased alkaloids (0.61±0.00 mg/100g to 0.53±0.02 mg/100g), tannins (2.06±0.02 mg/100g to 1.08±0.02 mg/100g) and flavonoids (56.80±0.01 mg/100g to 35.88±0.02 mg/100g) among others. Among the naturally ripe (control), unripe and artificially ripened mango fruits, there were no significant differences ($p<0.05$) in vitamins A, B1, B2, and B3. No significant difference ($p<0.05$) was obtained in the values of B6 for the naturally and artificially ripened mangoes which ranged from 0.20±0.01 mg/100g in calcium carbide group to 0.28±0.06 mg/100g in hot water group, but each was significantly higher than the value for the unripe mango group (0.13±0.02 mg/100g) The levels of vitamin C (mg/100g) was highest in the unripe fruits (51.06±0.05 mg/100g) followed by hot water treated fruits (50.06±0.05 mg/100g) which did not differ significantly ($p<0.05$) from the values for polybag treated fruits (49.54±0.19 mg/100g) but each was significantly higher than the value for the naturally ripe fruits (30.90±0.14 mg/100g). In general, it may be concluded that artificial ripening methods increased the phytochemical constituents and vitamin levels in the fruits investigated.*

Keywords— Phytochemical, vitamin, artificial ripening, mango fruits, unriped.

I. INTRODUCTION

The role of plants in folklore medicine is ascribed to the presence of various phytochemicals like carotenoids, phenols, flavonoids, alkaloids, tannins, saponins, glycosides and phytosterols (Schreiner and Huysken-keil, 2006; Basu *et al*., 2007) which are non-nutritive plant chemicals that have disease averting and curative properties (Duyn & Pivonka, 2000).

Information from emerging data posits that consumption of phytochemical rich foods such as fruits may provide protection against neurodegenerative diseases such as Alzheimer's, and Parkinson's diseases (Davinelli *et al*., 2012; Gao *et al*., 2012; Jones *et al*., 2012), promote cardiovascular health, (Dauchet *et al*., 2006), lower the risk of breast, colon and lung cancer among others (Hung *et al*., 2004) and may improve insulin sensitivity leading to decreased risk of type 2 diabetes (Arts & Holman, 2005).

Vitamins which might be water soluble (C, B) or fat soluble (A, D, E, K) are vital food nutrients, critical for sustaining cellular function. Vitamins are vital for the progression and support of most biological processes fundamental for human survival (Rossato *et al*., 2009) as they promote wellbeing in various ways including the enhancement of cell capacities, mediating an immense number of biological processes, disease prevention, promoting bone structure and strength, reducing inflammation, promoting cardiovascular health and improving endothelial cell function (Naidu, 2003).

Example of fruits include; mango, apple, cashew, orange, grapes, water melon, lemon and pineapple. Among the fruits, mango (*Mangifera indica*) in the *Anacardiaceae* family broadly found in tropical and subtropical districts is known as the lord of fruits (Onyeani *et al*., 2012) for its revivifying and exciting savour (Zewter *et al*., 2012; Farina *et al*., 2013).

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Fruit intake is not as high as it ought to be because of unavailability during off seasons for reasons such as rapid ripening, vulnerability to diseases (Onyeani *et al*., 2012) and rapid post-harvest decay because of ripening and softening which limit the storage, handling and transportation of the fruit (Amarakoon *et al*., 1999). In other to minimize post harvest loss, fruit vendors therefore harvest them prior to ripening and ripening is induced artificially (Goldman *et al*., 1999) by the use of various chemicals such as ethylene gas, ethephon and calcium carbide (Singal *et a.l*, 2012; Sogo –Temi *et al*., 2014; Gbakon *et al*., 2018). Iroka *et al*., (2016) has also reported other methods such as dipping into hotwater and wrapping in dark polyethylene bags. The use of these ripening agents and ripening techniques may successfully reduce or minimize post harvest losses but such activities may lead to the exposure of these fruits to food contamination (Orisakwe *et al*, 2012) thereby exposing consumers to numerous health conditions such as diarrhea,, digestive disorders, dementia, oedema, liver and kidney dysfunction, as well as cardiovascular diseases (Kader, 2007; Kjuus, *et al*., 2007; Pandarinathan and Sivakumar, 2010; Dhembare *et al*., 2013). The peremptory request for food safety (Ruchitha 2008) has inspired this research work which is geared toward exploring possible hazards associated with artificial ripening of fruits. More so, considering the importance of plant constituents to the overall wellbeing of man, this investigation was conducted to ascertain possible changes in the phytochemical and vitamin contents of mango fruit traceable to these artificial ripening methods.

II. MATERIALS AND METHODS

Collection and preparation of samples

Ripe and unripe mango fruits were sourced from Ibeku Community in Aboh Mbaise Local Government in Imo State, Nigeria. The ripe and a set of the unripe mango fruits were left untreated while the remaining unripe mango fruits were cleaned and given the following treatment; a set of ten was left under the sun for 4 hours after which the fruits were tied in a clean empty dark poly bag for 3 days. A second set was soaked in hot water (100°C) for five minutes and was covered with a thin cloth for 2 days while the third set was placed in a plastic bucket containing ground calcium carbide (2g/100g of mango fruit) for twenty-four hours. After ripening was induced in all treated sets, fruits were sliced, air dried, ground and used for the various analysis (Iroka *et al*., 2016).

Phytochemical analysis: Calibration, identification and quantification

Standard solutions were prepared in methyl alcohol for alkaloids, flavonoids and simple phenolics; acetone for carotenoids; dichloromethane for phytosterols and simple terpenes; ethanol for, glycosides and saponins. The linearity of the dependence of response on concentration was confirmed by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for every compound determined, using the standards.

Phytochemical tests were conducted on the dry ground sample using standard methods to quantify carotenoids (Rodriguez-Amara and Kimura, 2004), phenols (Provan *et al*., 1994), terpenoids (Ortan *et al*., 2009), alkaloids (Tram *et al*., 2002), tannins (Luther 1992), saponins (Guo *et al*., 2009), Phytosterols (AOAC International, 2006), flavonoids (Millogo *et al*., 2009) and glycosides (Oluwaniyi and Ibiyemi 2007).

Vitamin analysis

Samples were extracted following the method of Zhao *et al*., (2004)

Procedure

The weights of the samples were determined and samples were ground into powder, using Janice and Kunkel grinder. One gram of the sample was homogenized in I ml of ethanol, and separated by refluxing with 10 ml of re-refined methanol, for 6 hours at very low temperature. To guarantee removal of the pulverized vitamins, this procedure was duplicated using new solvents. Another 1g of the pulverized sample was homogenized in I ml of ethanol and extracted by refluxing with 10 ml of Chlorofoam for an additional six hours still at very low temperature. After which the two extracts were evaporated to dryness with the aid of a rotary evaporator and their residues were then combined. 4.00 ml of 7% BF3 Reagent was included into the blend which was then warmed in the oven for 45 minutes at 100°C after which it was cooled to room temperature before 1.0g of anhydrous Na_2SO_4 was added so as to guarantee the removal of water. It was then exposed to gas chromatographic investigation using pulse fire photometric detector for Vitamin determination.

Standard solutions were prepared and the linearity of the dependence of response was checked by regression analysis. Identification was based on comparison of retention times and spectral data with standards.

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Quantification was performed by setting up calibration curves for each compound determined, utilizing the standards.

Statistical analysis

The data were analyzed by the analysis of variance (ANOVA). The differences between the groups were compared using the Duncan multiple range test. The results are expressed as mean ± standard deviation. Significance was accepted at $p \leq 0.05$.

III. RESULTS AND DISCUSSION

The concentrations of carotenoids, phenols and terpenes were significantly ($p>0.05$) higher (11.46±0.04mg/100g), (25.27±0.02 mg/100g) and (1.77±0.02 mg/100g) in the naturally ripe (control) but lower in the unripe (6.53±0.02mg/100g), (15.13±0.02 mg/100g) and (0.08±0.02 mg/100g) groups respectively whereas values obtained for alkaloids, tannins, phytosterol, flavonoids and glycosides were higher in the unripe group (0.61±0.00 mg/100g), (2.06±0.02 mg/100g), (85.77±0.23), (56.80±0.01 mg/100g) and (3.84±0.02) but lower (0.49±0.06 mg/100g), (0.93±0.02 mg/100g), (54.30±0.07), (36.09±0.02 mg/100g) and (2.61±0.02) in the hot water groups respectively. The concentration of Saponins ranged from (0.19±0.02mg/100g) in hot water group to (0.47±0.03mg/100g) in polybag group.

Table 2 shows the vitamin contents of mango (*mangifera indica)* subjected to different ripening methods. The concentrations of vitamins A, B1, B2 and B3 followed the same trend and revealed that there were no significant ($p \leq 0.05$) differences in all groups under comparison and values were higher in the hot water group but lowest in the unripe group. No significant difference ($p<0.05$) was obtained in the values of B6 for the control and the artificially ripe mangoes which ranged from 0.20±0.01 mg/100g in calcium carbide group to 0.28±0.06 mg/100g in hot water group, but each was significantly higher than the value for the unripe mango group (0.13±0.02 mg/100g). The levels of vitamin C mg/100g was highest in the unripe fruits (51.06±0.05 mg/100g) followed by hot water treated fruits (50.06±0.05 mg/100g) which did not differ significantly ($p<0.05$) from the values for polybag treated fruits (49.54±0.19 mg/100g) but each was significantly higher than the value for the naturally ripe fruits (30.90±0.14 mg/100g)

Table.1: Phytochemical composition (mg/100g) of mango (Mangifera indica) subjected to different methods of ripening.

	Carotenoids	Phenols	Terpenes	Alkanoids	Tannins	Saponins	Phytosterols	Flavonoids	Glycosides
Naturally (Ripe)	11.46 ± 0.04^a	25.27 ± 0.02^a	1.77 ± 0.02^a	0.53 ± 0.02^c	1.08 ± 0.02^c	0.37 ± 0.02^b	59.13 ± 0.02^c	35.88 ± 0.02^c	2.90 ± 0.20^b
Unripe	6.53 ± 0.02^d	15.13 ± 0.02^d	0.08 ± 0.02^c	0.61 ± 0.00^a	2.06 ± 0.02^a	0.19 ± 0.02^d	85.77 ± 0.23^a	56.80 ± 0.01^a	3.84 ± 0.02^a
Polybag	9.73 ± 0.04^c	16.27 ± 0.02^c	0.17 ± 0.02^b	0.57 ± 0.02^b	1.38 ± 0.02^b	0.47 ± 0.03^a	65.26 ± 0.12^b	41.03 ± 1.71^b	2.89 ± 0.02^b
Carbide	9.94 ± 0.06^c	16.37 ± 0.04^c	0.18 ± 0.14^b	0.51 ± 0.00^b	1.28 ± 0.05^b	0.46 ± 0.06^a	66.28 ± 0.03^b	41.72 ± 0.57^b	2.88 ± 0.05^b
Hot Water	8.90 ± 0.21^b	17.30 ± 0.20^b	0.18 ± 0.03^b	0.49 ± 0.06^d	0.93 ± 0.02^d	0.24 ± 0.02^c	54.30 ± 0.07^d	36.09 ± 0.02^c	2.61 ± 0.02^c

Values are Mean ± standard deviations of triplicate determinations. Values in the same column having the same superscript letters are not significantly different at the 5% level.

Table.2: Vitamin contents (mg/100g) of mango fruits (mangifera indica) subjected to different methods of ripening.

Groups	Vitamin A	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B6	Vitamin C
Naturally Ripe	0.30 ± 0.06^a	0.06 ± 0.06^a	0.07 ± 0.03^a	0.76 ± 0.04^a	0.26 ± 0.04^a	30.90 ± 0.14^a
Unripe	0.26 ± 0.03^a	0.05 ± 0.02^a	0.04 ± 0.01^a	0.65 ± 0.04^a	0.13 ± 0.02^b	51.06 ± 2.00^b
Polybag	0.33 ± 0.05^a	0.06 ± 0.07^a	0.06 ± 0.03^a	0.68 ± 0.11^a	0.28 ± 0.14^a	49.54 ± 0.19^b
Carbide	0.29 ± 0.06^a	0.06 ± 0.03^a	0.05 ± 0.02^a	0.67 ± 0.10^a	0.20 ± 0.01^a	41.54 ± 0.16^c
Hot water	0.34 ± 0.06^a	0.08 ± 0.03^a	0.09 ± 0.04^a	0.78 ± 0.09^a	0.28 ± 0.06^a	50.06 ± 0.05^b

Values are Mean ± standard deviations of triplicate determinations. Values in the same column having the same superscript letters are not significantly different at the 5% level.

IV. DISCUSSION

Phytochemical investigation revealed that carotenoids, phenolics, terpenes decreased significantly ($p<0.05$) in the artificially induced ripening groups compared with the control. Carotenoids have antioxidants, anti-carcinogenic and anti-mutagenic properties, giving protection against various diseases such as different types of tumors, cardio vascular diseases as well as age related illnesses. (Dauchet *et al*., 2006; Davinelli *et al*., 2012). Carotenoids are vital in their role as precursors of important vitamins such as vitamin A (Schreiner and Huysken-keil, 2006). They also play extremely vital role as immune system booster aiding the body's ability to combat diseases and infections (Basu *et al*., 2007; Blanchflower *et al*, 2013). Menichini *et al*., (2009) in a similar work using *capsicum Chinese pepper* submitted an increase in carotenoids with ripening but Alagbaoso *et al*., (2017) submitted an insignificant difference in the carotenoid content of Avocado pear with ripening. Difference may be ascribed to the fruit type or maybe due to the ripening methods employed.

Polyphenols enhance insulin sensitivity thereby lowering the risk of type 2 diabetes by inhibiting carbohydrate digestion and glucose absorption in the intestine; stimulation of insulin secretion from the pancreas and the modulation of glucose release from the hepatic cells (Carter *et al*., 2010). The observed increase in the phenol content of the naturally ripe compared with the artificially ripened mango fruit, does not agree with earlier report (Rodríguez *et al*., 2016) who submitted a decrease in the phenolic contents of palm fruit with ripening. Variation might be ascribed to differences in the fruits used.

Terpenes are the main constituents of the essential oils of many plants products. They function as natural flavor additives for food, as fragrances in perfumery, and in medicine and alternative medicines such as aromatherapy.

The observed decrease in the contents of carotenoids, phenols and terpenes in the artificially ripened mango fruit, in the current study connotes a likely reduction in disease prevention, antioxidant and flavor enhancing potentials of the fruit.

Flavonoids were observed to be higher in the unripe mango group compared to the naturally ripe as well as the artificially ripened mango groups suggesting that ripening depleted this bioactive compound. According to Herrmann (1991), changes in the amount of flavonoids during fruit ripening might be connected to the development of anthocyanin pigment in the fruit. The antioxidant property of plant products is thought to correlate with the absolute flavonoid content in the fruits, demonstrating that flavonoids could be the determinants of the anti-oxidant capacity of the fruit as reported by Izundu *et al*., (2016). In recent times, there has been a surge of interest in bioactive constituents like flavonoids, owing to their antioxidant capacity which is associated with the redox properties of their hydroxyl side chain as well as the structural relationship between their different functional groups thereby empowering them to function as metal chelators, reducing agents, hydrogen donors, (Amic *et al*., 2003), singlet oxygen quenchers (Gomez-Alonso *et al*., 2003, Materska & Peruka, 2005) and free radical chain breakers (Oboh *et al*., 2008) Flavonoids decreased in the artificially ripened groups thus connoting a decrease in the antioxidant capacity of the fruit. The fact that unripe mango fruit has higher flavonoid content suggests that it might be recommended for diabetic and hypertensive patients as dietary intervention in the management of their pathological conditions (Ibukun *et al*., 2012).

Present study revealed significantly lower concentrations of flavonoids, tannin, alkaloids, phytosterol and glycosides in the artificially ripened mango groups compared with the unripe but the values for each (except glycosides) were significantly higher in the calcium carbide and the polybag ripened mangoes compared to the naturally ripe mangoes. The hotwater ripened mangoes were significantly lower in alkaloids, tannins, saponins, phytosterols and glycosides which agree with earlier report (Negi, 2012) that many phytochemicals are lost by heat processing. Increase in tannin content of the artificially ripened mangoes compared with the naturally ripe ones may be connected to their role as flavor contributors (Aina, 1990) adduced by the observed higher tannin content of the unripe mangoes compared to the rest of the group. Information from emerging data suggest that tannin depletes blood cholesterol (Basu *et al*., 2007) connoting that the consumption of unripe mangoes might be better preferred for the management of hypertension and Ischemic diseases. Saponins are high molecular weight plant constituents that have a sugar moiety associated with a steroid glycone (Price *et al*., 1987). They have cleansing, pesticidal, antilipideamic and anticancer properties (Gurfinkel & Rao, 2003; Kim *et al*., 2003) and are believed to be valuable in the treatment of polluted water (Hall & Walker, 1991). Alkaloids are low atomic weight basic (nitrogenous) compounds. They exert strong defensive impact against pathogens and herbivores and are thought to have pain relieving, antimalarial, antibacterial and antihypertensive properties (Dangi *et al*., 2002). Glycosides are triterpenoids which have cardio active properties (Brian *et al*., 1985). They exert various impacts on the mechanical and electrical activities of the heart just as on the neural tissue (Olaleye, 2007). Present study revealed that flavonoids, tannins, alkaloids, phytosterols and glycosides contents were higher in the artificially ripened mangoes compared to the naturally ripe ones.

Vitamin analysis showed a significantly higher value of vitamin C in the artificially ripened mango fruits compared with the control which did not vary with the unripe mango group. This report agrees with previous report (Mamiro *et al*.,2007, Appiah, *et al*., 2011) that presented significant increase in Vitamin C with induced ripening but disagrees with the report of (Ralman *et al*., 2007) who reported that fruit nutrients like Vitamin C were higher in naturally ripened fruits. Variations might be attributed to ripening stage or method of artificial ripening employed in the fruit ripening processes (Gbakon *et al*., 2016). Subjection of fruits to light directly impacts on the chemical composition of fruits implying that the higher the light intensity, the higher the vitamin C content in the fruits since light is required for energy generation during photosynthesis which is later used as glucose to synthesize more ascorbic acids in the fruits (Stumpf *et al*., 1988), this agrees with the report of lower vitamin C content observed in the polybag group in current study.

The observed increase in the concentration of vitamin C in the artificially ripened mango fruits is important since vitamin C is regarded as essential to human health (Appiah *et al*., 2011) for various reasons, including, prevention of scurvy and cancer, relief from common cold, formation of bile salt from cholesterol, stimulation of collagen synthesis and also for its critical role in the wound healing process (Iqbal *et al*., 2004). More so, vitamin C improves absorption of iron from non-heme sources (Teucher, *et al*., 2004). Vitamin C is a water-soluble, free radical scavenging antioxidant found in the cytoplasm of cells, (Mckee and Mckee 1999). Production of free radicals in living organisms gives rise to oxidative stress in the living cells (Nwaogu, *et al*., 2011). Vitamins A, C, and E and some enzymes such as catalase, glutathione peroxidase, and superoxide dismutase help to terminate free radical chain reactions in a living cell which if left unchecked, may prompt onset of numerous diseases (Nwaogu, *et al*., 2008). Vitamin C is commonly utilized as food additive acting as antioxidant (Whitney & Rolfes, 2008). Humans are unable to synthesize their very own vitamin C since human cells cannot perform the last but crucial step in vitamin C biosynthesis which is the change of L-gulono-g-lactone into ascorbic acid catalyzed by gulonolactone oxidase enzyme (Muhamed *et al*., 2014; Eze *et al*., 2017). Therefore, vitamin C is required for maintaining physiological capacities and to meet this requirement, vitamin C must be consumed from diet.

The adverse effect of calcium carbide as a ripening agent has long been established by various researchers (Singal *et al*., 2012; Dhembare *et al*., 2014). Also, the migration of lead from black polythene bag into cooked green banana has been reported by Banadda *et al*., (2011). They reported that lead (Pb) and cadmium (Cd) which are residues from the polymerization process of polythene can migrate into food materials at high temperature and under long storage period. It is therefore, pertinent to note that though artificial ripening of fruits may increase vitamin C and many of the disease preventing phytochemical content of the fruit than the naturally ripe fruits, such practice may increase the risk of contamination of fruit with toxicants such as arsenic and lead found in the chemical materials utilized in these artificial ripening processes (Sogo-Temi *et al*., 2014.

V. CONCLUSION

Findings have shown that artificial ripening of fruits increased many of the Phytochemicals investigated and also increased vitamin C content of the mango fruits. Owing to the importance of these food constituents in maintaining optimal health, biological methods, which would not pose any threat to human health should be discovered and encouraged.

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Production Function Analysis of Non member of dairy Cooperative Society for Milch Buffalo in District Etawah of U.P.

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***Abstract*— *This study covered Cobb douglas production function, Tukey and Kramer analysis on Non members dairy cooperative society for milch Buffalo in district Etawah of U.P. In study researchers have taken post-stratified into Landless, Marginal, small, medium and large herd size categories. The study effect of various factors of production in (Rs.) like Feeding cost included (dry fodder + green fodder), expenditure of concentrate included (grain + khali + mineral material and chunni / choker) and miscellaneous expenses included (labor charge and fixed cost) on milk produced by the Buffalo of dairy cooperative society non members in annual in different categories of farmers. Further, the researchers have found out the comparative analysis of all the categories of dairy cooperative society non members. At last Tukey and Kramer test was applied on all the category of dairy cooperatives society members in milchBuffalo to get into the depth of the problem under investigation. This study is helpful to find out the elasticity of different factors of milk production and comparative analysis in all categories of members dairy cooperative society in milchBuffalo by Cob douglas production function analysis.*

***Keywords*— Elasticity of fodder, Elasticity of concentrate, Elasticity of miscellaneous, Return to scale, Classification Code: Agriculture Management.**

I. INTRODUCTION

FAO predicting a 2% increase of world milk production from 805 million tonnes in 2015 to 827 million tons in 2020. Most of this increase is expected to come from developing countries such as India, China, Pakistan and Turkey, where it will be used to meet growing demand. The FAO forecasts also show some supply growth in Europe, Australia and the US, although at much lower rates while they predict New Zealand‟s 2015 production to be roughly the same as last year. As consumption levels in developed countries such as Europe, Oceania and North America are unlikely increase fast enough to use up the additional milk supplies, this will lead to an increase in exports during 2015.

Uttar Pradesh is the highest milk producing state 23.33 Million Tonnes and hold a share of more than 17% in the total milk production in India. Apart from being the largest milk producer, Uttar Pradesh also has the largest number of cows and buffaloes, which is more than 1.8 Crore. in 2014-15. Kherigarh, Ponwar, Gangatiri and Kenkatha are some of the cow breeds found in Uttar Pradesh. These cow breeds are mainly found in Uttar Pradesh and known for producing milk in high quantity. Uttar Pradesh has more than 40 dairy cooperatives, which supply milk to many states in the country. On the basis of per capita milk consumption, Uttar Pradesh continued to remain the leading milk producer, followed by Rajasthan and Gujarat, and whereas, the per capita demand was maximum in Punjab followed by Haryana.

Milk is an essential as well as popular food of the Indian diet. It is highly nutritious and occupies 15 percent of the total consumed dietary protein in the industrialized world. Grossly speaking milk constitutes 3.1 percent protein 4.0 percent fat, 5.0 percent lactose 0.74 percent minerals and sizeable amount of vitamins, milk is also a close substitute for nonvegetarian food.

"As per an assessment made by the Planning Commission Report- 2012, the domestic demand for the milk by 2020-21 is expected to be 172.20 million tons. India would have sufficient production to meet such demand. The international body on the farm sector in its latest „Food Outlook‟ report also estimates global milk production in 2020 grow by 2% to 827 million tones. The National Dairy Development Board (NDDB) had published a report in "Perspective 2010" in which to enable the co-operatives to meet the new challenges of globalization and trade liberalization. Like other major dairying countries of the world, the Indian co-operatives are expected to play a predominant role in the dairy industry in future as well. However, India is in the meantime, attaining its past glory and is once again becoming "DoodhKaSagar". But what percentage of this Sagar is handled by the co-operatives is just a little over 7 per cent. Since liberalization of the dairy sector in 1991, established of the dairy factories in the country but their share of total milk is hardly 5 per cent. Therefore, the total share of the organized sector in India, both co-operatives as well as the private sector is hardly 12 per cent. Besides, growth in milk production is likely to continue at present * (Department of Animal Husbandry, Dairying& Fisheries, Ministry of Agriculture, GOI-2014-15) rate of 4.4 % in the near future. Who will handle this increment in milk production in India? Demand for milk at current rate of income growth is not sufficient; India needs to grow at-least 7 per cent per annum to full fill the demand. The study analyzed various factors of production in (Rs.) like Feeding cost included (dry fodder + green fodder), expenditure of Concentrate included (grain + khali + mineral material and chunni / choker) and miscellaneous expenses included (labor charge and fixed cost) on milk produced by the cow of dairy cooperative society members in annual in different categories of farmers i.e, landless, marginal, small, medium and large on the basis of land holding capacity. Analyses of Cobb Douglas production function, researchers find out elasticity of fodder, concentrate and miscellaneous factors of milk production. Further, the researchers have identified percentage of data variation on different category members of dairy cooperative society. At last Tukey and Kramer test was applied on all the category of dairy cooperatives society members in milch cow to get into the depth of the problem under investigation. This study is helpful to find out the comparative analysis in all categories of members dairy cooperative society in milch Cow.

"Etawah" in Uttar Pradesh is famous for its Bhadawari breeds of buffalo and Jamunapari breed of goats. The said breed of buffalo were also known for consuming less fodder relative to production of high fat content milk. However, all the milch animals such as buffalo, cow and goats are grazed in the ravines and the forest area between Jamuna and Chambal rivers of Etawah district of U.P. The numbers of milch livestock of Etawah district during 2012 were reported as total number of female adult cows 1, 10,825 total number of adult females' buffaloes 92065 and total female adult goats were 2, 41, 61.

II. REVIEW OF LITERATURE

Murithi, Festus Meme,(2002), study was motivated by the need to find means of increasing milk supply in Kenya in order to meet an expected rise in demand. The study was concerned with the efficiency of resource use in smallholder milk production. The major objective of the study was to determine whether tnere are possibilities of increasing milk production through re-allocation of the resources used in milk production~ The problems encountered by farmers involved in milk production were also examined. The data used in the study were collected from 60 smallholders who are members of five Dairy Co-operative Societies which are affiliated to the Meru Central Farmers Co-operative Union. A Cobb-Douglas milk production function was fitted using the inputs used in milk production. The results showed that concentrates significantly

Influenced milk yields. The test for efficiency of resource use revealed that there was inefficiency in the use concentrates. Profit maximization I~equires that the marginal value product of an input be equated to the price. If this condition is fulfilled in the study area with respect to concentrates, the average milk yeild per animal per year would increase by 73% above the current levels. An important conclusion of the study is that there could be substantial in milk output and consequently gains in farm profits if the amount of concentrates fed to the animals is increased above the cur-r-errt level s. It is recommended that:- (i) effot'ts be intensified to educate the benefits of increased feeding of concetrates to the (ii) animals, constraints which contribute to the unavailability of concentrates when farmers need them be removed, (iii) farmers be educated on how they can the excess animal feeds which is produced in the winter season to feed the animal and educated on how best season, they can utilize the farm by-products while they are of high nutrition value to feed the animals.

Sharma, P.K. & Singh, C.B. (1984), conducted a study in the intensive cattle development project and observed an increasing trend of human labor employment per household. The dairy enterprise on an average generated 250 days of employment on both category of beneficiary and non-beneficiary households . The family labor income of Rs.1076 obtained from cross bred cow was much higher than that of a buffalo and local cow. Further the beneficiary households recorded higher income from different types of milch animal as compared to that of non beneficiary households. Therefore, they concluded that the project has been able to generate additional gainful employment in the study area and thus it can go a long way in boosting up income and employment levels specially an small cattle holdings.

Sharma, P.K. & Singh, C.B. (1986), studied the impact of I.C.D.P. Karnal on production, consumption and marketed surplus of milk in rural Karnal. The study revealed that production of milk was relatively higher on the beneficiary households than that of nonbeneficiary households of cattle owners with rise in production of corresponding increase in milk being marketed by the beneficiary households. The overall marketed surplus of milk on beneficiary and non beneficiary households was about 44 and 28 per cent respectively. The project could, therefore be expected to provide a better source of income through milk production. Interestingly a positive impact of project was seen as consumption of milk. The per capita per day milk consumption of 729 and 623 gm on beneficiary and non beneficiary households respectively. It was much higher than the national average of 121 gm only

Hirevenkanagoudar, L.V. et .al., (1988), studied the impact of dairy development programmes of the Karnataka Dairy Development Cooperation (KDDC) on the selected economic aspects of small and marginal farmer and agricultural labours. The study revealed that over 56 per cent of KDDC beneficiaries were getting 50-75 per cent of their family income from dairy enterprises whereas, 60-87 per cent of non KDDC farmers getting 25 per cent of their income from dairy enterprises. All KDDC farmers were selling milk to dairy co-operative societies. Mostly small farmers, marginal farmers and agricultural labors in the KDDC programme and 60 per cent of the non KDDC category through that dairy co-operative societies were the best agencies for milk marketing. More than 64 per cent of KDDC farmers had repaid 75 per cent to 100 per cent of the dairy loan, whereas only 10-25 percent at nonKDDC farmer had repaid 75-100 per cent of their dairy loan.

Dass, B. et. al., (1990), studied performance of dairy co-operative. involved in production of dairy co-operative involved in production and distribution of milk in Tarai region of district Nainital (Uttar Pradesh) during the year 1986. The study revealed that the co-operative societies had a positive and significant impact on the size of milch breed, level of milk production and marketed surplus of milk per member household. The size of milch herd increased by 55 per cent, the level of milk production by 65 per cent and marketed surplus of milk by 72 per cent in the societies group as compared the non-societies group. The income generated through dairying was 30 per cent of the total cash income in the societies group as against 21 per cent in the non-societies group.

Jitendra, K. &Shankara, M. (1992), studied the impact of dairy co-operative and income and employment in chittordistrict,Andhra Pradesh. It was found that agricultural labour and non-agricultural labour earned more income from dairying than small farmers who were earned more in crop production. The employment created to members (121.5 days in area-I and 112.2 days in area-II) was significantly more compared in non-members (76 days in area-I and 53.5 days in area-II) in the study area. Thus, the dairy co-operative have contributed in generating more income and employment to the dairy farmers.

Prajneshu,(2008), the set of Cobb-Douglas production functions is usually fitted by first linear zing the models through logarithmic transformation and then applying the method of least squares. However, this procedure is valid only when the underlying assumption of multiplicative error-terms is justified. Unfortunately, this assumption is rarely satisfied in practice and accordingly, the results obtained are of doubtful nature. Further, nonlinear estimation procedures generally yield parameter estimates exhibiting extremely high correlations, implying thereby that the parameters are not estimated independently. In this paper, use of expected-value parameters has been highlighted and the advantages of their use have also been discussed. Finally, the developed methodology has been illustrated by applying it to the wheat yield time-series data of Punjab.

Venkatesh P. and Sangeetha V.,(2011), a study was conducted to examine the cost structure and resource use efficiency of dairy farms in the Madurai district of Tamil Nadu. The dairy farmers were selected by using multi stage random sampling technique. Tabular analysis and Cobb-Douglas production function were used in this study. Total costs per lactation per animal estimated were of the order of Rs.12776.09, Rs 11791.20 and Rs.12079.28 and returns per rupee of investment 0.78, 1.08 and 0.95 respectively on small, large and pooled farms. Feed cost was the higher input cost in dairy farming (61.6%). The cost of production milk per litre was less in case of large farms (Rs. 4.62) compared to small farms (Rs. 5.39). Results indicated the inverse relationship with the size and the herd of the total costs, due to economies of scale. Functional analysis showed barring human labour on small farms all the selected input variables such as green fodder, dry fodder, concentrates and health care were positive and significant impact on the production of milk indicating the potentiality of their further use.

Meena G. L. et.al.,(2012), study was undertaken in Alwar District of Rajasthan with the objectives to examine the input-output relationships and assess the resource use efficiency in milk production. The study covered 75 cooperative member milk producers and 75 non-cooperative member milk producers. The results of Cobb-Douglas production function revealed that concentrate had positive and significant influence on returns from buffalo milk across all the household categories for both the member and non-member groups. Green fodder and dry fodder were also influenced the returns from milk significantly across all the household categories for both the member and non-member groups with the sole exception of large category of non-member group. D_1 (winter) and D_2 (Rainy) dummy variables were found to be positive and statistically significant. The results of Chow's test clearly revealed that the production functions between member and non-member groups differed significantly. The results of the resource use efficiency revealed that green fodder was over-utilized in small and medium categories for both the member and non-member groups, dry fodder was over-utilized by medium category of member group, concentrate was over-utilized by only medium category of member group and by small & medium categories of non-member group while it was under-utilized by large category of non-member group and labour was over-utilized by only small category of member group.

Makwana D. Girish et.al.(2016), suggested the dairy sub-sector occupies an important place in agricultural economy of India. As milk is the second largest agricultural commodity in contributing to GNP. Currently, more than 80 % of the milk produced in the country is marketed by the unorganized sector (private organization) and less than 20 % is marketed by the organized sector. But, both organized and unorganized sector in the dairy industry of the district face a lot of constraints relating to production and marketing constraints as well as – infrastructural , technical , socio-psychological, economical with high or low severity to expansion of milk production in the district, availability of green fodder and concentrate , knowledge of balance feeding, irregular sale of milk ,lack of time of marketing, less knowledge about of marketing strategies, no or less provision for advance payment for milk by society or vendors, delay in payment by unorganized sector, in ability to market for value added products, transportation. Processing availability of veterinary facilities , lack of awareness of animal health care and training facilities for scientific dairying etc. facing by cooperative and non-cooperative members in Kheda district of Gujarat.

Mahida D. et.al.(2018),suggested thatdairying has been a prominent supplementary enterprise and regular source of income to the farmers. Indian dairy sector has progressed commendably well with seven-fold increase in milk production since independence, but progress in terms of yield per animal is still low which is quite unsustainable. Literature suggests two approaches for productivity growth viz., through technical progress and improved efficiency. The present study is an attempt to determine the factors affecting the technical efficiency of dairy farmers in Gujarat state with a special emphasis on the role of milk cooperatives. Multiple regression analysis and regression tree approach were used for arriving valid conclusions. Results indicated that socio-economic factors i.e. membership in dairy cooperative society, non-farm annual income, access to information, and herd size significantly influenced the technical efficiency of farmers. Dairy cooperatives provide several inputs in the form of dairying resources as well as technical information to the farmers which significantly influenced their efficiency. The study concludes with policy prescriptions for enhancing milk production and shift towards sustainable dairying.

III. RESEARCH METHDOLOGY

District Etawah milk producers' cooperative union was purposively selected from the state of Uttar Pradesh. Exhaustive lists of all the milk producers' cooperative societies in Etawah district milk producers' cooperative union were prepared. Researchers have selected randomly 150 non member of dairy cooperative society & 150 members of dairy cooperative society from 10 Villages of 2 blocks selected in district Etawah. All the milk producing household members and non members were classified into five categories, viz., Landless, Marginal, Small, Medium and Large farmers on the basis of land holding capability. Thus, in all, 300 households were interviewed during the year 2008-09. The primary data were collected to help of well structured pre-tested schedule by the personal inquiry method. The data collected were subjected to tabular analysis in order to study the comparative economics of milk production. Cobb-Douglas type Production Functional analysis was applied on cow milk production with three variables like-fodder, concentrate and miscellaneous of different categories landless, marginal , small, medium and large member farmers of dairy cooperative society.

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The study effect of various factors of production in (Rs.) in case of milk cooperative societies non members in annual in different categories.

$$y = a x_1^{b_1} x_2^{b_2} x_3^{b_3} \quad \ldots\ldots (1)$$

log y = log a+ b_1 log X_1 + b_2 log X_2 +b_3 log X_3 …(2)

Where

Y	=	Production of milk in (Rs.)
X_1	=	Feeding cost included (dry fodder + green fodder)
X_2	=	Expenditure of Concentrate included (grain + khali + mineral material and chunni / choker)
X_3	=	Miscellaneous expenses included a labor charge and fixed cost.
b_i	=	Respective elasticity's of milk production
a	=	constant

Having estimated the cost of milk production, it is desirable to ascertain the reliability of these fodder costs, concentrate cost and miscellaneous expanses estimates. The most commonly used "t" test was applied to ascertain whether the cost of milk is significantly different from zero or not at some specified probability level.

"t" cal=b_j / standard error of $b_{j..}$

If calculated "t" value is greater than the table value of "t" at a specified probability level and "n-k-1" degree freedom, bj is said to be statistically significant.

IV. RESULT AND DISCUSSION

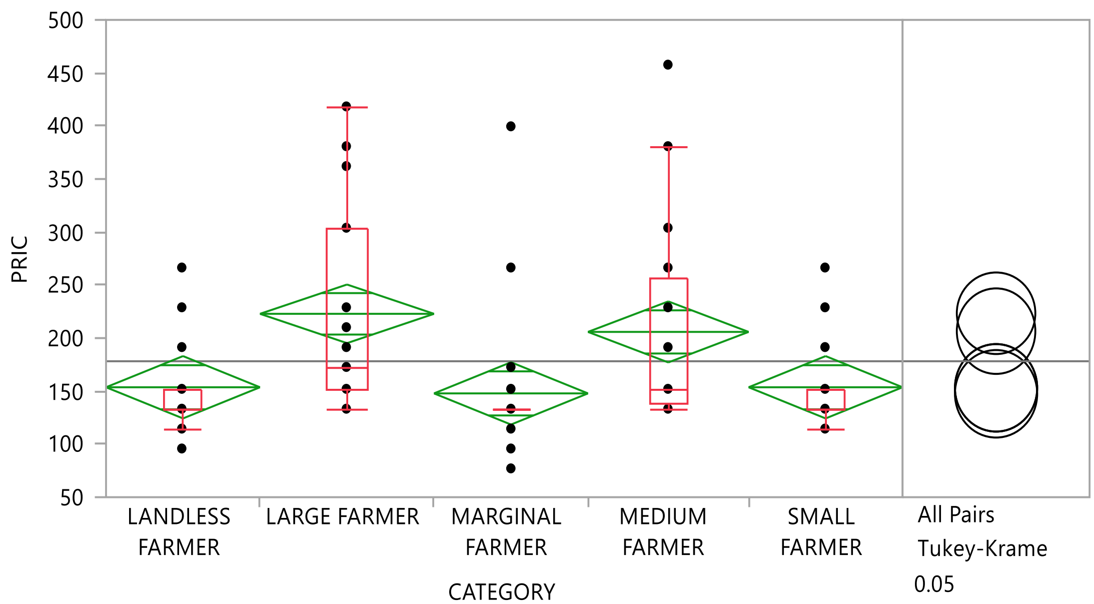
Summary of all categories of Non Member Dairy Cooperative Society for Milch Buffalo:

The analysis are revealed that mean of large farmers was observed Rs. 222.88 they were the most benefited in nonmember buffalo category followed by Medium farmers Rs. 205.88, Landless and Small farmers Rs.163.65 each and the least for Marginal farmer Rs.147.87.

Tukey test was applied to see to get in to the depth of the problem under investigation. This indicated that there is non-significance difference between Medium, Landless and Small farmers. At the last there is non-significance difference among Landless, Marginal and Small farmers for milch Buffalo. The other report indicated the fact that fact P value for Large farmer-Marginal farmer, Large farmer-Landless farmer, Large farmer-Small farmer and Medium farmer-Large farmer were observed significant at 5 % level of Probability (0.0032, 0.0081, 0.0081 and 0.0074) respectively

Summary of all categories of Non Members Dairy Cooperative Society for Milch Buffalo:

Oneway Analysis of PRICE by CATEGORY



Quantiles

Level	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
LANDLESS FARMER	95	114	133	133	152	228	266
LARGE FARMER	133	152	152	171	304	391.4	418
MARGINAL FARMER	76	102.6	133	133	133	228	399

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Level	Minimum	10%	25%	Median	75%	90%	Maximum
MEDIUM FARMER	133	133	137.75	152	256.5	380	456
SMALL FARMER	114	133	133	133	152	250.8	266

OnewayAnova

Summary of Fit

Rsquare	0.171147
AdjRsquare	0.142065
Root Mean Square Error	71.13633
Mean of Response	178.1849
Observations (or Sum Wgts)	119

Means for OnewayAnova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
LANDLESS FARMER	23	153.652	14.833	124.27	183.04
LARGE FARMER	26	222.885	13.951	195.25	250.52
MARGINAL FARMER	23	147.870	14.833	118.49	177.25
MEDIUM FARMER	24	205.833	14.521	177.07	234.60
SMALL FARMER	23	153.652	14.833	124.27	183.04

Std Error uses a pooled estimate of error variance

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.77192	0.05

LSD Threshold Matrix

Abs(Dif)-HSD	LARGE FARMER	MEDIUM FARMER	LANDLESS FARMER	SMALL FARMER	MARGINAL FARMER
LARGE FARMER	-54.689	-38.766	12.788	12.788	18.571
MEDIUM FARMER	-38.766	-56.922	-5.356	-5.356	0.426
LANDLESS FARMER	12.788	-5.356	-58.147	-58.147	-52.364
SMALL FARMER	12.788	-5.356	-58.147	-58.147	-52.364
MARGINAL FARMER	18.571	0.426	-52.364	-52.364	-58.147

Connecting Letters Report

Level				Mean
LARGE FARMER	A			222.88462
MEDIUM FARMER	A	B		205.83333
LANDLESS FARMER		B	C	153.65217
SMALL FARMER		B	C	153.65217
MARGINAL FARMER			C	147.86957

Levels not connected by same letter are significantly different.

Return to Scale for the Dairy Cooperative Society Non-Members (Buffalo):

Table

S.N.	Category	β_1	β_2	β_3	Total $\beta_1+\beta_2+\beta_3$	Return to Scale ≥1
1	Landless	1.13099	7.24889	1.737800	10.117	**≥1**
2	Marginal	101.2977	680.9261	-5394.2367	-4612.0129	≤1

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3	Small	-1.04399	28.6483	-1.42134	28.270	**≥1**
4	Medium	1.33266	502.157	-24.7964	478.693	**≥1**
5	Large	1.9923	19.000	-3.82199	17.17031	**≥1**

β_1= Elasticity of Fodder

β_2= Elasticity of Concentrate

β_3= Elasticity of Miscellaneous expanses

The above table no.31 reveal that Elasticity of milk production for all the five categories of non member farmers of dairy cooperative society in buffalo namely Landless, marginal, small, medium and large farmers. The last column indicates their economies of scale. Their respective value were observed 10.117, -4612.0129, 28.270,478.693 and 17.17031 respectively, out of these five categories namely marginal farmers were observed have decreasing return to scale with value -4612.0129.

The remaining four categories i.e., landless, small, medium and large exhibited increasing return to scale with the value of 10.117, 28.270, 478.693 and 17.17031 respectively. Analysis further reveals that return to scale was the highest for medium farmers followed by small, large and landless non member farmers of dairy cooperative society in case of buffalo.

V. CONCLUSION

Study reveal that Elasticity of milk production for all the five categories of non member farmers of dairy cooperative society in buffalo namely Landless, marginal, small, medium and large farmers. Out of these five categories namely marginal farmers only were observed have decreasing return to scale.

The remaining four categories i.e., landless, small, medium and large exhibited increasing return to scale and analysis further reveals that return to scale was the highest for medium farmers followed by small, large and landless nonmember farmers of dairy cooperative society in case of buffalo.

The analysis are revealed that mean of large farmers was observed highest they were the most benefited followed by Medium farmers, Landless and Small farmers and the least for Marginal farmer in nonmember of Buffalo category.

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Association of seed mycoflora with peas *Pisum sativa* L. seeds

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***Abstract*—*Seed is a vital source for producing optimum yield. Seed-borne pathogens may cause losses by reducing seed germination, developing seed-borne diseases and mortality of seedlings in nursery beds. However, knowledge of the about the biology and extent of seed-borne pathogens and thereafter practices for their management can help to reduce seed and seedling losses. Thus, present studies were conducted to find out the association of seed associated mycoflora with major vegetable peas in the laboratories of Department of Plant Protection, Sindh Agriculture University and Federal Seed Certification and Registration Department, Karachi, Sindh, Pakistan from February to October 2017. Total of 10 different fungal species belonging to different genera was isolated from peas seeds through blotter paper and agar plate methods. Significantly highest frequency percent for Alternariaalternata (50%), Aspergillus fumigates (48.33%), A. niger (36.67%), A. flavus (23.33%), C. lunata (17.5%) and F. oxysporum (17.5%). However, the lowest frequency was recorded for T. viride (4.17%), R. stolonifer (5%), Penicillium sp. (5.83%) and Stemphylium (11.67%) through the agar plate method. A similar trend of frequency was noticed for blotter paper in all recorded fungi; however, the extent was lower compared to the agar plate method. The results of the present studies provide the baseline information about seed mycoflora for further studies and management of seed-borne diseases associated with peas seeds.*

Keywords*—*Pisum sativa, Seed Mycoflora, Isolation methods.

I. INTRODUCTION

Pea, *Pisum sativa* L is the most important vegetable and is cultivated throughout the world for consumption of human being and animals. It is one of the richest, cheapest and readily available sources of minerals, vitamins, protein, and carbohydrates. It is a valuable food product due to the rich source of nutrients that capable of meeting the dietary needs of approximately eight hundreds to nine hundred million undernourished peoples throughout the world **[1]**.

However, the yield is influenced by the number of foliar and soil-borne diseases. Moreover, the pea is the most sensitive to damping off and root and foot rots caused by seed and soil-borne fungal pathogens **[2].** The infestation in the seed may results occurrence of severe diseases in subsequent crop **[3].** Varying degrees of discoloration and shriveling can be seen in infected seeds; however, sometimes other even infected seeds remain symptomless **[4, 5, 6].**

In pea, Ascochyta blight disease is caused by three seedborne fungal species such as *Ascochytapisi*, *Phomapinodella*, and *Mycosphaerellapinodes*. These species can also survive on plant residues in soil. Generally, such fungi occur individually or some time in combination and then referred to as the Ascochyta complex **[7, 8].** Seed-borne infestation by *P. pinodella* and *M. pinodes* generally causes more severe and widespread seedling diseases compared to an infestation of *A. pisi***[3].** In addition to Ascochyta complex, several other seed-borne pathogens such as *Alternariaalternata*, *A. tenuissima*, *Penicillium* spp., *Fusarium* spp. and other fungi that may also cause root rots and reduce the germinating power, deteriorate seedlings', affect health and growth of pea **[9, 10]**.

It is obvious that most of the important seed borne diseases caused by pathogens are disseminated by seeds; thus, it is known as passive carriers, often cause significant losses in yield. So for sustainable production and quality yield, use of disease-free and healthy seeds have been recognized best approach. Therefore, knowledge about seed health and quality, proper seed testing is essential to get tasks. Thus, the present investigation was conducted to analyze the seed associated mycoflora of peas vegetable.

II. MATERIAL AND METHOD

2.1 Study locations

The present study was conducted in the laboratories of the Department of Plant Protection, Sindh Agriculture University and Federal Seed Certification and Registration Department, Karachi, Sindh, Pakistan.

2.2 Collection of Seed Samples

Seed samples of peas kindly provided by Federal Seed Certification and Registration Department, Karachi, Sindh, Pakistan for isolation and identification of seed mycoflora and seed health analysis. Seed samples assessed at the laboratory of Federal Seed Certification and Registration Department and brought to the laboratory of Department of Plant Protection, Sindh Agriculture University, Tandojam, Pakistan for further studies.

2.3 Isolation and identification of seed mycoflora

Seed samples of peas were randomly collected for isolation purpose. Samples were placed in paper bags and brought for isolation and identification of seed mycoflora in the laboratories of Federal Seed Certification and Registration Department and Department of Plant Protection, Sindh Agriculture University, Tandojam.

2.3.1 Isolation of Seed mycoflora

Isolation of seed-borne mycoflora associated with peas was conducted by using isolation techniques described by the International Rules for Seed Testing Association (ISTA, 2015) as under:

2.3.1.1 Standard blotter method

Seed samples of peas were tested by standard blotter method for the isolation of associated mycoflora. Three layers of blotter papers circular in shape and 150 and 90 mm diameter according to the size of Petri dishes were cut with the help a scissor. The blotters were dipped and moistened with sterilized distilled water and excess water was removed from the Petri dishes. The moistened blotter papers were transferred to sterilized Petri dish with the help of sterilized forceps. Randomly collected seed samples were thoroughly washed with tap water and then dried. These seeds were surface sterilized in 0.1 percent mercuric chloride solution (HgCl2) for 30 seconds followed by three washing with sterilized distilled water in beakers under aseptic conditions using laminar air flow. All seeds were then completely dried by placing on sterilized blotting paper. A total of 12 seeds were placed in such a manner that 11 were in an outer circle and one in the center. The seeded Petri dishes were incubated in the chamber. Seeds were examined on 3rd days, 5th days and 07th days after incubation (DAI).

2.3.1.2 The standard agar plate method

In the standard agar plate method, seeds were surface sterilized as described earlier. Five seeds were transferred aseptically to the Petri plates containing sterile potato dextrose agar (PDA) medium amended with an antibacterial agent and filled up to quarter strength in a manner that 4 seeds were kept at the outer circle and 01 seed at the center of Petri dish. The inoculated plates were incubated at 25±20ºC.

All the plates were monitored regularly and growing colonies were subjected to different laboratory codes for frequency percentage and further analysis.The culture, thus, obtained was subjected to purification. A single spore culture technique was used to purify the isolates. Sub-culturing of isolates were made time to time to maintain the fresh culture for further analysis until the end of experiments.

2.3.2 Identification of pathogens

Temporary slides of fungal isolates from pure cultures were made and observed under a light microscope. Morphological and cultural characters of isolated fungi were recorded and compared with standard keys for establishing their identity (Barnett and Hunter 1972; Booth and Sutton 1984; Nelson *et al.* 1983). In addition, internet databases were also used to compare the morphological characteristics of isolates.

2.4 Determination of extent of seed mycoflora

Determination of extent of seed associated mycoflora with major vegetable crops viz; peas, bottle gourd, cucumber, and tomato were evaluated from both methods; standard blotter paper and agar plate method. The extent of seed associated mycoflora from each vegetable was calculated by using the following formula:

$$\text{Frequency (\%)} = \frac{Number\ of\ Pieces\ colonized}{Total\ Number\ of\ Pieces\ studied} \text{X100}$$

2.5 Statistical analysis

The data obtained in present were statistically analyzed by using the standard procedures for analysis of variance, ANOVA (linear model), and mean separation (least significant difference, LSD) of all parameters by using the computer software Statistix 8.1 **[15].**All differences described in the text were significant at the 5% level of probability.

III. RESULTS

The results so far achieved are summarized here and hope that will be helpful for pre-sowing seed treatments and sustainable management of seed associated mycoflora.

3.1 Isolation and identification of seed mycoflora

The association of seed associated fungi isolated through blotter paper and the agar plate method showed great variability. It was observed that higher numbers of fungal isolates were preferably grown on agar plates compared to blotter papers. A total of 09 fungi viz; *Alternariaralternata, Aspergillusflavus, A. fumigates, A. niger, Curvularialunata, F. oxysporum, Penicillium, Rhizopusstolonifer*and *Stemphylium*. However, *Trichodermaviride* was the only fungus isolated through the agar plate method Fig. 1.

3.2 The extent of seed associated mycoflora

The frequency percent of different fungi showed significant variation (P<0.05 = 0.0000 and0.0000) among different isolates recorded for both blotter paper and agar plate method, respectively. However, no significant difference was found observed among the different seed samples of two years (P<0.05 = 0.8918 and 0.5087) for both blotter paper and agar plate method, respectively (Table 1).

The frequency percent of seed mycoflora with peas seed showed significantly highest percent for *A. alternata* (28.33 and 36.66%) followed by *A. niger* (25 and 31.66%), *C. lunata* (20 and 10%), *R. stolonifer* (18.33 and 13.33%), *F. oxysporum* (15 and 11.66%) and *A. fumigates* (15 and 16.667%) from the samples of 2016 and 2017, respectively (Figure 2). However, the frequency percent of *A. fumigates* from the seeds of 2017 was a bit higher (16.667%) compared to *R. stolonifer* (13.33%), *F. oxysporum* (11.66%) and *C. lunata* (10%). Whereas significantly lowest frequency percent was recorded for *Stemphylium* sp. (0 and 3.333) followed by *Penicillium* (8.33 and 5%), *A. flavus* (11.67 and 16.67%) from the seeds of 2016 and 2017, respectively (Fig. 2) through blotter paper.

The frequency percent of seed mycoflora isolated through the agar plate method from the peas seed samples of 2016 and 2017 showed significantly highest percent for *A. fumigates* (60 and 36.67%) followed by *A. alternata* (45 and 55%), *A. niger* (33.33 and 40%), *C. lunata* (25 and 10%) and *F. oxysporum* (20 and 15%), respectively (Figure 3). However, the frequency percent of *A. alternata* (55%)was higher than *A. fumigates* (36.67%). Significantly lowest frequency percent was recorded for *R. stolonifer* (5 and 5%) followed by *Penicillium* sp. (6.67and 5%), *T. viride* (8.33 and 0), *Stemphylium*sp. (10 and 13.33%) and *A. flavus* (16.67 and 30%) from the seeds of 2016 and 2017, respectively. However, *T. viride*was not recorded from the seed of 2017 through agar plate method as well through blotter paper methods during both years of seed (Fig. 3).

On an overall basis, the mean of two years for the frequency of fungi showed significant (P<0.05 = 0.0000 and 0.0001) difference among the different isolates and methods, respectively. However, no significant difference (P<0.05 = 0.7690) was found observed among the different seed samples of two years for overall frequency percent (Table 1). Significantly, highest frequency percent was recorded for *A. alternata* (32.5%) followed by *A. niger*(28.33%), *A. fumigates* (15.83%), *R. stolonifer*(15.83%) and *C. lunata* (15%) isolated through blotter paper method from peas seed. However, *Stemphylium* sp. (1.67%) followed by *Penicillium* sp. (6.67%) and *F. oxysporum* (13.33%) and *A. flavus* (14.17%) were recorded with the lowest frequency in blotter paper method (Table 2). The frequency in the agar plate method of peas seed showed little variation compared to blotter paper method. The highest frequency was recorded for *A. alternata* (50%), *A. fumigates* (48.33%), *A. niger* (36.67%), *A. flavus* (23.33%), *C. lunata* (17.5%) and *F. oxysporum*(17.5%). However, the lowest frequency was recorded for *T. viride* (4.17%), *R. stolonifer* (5%), *Penicillium* sp. (5.83%) and *Stemphylium* (11.67%) through agar plate method (Table 2).

Moreover, when the interaction was assessed for the frequency percent of both fungi and methods revealed a significant difference (P<0.05 = 0.0005). Majority of the fungal isolates prefer to grow on agar plates instead of blotter paper; thus, the significantly maximum frequency of a majority of fungi was recorded from agar plate methods except for two fungi, *R. stolonifer*, and *Penicillium* sp. that produce higher frequency on blotter paper. The interaction of fungi and years revealed no significant difference (P<0.05 = 0.0935) among the different isolates for 2016 and 2017 seeds of peas (Table 2).

IV. DISCUSSION

Seeds play an important role in producing optimum yield through healthy crop production. Healthy seeds, particularly pathogen-free seeds, are necessary for the maintenance of optimum plant populations and production. Approximately 16% of crop losses occur due to plant diseases annually, however, out of which about 10% loss takes place due to seed-borne diseases has been reported by Fakir (1983). Seed-borne pathogens may cause losses by reducing seed germination, developing damping-off diseases, and mortality of older seedlings in nursery beds. Sometimes seed fails to germinate (Mahmoud *et al.* 2013; Haikal, 2008) or losses have occurred in containers during transportation (Campbell and Landis, 1990). However, knowledge of the about the biology and extent of seed-borne pathogens and thereafter practices for their management can help to reduce seed and seedling losses. Thus, present studies were conducted to find out the association of seed associated mycoflora with major vegetable crops in the laboratories of Department of Plant Protection, Sindh Agriculture University and Federal Seed Certification and Registration Department, Karachi, Sindh, Pakistan from February to October 2017.

The association of seed associated mycoflora revealed the diversity of fungal species are associated with seeds that may cause ultimately severe losses to peas vegetable. A total of 10 fungal species were isolated from the seeds of peas. With reference to seed associated fungi with different vegetable seeds, several studies have been conducted; however, the number and species reported mycoflora were different compared to present studies that might be due to the genetic potential of seeds and climatic conditions of the areas.Sheela (2015) determined the association of 15 genera and 29 species of fungi from the seeds of Pigeon pea. Avinash and Rai (3013) also isolated seed-borne fungi of the Cucurbitaceae family using the standard blotter method and deep-freeze method. They isolated and identified about 26 genera and 39 species of fungal colonies. The association of seed associated fungi isolated through blotter paper and agar plate method in the present study showed great variability. It was observed that a higher number of fungal isolates was preferably grown on agar plates compared to blotter papers. Our studies are inconsistent with Sheela (2015), she reported both blotter method and agar plate method as the most suitable technique for the detection of fungi. Though both methods in current remained conducive, however, higher frequency of fungi was recorded with the agar plate method. A total of 10 fungi were found associated with peas seeds, including 09 fungi viz; *Alternariaralternata*, *Aspergillusflavus, A. fumigates, A. niger, Curvularialunata*, *F. oxysporum, Penicillium, Rhizopusstolonifer* and *Stemphylium* were isolated through blotter paper methods. However, one additional fungus, *Trichodermaviride* was isolated through the agar plate method in addition to the above nine fungi. Avinash and Rai (3013) isolated seed-borne fungi of the Cucurbitaceae family using the standard blotter method and deep-freeze method. They isolated 08 fungi from bottle and ridge gourd such as *Fusariumverticillioides*, *F. oxysporum*, *Alternariacucumerina*, *A. alternata*, *Chaetomiumglobosum*, *C. indicum*, *C. crispatum* and *C. funicola*. *F. verticillioides* and *F. oxysporum*. Whereas from pumpkin seeds only *F. oxysporum* and *Cladosporiumcucumerinum;* and *Alternariacucumerina* and *Alternariaalternata* were isolated from bitter gourd seeds.

The great variability was found observed for the extent of associated fungi isolated through blotter paper and agar plate methods from seed samples of years, 2016 and 2017. The extent of seed associated fungi revealed significant variation (P<0.05 = 0.0000 and0.0000) among different isolates recorded for both blotter paper and agar plate method, respectively. However, no significant difference was found observed among the different seed samples of two years (P<0.05 = 0.8918 and 0.5087) for both blotter paper and agar plate method, respectively. The frequency percent of seed mycoflora with peas seed showed significantly highest percent for *A. alternata* (28.33 and 36.66%) followed by *A. niger* (25 and 31.66%), *C. lunata* (20 and 10%), *R. stolonifer* (18.33 and 13.33%), *F. oxysporum* (15 and 11.66%) and *A. fumigates* (15 and 16.667%) from the samples of 2016 and 2017, respectively. Whereas significantly lowest frequency percent was recorded for *Stemphylium* sp. (0 and 3.333) followed by *Penicillium* (8.33 and 5%), *A. flavus* (11.67 and 16.67%) from the seeds of 2016 and 2017, respectively, through blotter paper. The frequency percent through agar plate was almost similar to blotter paper method, only litter variation was found observed in the extent and number of isolates. On an overall basis, the mean of two years for the frequency of fungi showed a significant difference among the different isolates and methods, respectively. However, no significant difference was found observed among the different seed samples of two years for overall frequency percent. Significantly, highest frequency percent was recorded for *A. alternata* (32.5%) followed by *A. niger*(28.33%), *A. fumigates* (15.83%), *R. stolonifer*(15.83%) and *C. lunata* (15%) isolated through blotter paper method from peas seed. However, *Stemphylium* sp. (1.67%) followed by *Penicillium* sp. (6.67%) and *F. oxysporum* (13.33%) and *A. flavus* (14.17%) were recorded with the lowest frequency in blotter paper method. The frequency in the agar plate method of peas seed showed little variation compared to blotter paper method. The highest frequency was recorded for *A. alternata* (50%), *A. fumigates* (48.33%), *A. niger* (36.67%), *A. flavus* (23.33%), *C. lunata* (17.5%) and *F. oxysporum*(17.5%). However, the lowest frequency was recorded for *T. viride* (4.17%), *R. stolonifer* (5%), *Penicillium* sp. (5.83%) and *Stemphylium* (11.67%) through the agar plate method. Moreover, when the interaction was assessed for the frequency percent of both fungi and methods revealed a significant difference

(P<0.05 = 0.0005). Majority of the fungal isolates prefer to grow on agar plates instead of blotter paper; thus, the significantly maximum frequency of a majority of fungi was recorded from agar plate methods. The interaction of fungi and years revealed no significant difference (P<0.05 = 0.0935) among the different isolates for 2016 and 2017 seeds of peas. In the literature, very few studies are available with regards to seed associated mycoflora with peas vegetable. In one study, Wilman*et al*. (2014) identified the pathogens present in the seeds of the tested cultivars viz; *Alternaria* spp. as predominant followed by *Fusarium* spp., *Stemphylium* spp., *Ulocladium* spp., *Botrytis cinerea* Pers., *Epicoccumnigrum* Link., and *Phomapinodella*. Ozgonen and Gulcu (2011) also determined mycoflora of pea seeds and found some most common fungi viz; *Fusarium* spp., *Alternaria* spp., *Macrophominaphaseolina*, *Phytophthoramegasperma*, *Rhizoctoniasolani* and *Sclerotiumrolfsii* from peas seed. Marcinkowska (2010) conducted a study during 2004 and 2006 in *Pisumsativum* seed; maximum fungal species (27) were noticed in 2004, while the minimum (16) during 2006. Significantly, the highest frequency was recorded for *A. alternata* compared to other fungal species viz; *Fusarium*sp., *Penicillium,Phoma, Stemphylium Mycosphaerella*, and *Ascochyta*. Our studies are also in agreement with Marcinkowska (2010) with regard to the association of seed mycoflora in older seeds was higher compared to newer seeds. Moreover, in the current study *A. alternata* was also isolated with the highest frequency.

V. CONCLUSION

It is obvious that seed-borne pathogens can cause severe losses to the crop by reducing seed germination, developing seed-borne diseases and mortality of seedlings in nursery beds. A total of 10 different fungal species belonging to different genera were isolated from peas seeds through blotter paper and agar plate methods revealed the association fungal pathogen with peas seeds. The results of present studies provide the baseline information about seed mycoflora for further studies and management of seed-borne diseases associated with Peas seeds in Sindh Province of Pakistan

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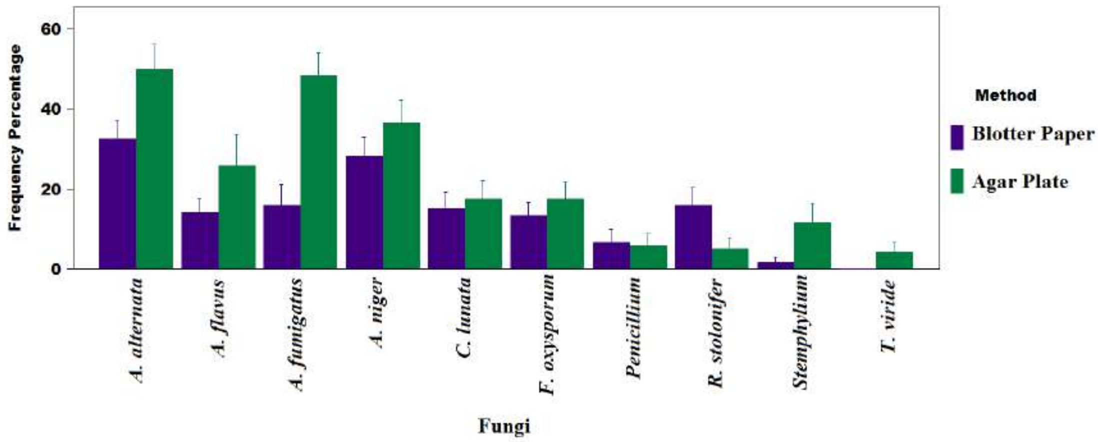

Fig 1. Comparison of blotter paper and agar plate methods for frequency percent of seed associated fungi isolated from the peas seed sample of 2016 and 2017.

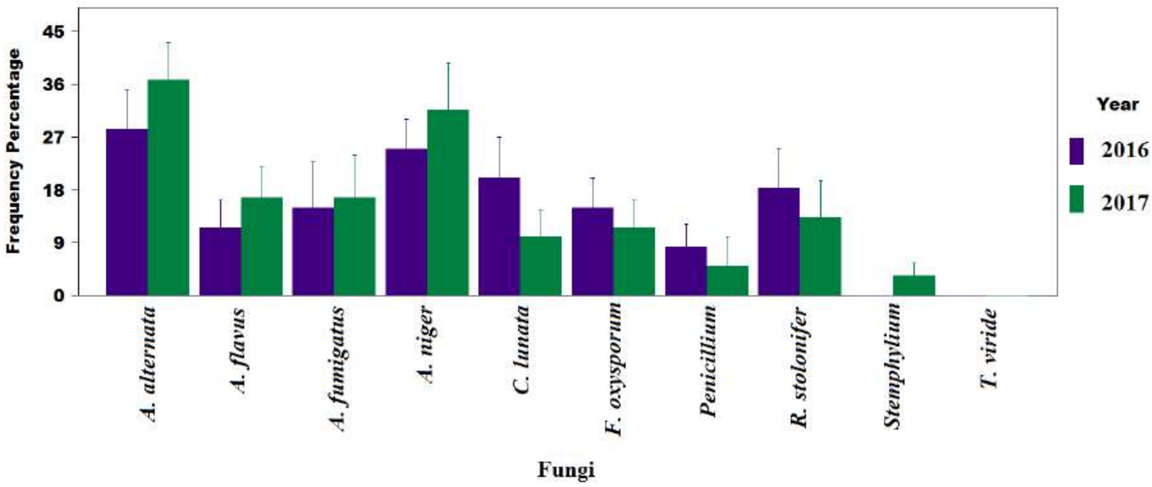

Fig 2. Frequency percent of seed associated fungi isolated through the blotter paper method from the peas seeds sample of 2016 and 2017.

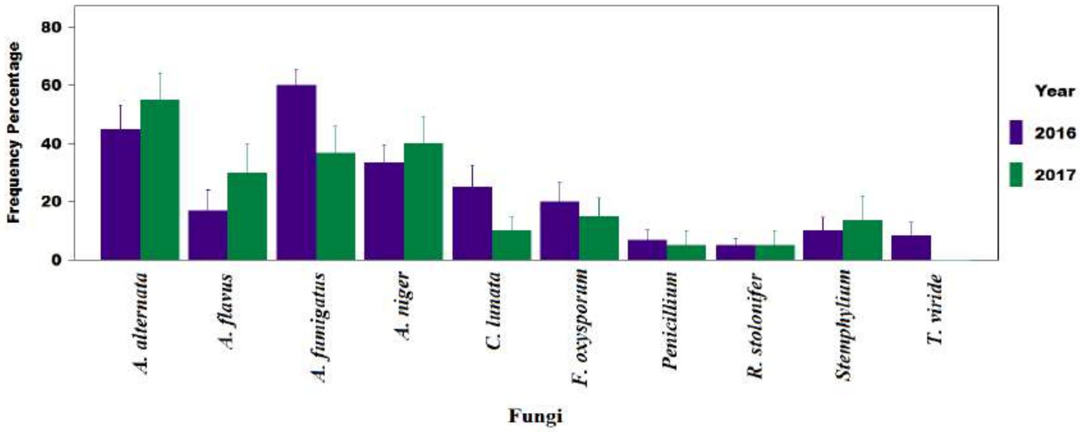

Fig 3. Frequency percent of seed associated fungi isolated through the agar plate method from the peas seed samples of 2016 and 2017.

Table 1. Analysis of variance for combine frequency percent of fungi isolated through blotter paper and agar plate methods from pea vegetable seeds

Source	DF	SS	MS	F	P	Remarks
Fungi	9	73891	8210.09	17.36	0.0000	**
Year	1	41	40.83	0.09	0.7690	-
Plate	11	2309	209.92	0.44	0.9357	-
Method	1	7521	7520.83	15.9	0.0001	-
Fungi*Year	9	7118	790.83	1.67	0.0935	-
Fungi*Method	9	14404	1600.46	3.38	0.0005	-
Fungi*Year*Method	10	2792	279.17	0.59	0.8223	-
Error	429	202924	473.02	-	-	-
Total	479	310999	-	-	-	-
CV	118.9	-	-	-	-	-

**** Highly Significant**

Table 2.The extent of seed associated mycoflora isolated through blotter paper and agar plates methods from peas seeds.

Fungi	Overall Frequency Percent*	
	Blotter Paper Method	Agar Plate Method
A. alternata	32.5 a	50.00 a
A. niger	28.33 a	36.67 b
A. fumigates	15.83 b	48.33 ab
R. stolonifer	15.83 b	5.00 de
C. lunata	15.00 b	17.50 cd
A. flavus	14.17 b	23.33 c
F. oxysporum	13.33 b	17.50 cd
*Penicillium*sp.	6.67 bc	5.83 de
*Stemphylium*sp.	1.67 c	11.67 cde
T. viride	-	4.17 e
SE	5.4743	6.7562
LSD *P* <0.05	10.792	13.319

Note: Figures following the similar letter within a column are not significantly varied according to the LSD (least significant difference) test at *P*<0.05.
*Overall mean frequency percent of two years 2016 and 2017 seeds samples.

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DNA Binding Studies of Ternary Copper (II) Complexes of Doxycycline with Polypridyl Ligands

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Abstract— *Tetracyclines are a family of drugs with interesting pleiotropic properties. The recognition by scientists of the therapeutic properties and safety profile of this class of drugs has led to the continuous derivatization of these compounds and implementation of clinical trials to explore their potential applications for a wide range of diseases. To this end, this paper review literature on tetracyclines from the discovery of the first tetracycline to a new class of tetracyclines called glycylcyclines. The DNA binding propensities of some copper (II) complex of doxycycline and mixed ligand copper (II) doxycycline complexes with polypyridyl ligands were also investigated by UV-Vis titration, viscosity and thermal melting experiments. The mixed ligand copper (II) doxycycline complexes with polypyridyl ligands were found to bind CT DNA in the major groove in a similar fashion to methyl green causing no change in the viscosity and negligible increase in thermal melting of CT DNA.*

Keywords— copper (II), doxycycline, polypyridyl, DNA binding, complex.

I. INTRODUCTION

7-chlorotetracycline was the first tetracycyline discovered, extracted from Streptomyces aureofaciens present in soil by Benjamin M Duggar in 1948 and named aureomycin [1-3]. Two years later Terramycin (5-hydroxytetracycline) was isolated by fermentation of the actinomycete, *Streptomyces rimosus*by Finlay and coworkers [4,5]. Catalytic hydrogenolysis of aureomycin was reported to produce the parent tetracycline [6,7] which was later prepared by fermentation of certain strains of *Streptomyces albo-niger*[8].In 1955, Conover patented tetracycline which became the most prescribed broad-spectrum antibiotic in the U.S within three years, and because of their great therapeutic value and wide application the tetracyclines have received a great deal of attention and is the object of several reviews [9-13]. The studies on tetracyclines include synthesis of novel tetracyclines and improved methodology [14-20], their clinical applications [10,11,21]**,** uptake, mechanisms of action [9, 21-22], resistance [23-28], metal chelation [29-34], environmental impact, detection and removal [35-38]. The rise in drug-resistant bacteria has also led to new approaches to antibiotic development including the development of novel pharmaceuticals such as doxycycline and novel tigecycline [39], the first member of a new class of tetracyclines named glycylcyclines.

Besides the use of tetracyclines as broad-spectrum antibiotics new uses have emerged including their potential use in anticancer therapy [40-49]. Tetracyclines inhibit matrix metalloproteinases MMPs [44,50] and are also effective but slow-acting anti-malarial drugs [41,51]. At concentrations within the therapeutic levels, tetracyclines inhibited production of reactive oxygen species (ROS) which account, in part, for their anti-inflammatory action such as in acne or vulgaris [52]. Minocycline is a proven antioxidant with radical scavenging potency similar to vitamin E [53,54] because of its many substituted phenol rings.Of the tetracyclines, doxycycline is the most commonly used [44], a more potent MMP inhibitor, better absorbed and with longer half-life than the parent compound, tetracycline [55].It has been found that doxycycline reduces the *in vitro* growth of human breast cancer by 70% [55,56] as well as prostate cancer cells [57].

The binding of tetracyclines with proteins was found to be greatly enhanced when complexed with divalent metal ions [58,59]. The potency of tetracyclines as MMP inhibitors is also positively related to their chelation to metal ion at the active site of the MMPs [60-61].

Moreover, several metal complexes have been used as artificial chemical nucleases among which copper nucleases

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especially copper diimine complexes have been a well-researched class of syntheticmetallonucleases and widely tested anticancer agents. The therapeutic properties of copper, the wide application of doxycycline, and DNA binding and cleavage abilities of diimine ligands prompted us to synthesize and investigate biological activities of mixed copper complexes containing doxycycline and polypyridyl ligands.

II. EXPERIMENTAL SECTION

Materials and Measurements

Doxycycline hyclate was obtained from Neimeth International Pharmaceuticals Plc, Nigeria and fresh solutions were used to ensure stability. Copper nitrate trihydrate and Tris(hydroxylmethyl) aminomethane-HCl (Tris-HCl) were from Qualigens while 2,2'-bipyridine and 1,10-phenanthroline monohydrate were from SDFCL, India. The ligands dipyrido[3,2-d:2',3'-f]quinoxaline (dpq) [67] and dipyrido[3,2-a:2',3'-c]phenazine (dppz) [68] were synthesized according to reported procedure [1]. Calf thymus DNA sodium salt (CT DNA) was used as obtained from SRL, India. All other chemicals and reagents are of analytical grade and used without further purifications. The metal complexes were synthesized according to previous published reports [69]. UV-Vis absorption measurements were carried out on Jasco V-630 spectrophotometer. 2.0×10^{-5} mol L^{-1} of doxycycline and complex concentrations were used.

DNA Binding Studies

The DNA binding experiments were performed in Tris-HCl/NaCl buffer (5 mol L^{-1} Tris-HCl/ 50 mol L^{-1}NaCl, pH 7.2). The concentration of CT-DNA was calculated from its known extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}). Solutions of CT-DNA in buffer gave a ratio of UV absorbance at 260 nm and 280 nm (A_{260}/A_{280}) 1.8-1.9 indicating that the DNA was sufficiently free of protein.

Electronic absorption titration. The electronic absorption spectra of 2×10^{-5} mol L^{-1} of the complexes with increasing concentration of nucleotide (1.0×10^{-5}-1.5×10^{-4} mol L^{-1}) were recorded in buffer following reported procedures [70-71]. The solutions were allowed to equilibrate for 20 minutes at room temperature after addition of DNA to the metal complex before absorption readings were taken. Binding constants of complexes were determined according to equation 1 [72-73]

$$[DNA] / [\varepsilon_a-\varepsilon_b] = [DNA] / [\varepsilon_b-\varepsilon_f] + 1 / K_b [\varepsilon_b-\varepsilon_f] \quad (1)$$

Where [DNA] is the concentration of DNA in base pairs, $\varepsilon_a$is the extinction coefficient observed for the MLCT absorption band at the given DNA concentration, $\varepsilon_f$is the extinction coefficient of the free (unbound) complex and $\varepsilon_b$is the extinction coefficient of the fully bound complex. The ratio of the slope $1 / [\varepsilon_a-\varepsilon_f]$ and the intercept $1 / K_b [\varepsilon_b-\varepsilon_f]$ obtained from a plot of $[DNA] / [\varepsilon_a-\varepsilon_f]$ versus [DNA] gives the intrinsic binding constant [73].

1×10^{-3} mol L^{-1} stock solutions of the complexes **1,2,3** were prepared in buffer while complexes **4** and **5** were prepared in doubly distilled water and diluted suitably with buffer to required concentrations.

Thermal melting experiments

DNA melting experiments were carried out by monitoring the absorption at 260nm of CT DNA (1.0×10^{-4} mol L^{-1}) with a JASCO V-630 spectrophotometer equipped with a Peltier temperature controlling programmer ETC-717 ().1ºCfrom 28 ºC to 95 ºC in the presence and absence of the complexes.

Viscosity measurements

Viscosity measurements were carried out using a semimicroviscometer. The flow time was measured with a digital stopwatch, and each sample was measured four times and an average flow time was calculated. Data are presented as $(\eta / \eta^0)^{1/3}$ versus the ratio [complex]/[DNA], where η is the viscosity of DNA in the presence of complex and η^0 is the viscosity of DNA alone.

Viscosity values were calculated from the observed flow time of DNA-containing solutions corrected for the flow time of buffer alone (t^0): $n=t-t^0$ [74]

III. RESULTS AND DISCUSSION

DNA Binding Studies

Upon the addition of a solution of calf thymus (CT) DNA to the copper (II) complexes, a decrease in the absorption intensities of the π - π^* absorption bands (250-400) of **2-5**was observed. As theextent of hypochromism is commonlyassociated with the strength of DNA interaction, this indicates strong DNA binding of the complexes.

In order to quantitatively compare the DNA binding affinities of the complexes, their intrinsic binding constants with CT-DNA were obtained by monitoring the intraligand band absorption with increasing concentration of DNA using equation 1 and were found to be 7.26×10^4, 6.7×10^4, 2.58×10^5 and 6.18×10^4 for complexes **5, 4,3** and **2** respectively. The fact that complex **3** has the highest binding constant shows that a binding mode other than intercalation is involved. The hypochromic shifts observed

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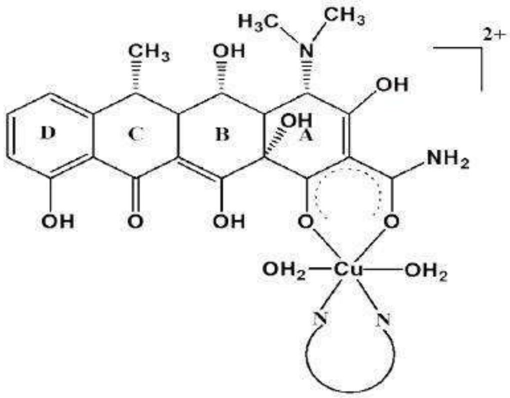
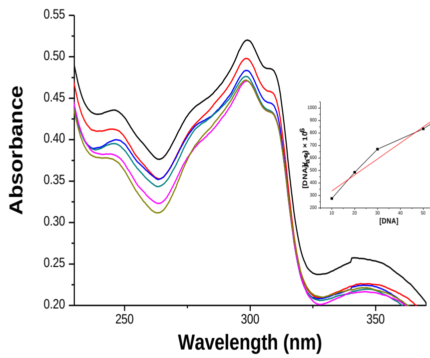
upon increasing concentration of DNA is likely to arise from surface aggregation of π-stacking interactions of the cationic complexes.

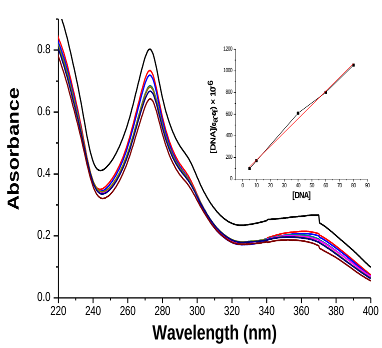


= dox (**1**), bpy (**2**), phen(**3**),dpq (**4**) and dppz (**5**)

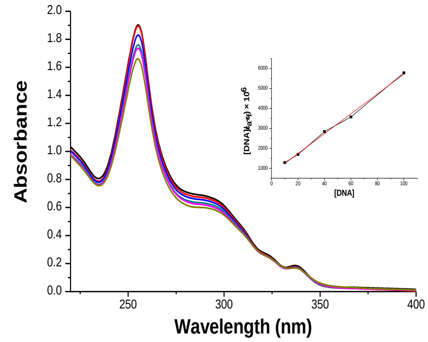
Fig.1: structures of the studied compounds[75]



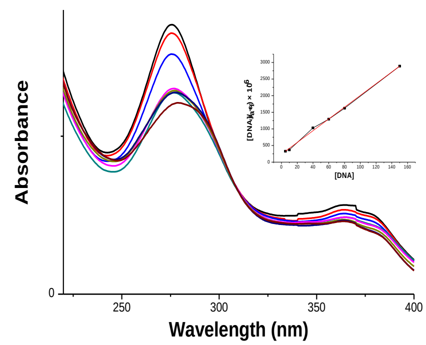
CubpyDox **2**



CubpyDox **3**



CubpyDox **4**



CubpyDox **5**

Fig.2: *Graphs showing the electronic absorption spectra of complexes 2,3,4 and 5 (20 μM) with increasing concentrations of CT-DNA in 50 mM NaCl and 5 mM Tris HCl buffer at pH 7.2.*

Thermal melting experiments

Thermal melting temperature (T_m) of DNA is the temperature at which half of DNA strands dissociates from double-stranded to single-stranded DNA state. The DNA melting experiment provides information about the stability of the DNA helix. The $T_m$of DNA in the absence of any external agent was found to be 62.7 ºC. Under the same experimental conditions and in the presence of complexes **2-5**, ΔT_m*values* are 0.7, 3.8, 2.0 and 2.4 ºC respectively. These values of $\Delta T_m$for the complexes obviates the possibility of intercalative binding of the complexes to DNA. Generally, the binding of metal complexes with DNA stabilizes the double helix structure which usually results in huge increase in T*m* of DNA for DNA intercallators. The modest values of ΔT_m (between +0.7 and +3.8 ºC) by complexes **2-5**support a non-intercalative binding mode for the complexes. Stabilization of +5.2°C was reported for poly[d(G-C)$_2$] by methyl green, a major groove binder. This result confirms that the complexes are major groove binders and is in agreement with the DNA binding constants obtained for the complexes.

Viscosity Measurements

Viscosity is an effective method to distinguish between modes of binding of small molecules to DNA [74]. For molecules that form bond with DNA, kinking/ bending of the DNA helix toward the minor or major groove leads to a decrease in the viscosity of DNA solution as a result of reduction in the end-to-end length of the DNA. However, groove binders do not alter the end-to-end length of DNA and therefore have negligible effect on viscosity of DNA solution while intercalation results in lengthening, unwinding and stiffening of the helix causing a significant increase in viscosity of DNA solution [74].

The addition of complexes **2-5** to CT-DNA did not alter its solution viscosity indicating that the mode of binding of the complexes is groove binding. Because changes in relative viscosity can be used in assigning DNA binding modes by intercalators [75-76], the data confirm that the complexes do not intercalate between the DNA bases.Other non-intercalative complexes of polypyridyl ligands have been previously reported [77-81].

IV. CONCLUSIONS

Five new single and mixed ligand copper (II) complexes of doxycycline and polypyridyl ligands have been tested as DNA binding agents by UV-Vis titration, viscosity and thermal melting experiments. Data from thermal melting profile and viscosity measurements reveal that the compounds moderately stabilize CT-DNA as major groove binders. The binding constants of the complexes are 7.26×10^4, 6.7×10^4, 2.58×10^5 and 6.18×10^4 for complexes **5, 4,3** and **2** respectively as evidenced by electronic titration.. This substantiates the non-intercalative binding abilities of polypyridyl ligands complexes to DNA through the major groove. The bulky ancillary ligand doxycycline must be responsible for the major groove binding abilities of these complexes.

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The use of promising entomopathogenic fungi for eco-friendly management of *Helicoverpa armigera* Hubner in chickpea

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Abstract**—***Gram pod borer, Helicoverpa armigera Hubner is known to be a major constraint of chickpea production which causes serious economic loess. The management of this pest in any crop is always been challenge to the growers, famers and researcher. Thus, present study evaluated some promising entomopathogenic fungi for the sustainable management of H. armigera to minimize the economic loss in chickpea. Five different fungal isolates viz; Beauveria bassiana, Trichoderma virens, Trichoderma hamatum, Trichoderma koningii, and Paecilomyces sp. were used as entomopathogenic against gram pod borer, through dipping and poison food methods under laboratory conditions. The entomopathogenic potential of different fungal strains revealed significantly ($P<0.05 = 0.0000$) highest mortality with B. bassiana (46.67%) followed by T. koningi (23.33%), T. virens (11.11%) and T. hamatum (8.33%) through dipping method. In case of poison food method significantly highest mortality was recorded with T. koningi (20%) followed by B. bassiana (6.66%) after 24 h. The mortality with B. bassiana after 96 h was become higher (41.667%) compared with other strains. No mortality was recorded with Paecilomyces sp. and control (dipped in simple water) in both methods. It is obvious that microbial control agents are very effective and the promising entomopathogenic fungi of current study are hoped would be helpful for eco-friendly and alternative to chemical pesticides for sustainable management of H. armigera in chickpea.*

Keywords— Entomopathogenic fungi, Helicoverpa armigera, management, chickpea.

I. INTRODUCTION

Chickpea, *Cicer arietinum* L., locally known as gram, is an ancient cultivated plant with varying names in different countries **[1]**.It is believed that chickpea is a great source of biomolecules such as proteins, carbohydrates, dietry fibre, minerals and vitamins and its use has been increased for reducing risk of human diseases **[2]**. It is also rich source of balanced amino acids in human diet and is highly enriched with sulphur containing amino acids e.g. methionine and cysteine **[3]**.

Despite the fact that the chickpea has great economic and nutritional value, the production of chickpea is far below than other countries where chickpea is commonly cultivated, which likely due to several biotic and abiotic factors. Chickpea plant is highly susceptible to various insect pests at different critical growth stages from seedling stage to maturity. Around 60 species of insect pests belonging to orders Lepidoptera, Hemiptera, Diptera and Thysanoptera are commonly found in chickpea crop **[4, 5]**. However, the gram pod borer, *Helicoverpa armigera*Hubner (Lepidoptera: Noctuidae) is known to be a major constraint of chickpea production which reduces economic loess around 6 to 20% **[6, 7, 8, 9]**. Moreover, infestation of gram pod borer has increased globally in last 50 years due to diversification of crops in agro-ecosystem **[10]**. Gram pod borer is a highly polyphagous pest due to its nature of diverse nature feeding habit such as leguminous and vegetables etc. **[11, 12]**. Since it possesses polyphagy nature (approximately has 180 host crops mainly chickpea, tomato, cotton, pigeon pea, cowpea, some flowers, vegetables and forest trees), high mobility and fecundity **[13, 14, 15, 16, 17]** and capability and adaptability to different environments due its facultative diapause, **[13, 18]**. Srivastava **[19]** reported that yield loss of chickpea can 10-60% in normal weather conditions and can increase upto 100% or complete crop failure **[20]**. In addition, a single larva of gram pod borer damaged 7- 10% pods, reduced 6.2% grains m^{-1} row^{-1} of the gram crop consequently overall declined 5.4% yield loss **[21]**.

The management of pests in any crop is always been challenge to the growers, famers and researcher. Various integrated pest management (IPM) practice for the control of insect pests of chickpea have been developed, tested and evaluated in farmers' fields **[22, 23, 24, 25, 26, 27].** In recent study **Ramesh and Rao [28]** advocated the IPM strategies. **Basha et al. [29]** compared IPM strategies such as resistant cultivars, intercropping, trap crop and border cropping for controlling insect pests of chickpea. They indicated that resistant cultivars, tillage practices, crop rotation, inter cropping and soil solarization were found effective measures for control of insect pest infestation on chickpea.

Nevertheless, the concern about the adverse effects of chemical pesticides on agriculture, human health and the environment has been increasing around the globe **[30, 31, 32, 33]**. It also has been reported about their adverse effects in many non-target organisms **[34, 35].** In the IPM programme, biological control with microbials is known to be a major component globally. Microbial control agents are believed an effective, environmental friendly and economic technique and alternative to chemical pesticides used for control of insect pest **[36, 37].** There are several microorganisms have been isolated for control of insect pests. Entomopathogenic fungi (EF) have been demonstrated to control Lepidopteran insect species **[37, 38]**. The EF play an important role in controlling the insect pests since there are 68% of EF based microbial pesticides **[39].** The EF has been recognized as important natural enemy of gram pod borer since long time. However, management is required a head of time prior to the onset of insect pest in field.

Based on aforementioned facts it was planned to use some promising entomopathogenic fungi for the sustainable management of *H. armigera* to minimize the economic loss in chickpea. To cater the need, present studies were conducted at Sindh Agriculture University Tandojam

II. MATERIAL AND METHOD

The evaluation of entomopathogenics was conducted in the Post Graduate laboratory of Department Plant Protection, Faculty of Crop Protection, Sindh Agriculture University, Tandojam, Pakistan. To evaluate the efficacy of entomopathogenic fungi (EPF) against the larval stages of H. armigera on chickpea crop series of experiments were conducted during 2015 and 2016under laboratory conditions

2.1 Fungal isolates

Five different fungal isolates viz; *Beauveria bassiana, Trichodermavirens, Trichoderma hamatum, Trichoderma koningii, Paecilomyces* sp. and *Penicillium* sp. were used as entomopathogenic against gram pod borer, *H. armigera* under laboratory conditions. The culture of all isolates were maintained by sub-culturing on Potato Dextrose Agar (PDA) medium and at the time of use freshly prepared culture was used as entomopathogenic.

2.2 Preparation spore suspension

The spores of all isolates were individually collected in 0.1 % Tween80 solution and final concentration was determined by hemocytometer. The stock solutions of different isolates were serially diluted to obtain the desired concentrations for bioassay. The desired amount of all strains conidia was put into distilled water and used to observe the efficiency against *H. armigera*.

2.3 Larval culture of *H. armigera*

The larvae of gram pod borer were collected before one day from the un-treated field of Pulses Sub-station, ARI, Tandojam, and culture was maintained in laboratory Plant Protection, Department, SAU, Tandojam on natural diet of chickpea tender shoots, leaves and pods. The five larvae were individually transferred in to plastic transparent cage.

2.4 Bioassay of entomopathogenic

The assessment of entomopathogenic fungi was conducted by two different methods such as: 1) Dip method and, 2) Poison Food method. All experiments were conducted with randomized complete block design under in vitro conditions. The details of methods are summarized here under:

2.4.1 Dip method

In this method different larval (2^{nd}, 3^{rd} and 4^{th}) instars were used to assess the efficacy of entomopathogenics against *H. armigera* under *in vitro* conditions. A total of five live larvae were dipped for 10 second in prepared concentration (1.381X 10^{-5}) first and then released in fresh chickpea food including tender leaves, shoots, and pods. The extra moisture of treated larvae was soaked on sterilized tissue paper. The experiments were conducted in the plastic transparent cage (measuring 30X30X30 cm) with randomized complete design with three replications. Moreover, experiments were repeated twice to further confirm the results.

2.4.2 Food poising method

In this method poison food method similarly different larval (2nd, 3rd and 4th) instar were used to assess the efficacy of entomopathogenics against. Initially, freshly collected food (chickpea tender leaves, shoots and pods) were sprayed individually with different entomopathogenic strains and then transferred in to plastic transparent cage (measuring 30X30X30 cm). The spore concentration was adjusted to 1.381X 10^{-5} by using distilled sterilized water before use. Five active larvae of different instar were released on to the contaminated food with fungal strains. The experiments were conducted under *in vitro* condition in randomized complete design with three replications. Moreover, experiments were repeated twice to further confirm the results.

2.5 Observations

After the application of entomopathogenics, observations were recorded on daily basis for mortality and survival of gram pod borer. Data was statistically analyzed by using the standard procedures for analysis of variance, ANOVA (linear model), by using the computer software Statistix 8.1 (Analytical Software, 2005). All differences described in the text were significant at the 5% level of probability.

III. RESULTS

The entomopathogenic potential of different fungal strains viz; *Beauveria bassiana, Trichoderma virens, Trichoderma hamatum, Trichoderma koningii* and *Paecilomyces* sp. were tested through two different methods, poison food and dipping methods in current study. The results of current study with five different entomopathogenic fungi (EPF) strains revealed significant differences among each other for their efficacy against the different larval stage (2nd, 3rd and 4th) of gram pod borer, *H. armigera*. The results so far achieved are further discussed here:

3.1 Effect of EPF through dipping method

The effect of four different EPF used through dipping method exhibited varied responses at different post treatment time intervals. Significantly highest mortality percent was recorded with *B. bassiana* (46.67%) followed by *T. koningi* (23.33%), *T. virens* (11.11%) and *T. hamatum* (8.33%). However, no any mortality was notice in case of *Paecilomyces* sp. after 24 h of treatment through dipping method (Table 1). It was further observed that after 48 h of treatment the larval mortality was further increased with *B. bassiana* (55.57%); however, in case of other strains it was decreased. Moreover, after 72 and 94 hs the mortality was observed moderately with *B. bassiana;* while the response of *T. virens* (25.00%) was remained better compared to *T. koningi, T. hamatum* and *Paecilomyces* sp.

The mean mortality percent of *H. armigera* larvae treated with different fungal strain through dipping methods indicates the obvious response of all strains. The highest mortality was produced by *B. bassiana* followed by *T. virens* (Figure 1). No mortality was recorded with *Paecilomyces* sp. and control (dipped in simple water). While the mean mortality percent of *T. koningi* and *T. hamatum* was moderate through dipping method under laboratory conditions (Figure 1).

3.2 Effect of EPF through poison food method

The efficacy of all tested fungal strains through poison food method was lower compared to dipping method. However, the trend of mortality was correlated with dipping method. After 24 h, significantly highest mortality percent was recorded with *T. koningi* (20%) followed by *B. bassiana* (6.66%). While, no any mortality was recorded with *T. virens, T. hamatum* and *Paecilomyces* sp. after 24 h of treatment through dipping method (Table 2).

It was further observed that after 48 h of treatment the larval mortality was increased with *B. bassiana* (46.667%); however, in case of *T. koningi* it was decreased (8.33%). Moreover, after 72 h mortality of larva was also observed with *T. virens* and *T. hamatum*. The mortality of larva with *B. bassiana* after 96 h was higher (41.667%) compared with other strains. Moreover, no any mortality was notice in case of *Paecilomyces* sp. after up to 120 h of treatment through dipping method (Table 2).

The mean mortality percent of *H. armigera* larvae treated with different fungal strain through poison food methods indicates the obvious response of all strains. The highest mortality was produced by *B. bassiana* followed by *T. koningi* (Figure 2). No mortality was recorded with *Paecilomyces* sp. and control (dipped in simple water); while the mean mortality percent of *T. virens* and *T. hamatum* was moderate through dipping method under laboratory conditions. It was further observed that mortality of larva with *T. virens* in case of poison food method was lower compared to dipping method (Figure 2).

IV. DISCUSSION

It is obvious that insect pest management is always dominated by the use of synthetic pesticides; thus results several health and environmental hazards that ultimately impacting all living beings. The entomopathogenic fungi (EPF) are fungal species that are pathogenic to insects. These fungal pathogen species play a vital role in reducing insect population dynamics comparatively earlier than other measures **[40].** The EPF has been recognized as important natural enemy of gram pod borer since long time. In the current study, different fungal strains viz; *Beauveria bassiana, Trichoderma virens, Trichoderma hamatum, Trichoderma koningii* and *Paecilomyces* sp. were tested through two different methods, poison food and dipping methods. Significant difference for the efficacy against the different larval stage (2nd, 3rd and 4th) of gram pod borer, *H. armigera* was observed among each other. Highest mortality percent was recorded with *B. bassiana* followed by *T. koningi, T. virens* and *T. hamatum*. However, no mortality was noticed in case of *Paecilomyces* sp. after 24 h of treatment through dipping method.The mean mortality percent of *H. armigera* larvae treated with different fungal strain through dipping methods has indicates the obvious response of all strains. The highest mortality was produced by *B. bassiana* followed by *T. virens*. No mortality was recorded with *Paecilomyces* sp. and control (dipped in simple water). While the mean mortality percent of *T. koningi* and *T. hamatum* was moderate through dipping method under laboratory conditions. In case of poison food method, lower mortality has been noticed compared to dipping method. Almost same trend of mortality was observed with dipping method. It was further observed that mortality of larva with *T. virens* in case of poison food method was lower compared to dipping method. In the previous study, fifteen fungal species, *Metarhizium anisopliae* (Metsch.), *M. flavoviride* (Metsch.), *Nomuraea rileyi* (Farlow) Samson, *Beauveria bassiana* (Balsamo) and *Paecilomyces farinosus* have been found which could be promising myco-insecticides **[41].** However, several studies focused on the use of *Beauveria bassiana* and their promising control on different insect pests. **Ebrahimi et al. [42]** conducted study on effect of entomopathogenic nematode, *Steinernema feltiae*, on survival and plasma phenoloxidase activity of *Helicoverpa armigera* (Hb) (Lepidoptera: Noctuidae) in laboratory conditions. Another study conducted by **Majeed et al. [43]** on pathogenicity of indigenous soil isolate of *Bacillus thuringiensis* to *Helicoverpa armigera* Hübner 1809 (Lepidoptera: Noctuidae). Specifically, **Mishra and Sobita [44]** evaluated the efficacy of *Beauveria bassiana* Balsamo against *Helicoverpa armigera* (Hubner) in field condition and revealed significant mortality of *H. armiger* at 1 and 5% level with different doses. In our study we also observed *Beauveria bassiana* as most promising EPF; however, our study also explored *T. koningi* and *T. virens*s trains that maybe further study through different methods. The bio-efficacy of *B. bassiana* studied by **Prasad et al. [45]** against *H. armigera* (Hubner) with four different concentrations which were sprayed topically against the most damaging 4th instar larvae and found up to 76.70 percent mortality with highest dose of 0.25ml x10^{8} spores/ml. Our study is consistent with findings of these lines and we found *Beauveria bassiana* as most effective EPF through both methods. **Savita et al. [46] (2015)** evaluated the effectiveness of *Metarhizium anisopliae, Beauveria bassiana, Nomuraea rileyi* with different concentration against *Helicoverpa armigera* (Hub.) infestation on chickpea under field conditions. They found *Metarhizium anisopliae* as most effective with minimum larval survival. However, our student is not in agreement with finding of this study; in our study we found *Beauveria bassiana* as most effective against different larval instar through both observed methods. The study of Aneela et al. **[47]** also supported our finding with reference to *Beauveria bassiana*. However, in addition to *Beauveria bassiana*, they also used jasmonic acid and the chlorantraniliprole (insecticide) either alone or combined form against gram pod borer. Significant decline was observed for larval population of gram pod borer and which was further reduced with increase in time of application. Moreover, our study also explored *T. koningi* and *T. virens* strains that maybe further explored for their entomopathogenic potential through different methods. These strains may also be explored to develop new myco-insecticides to be used against gram pod borer and other serious insect pests.

V. CONCLUSION

The use of entomopathogenic fungi (EPF) has been believed to be safe and an alternative to synthetic pesticides. Since many years, EPF has been recognized as important natural enemy of gram pod borer, *H. armigera*. In the current study, five different fungal strains viz; *Beauveria bassiana, Trichoderma virens, Trichoderma hamatum, Trichoderma koningii* and *Paecilomyces* sp. were tested through two different methods, poison food and dipping methods. Significant difference for the efficacy against the different larval stage (2nd, 3rd and 4th) of gram pod borer, *H. armigera* was observed among each other. Highest mortality percent was recorded with *B. bassiana* followed by *T. koningi*, *T. virens* and *T. hamatum*; however, no any mortality was noticed in case of *Paecilomyces* sp. through dipping and poison food methods under laboratory conditions. In our study we observed *Beauveria bassiana* as most promising EP; however, other strains such *T. koningi* and *T. virens* maybe further explored for their entomopathogenic potential through different methods. These strains may also be explored to develop new myco-insecticides to be used against gram pod borer and other serious insect pests.

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Table 1 Effect of different fungal strains on the mortality of H. armigeraused through dipping methods under laboratory conditions

Hours	**Mortality Percent of *H. armigera***											
	B. bassiana		***T. koningi***		***T. hamatum***		***T. virens***		***Paecilomyces* sp.**		**Control**	
24	46.67	ab	23.33	abcd	8.33	cd	11.11	bcd	00.00	d	00.00	d
48	55.57	a	13.33	bcd	6.67	cd	00.00	d	00.00	d	00.00	d
72	25.00	abcd	00.00	d	6.67	cd	25.00	abcd	00.00	d	00.00	d
96	24.44	abcd	8.33	cd	11.11	bcd	8.33	cd	00.00	d	00.00	d
120	40.00	abc	24.44	abcd	8.33	cd	16.67	bcd	00.00	d	00.00	d
SE	18.370											
LSD	36.745											

Note: Figures following the similar letter within a column are not significantly varied according to the LSD (least significant difference) test at P<0.05.

Table 2 Effect of different fungal strains on the mortality of H. armigeraused through poison food methods under laboratory conditions

Hours	**Mortality Percent of *H. armigera***											
	B. bassiana		***T. koningi***		***T. hamatum***		***T. virens***		***Paecilomyces* sp.**		**Control**	
24	6.6667	bc	20.00	abc	00.00	c	00.00	c	00.00	c	00.00	c
48	46.667	a	8.3333	bc	00.00	c	00.00	c	00.00	c	00.00	c
72	17.778	abc	00.00	c	6.6667	bc	6.6667	bc	00.00	c	00.00	c
96	41.667	a	6.6667	bc	8.3333	bc	00.00	c	00.00	c	00.00	c
120	33.333	ab	16.667	abc	6.6667	bc	6.6667	bc	00.00	c	00.00	c
SE	15.851											
LSD	31.707											

Note: Figures following the similar letter within a column are not significantly varied according to the LSD (least significant difference) test at P<0.05.

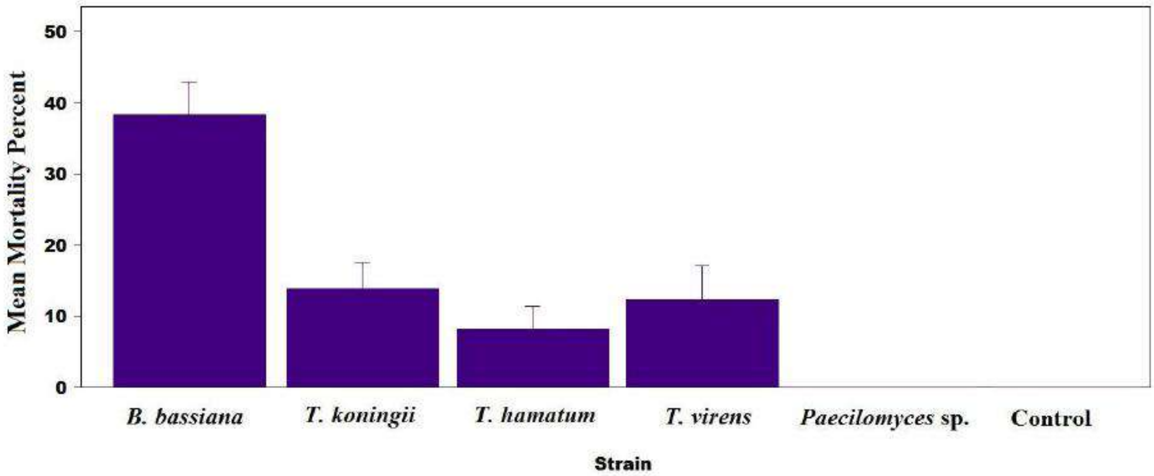

Fig. 1 Mean mortality percent of H. armigera treated with different fungal strain through dipping methods under laboratory conditions

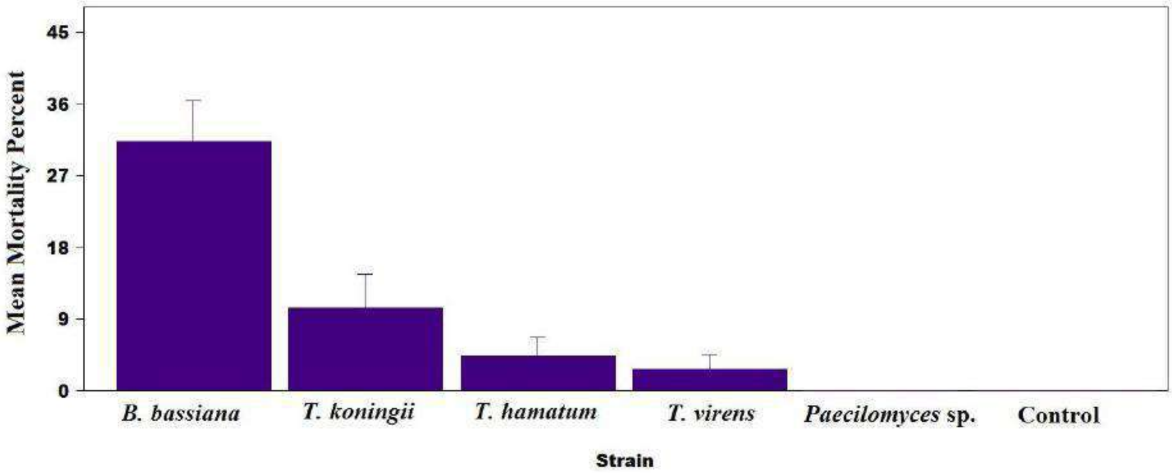

Fig. 2 Mean mortality percent of H. armigera treated with different fungal strain through poison food methods under laboratory conditions

Evaluating Problems of Waste Management in Tarakan City, North Kalimantan

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***Abstract*—*Various programs are implemented in the context of waste management in Tarakan City. The program does not provide optimal impact if you see the amount of waste generation that continues to increase. Waste generation in 2011 amounted to 98.7 tons/day and in 2018 it increased 79.05% to 176.73 tons/day with the level of solid waste service reaching 68%. This study aims to determine the main problems in waste management in Tarakan City. This type of research is descriptive research. The respondents were five people from five different agencies. Data collection techniques in the form of interviews to find out waste management problems. The qualitative data from the interviews are then converted into quantitative data so that hierarchical analysis can be done. Analysis of the data using the Analytic Hierarchy Process (AHP) method with Software Expert Choice 11. The main problem is obtained from the multiplication of indicator scores with priority aspect values. The results of the analysis show the main problem of solid waste management is the lack of a solid waste management budget (0.133); lack of garbage transport equipment (0,130); habit of residents in littering (0.118); sanctions in regulations are not implemented (0.114); and sanctions have less deterrent effect (0.107). The main problem of waste management in Tarakan City is related to the lack of solid waste management budget.*

***Keywords*— Analytic Hierarchy Process, PPSP, Solid Waste, Tarakan, Waste Management.**

I. INTRODUCTION

Based on Central Bureau of Statistics (*Badan Pusat Statistik*/BPS) projection results, the population of Tarakan City in 2017 was 253,026 inhabitants consisted of 120,609 female inhabitants and 132,417 male inhabitants. Compared with the population projection in 2016, the population of Tarakan City experienced a growth of 3.62%.[1]

This population growth is also directly proportional to the amount of waste generated in Tarakan City. From 2011 to 2018, the amount of waste generated in Tarakan City had increased by 79.05 percent. In 2018, the average amount of waste generated in Tarakan City was 176.73 tons per day with solid waste service levels reaching 68%.[2]

Actually, Tarakan City Government does not remain passive in handling the problem of managing this waste. Since 2011, the Tarakan City Government has joined 62 other regencies/cities in Indonesia to take part and play an active role in the Accelerated Development Program of Sanitation Settlements (*Program Percepatan Pembangunan Sanitasi Permukiman*/PPSP). This program has been running in two stages, namely the first phase starting in 2010 until 2014, then the second stage starting from 2015 to 2019.

In the early stage of Accelerated Development Program of Sanitation Settlements (PPSP), Tarakan City government has mapped the sanitation risk zone namely waste risk, the risk of waste water and the risk of drainage and clean water. One of the flagship programs of the Tarakan City Government in the first stage is educational activities based on waste management called Environmental Saving (*Tabungan Lingkungan*/Taling).

In this Taling activity, every elementary school student from grade 3 to grade 6, junior high school students and high school students are required to bring recyclable waste (paper, aluminum cans, plastic bottles, etc.) from their homes to school each day, predetermined to be exchanged for a number of points, this point if already collected can be exchanged into school equipment.[3]

In the second stage of Accelerated Development Program of Sanitation Settlements (PPSP), in addition to updating the sanitation risk zone map, Tarakan City Government also initiates a program called "Program Sampah Semesta (The Universe Waste Program, All Must Be Involved)" with the tagline "The trash is no longer visible and no smell exists". The implementation of the universal waste program is carried out by collecting waste directly from house to house using a waste cart (it can also be in form of a three-wheeled motorbike and alike), waste carts that have been filled with waste are then taken and collected to the waste terminal (temporary waste disposal) and then moved directly using dump truck to the Final Processing Site (TPA).[4]

Various programs and activities carried out by Tarakan City Government in terms of solid waste handling need to be immediately known for the root of the problem so that it is in line with its goal of reducing waste generation. It is not enough just to issue new programs/activities and even replicate from other regions without regarding to local characteristics, because the best solution in the form of integrated waste management is a combination of the application of solid waste technology by adapting local situations and conditions.[5]

An integrated waste management system requires cooperation from all parties and aspects, the sustainability of waste management will not work without the willingness and awareness of the community.[6] This study aims to determine the main problems in waste management in Tarakan City.

II. MATERIAL AND METHODS

This study is a descriptive study to describe an event and symptom that occurs at the present time. This study used respondents as sources of information; the number of respondents was five people from five different agencies (Regional Development Planning Board (*Bappeda*); Environmental Department; Final Processing Site Unit; Work Unit for Settlement Environmental Health System Development; and Village Office of Amal Beach) with purposive sampling method. Respondents were selected based on their expertise, experience and position in the research background.

Data collection techniques were in the form of interviews to find out waste management problems. Qualitative data from interviews were used as material for preparing questionnaires that would be used to assess the hierarchy of waste management problems. The qualitative data would then be converted into quantitative data so that hierarchical analysis could be carried out. Analysis of the data used the Analytic Hierarchy Process (AHP) method with the help of Expert Choice software 11.

III. RESULTS AND DISCUSSION

3.1 Hierarchy of Waste Management Problems

Unstructured and complex problems are broken down into homogeneous parts to be arranged in a hierarchy. The result of hierarchical arrangement is shown in Figure 1. It consists of two levels, namely the goal and the aspect that become the main problems along with its constituent criteria.

The structured aspects as shown in Figure 1 are in line with previous research which stated that the urban waste management system was basically seen as sub-system components that supported each other to achieve the goal of a clean, healthy and orderly city. The component includes the operational technical sub-system; organization and management (institution); laws and regulations; financing; and the role of the community (Human Resources).[7]

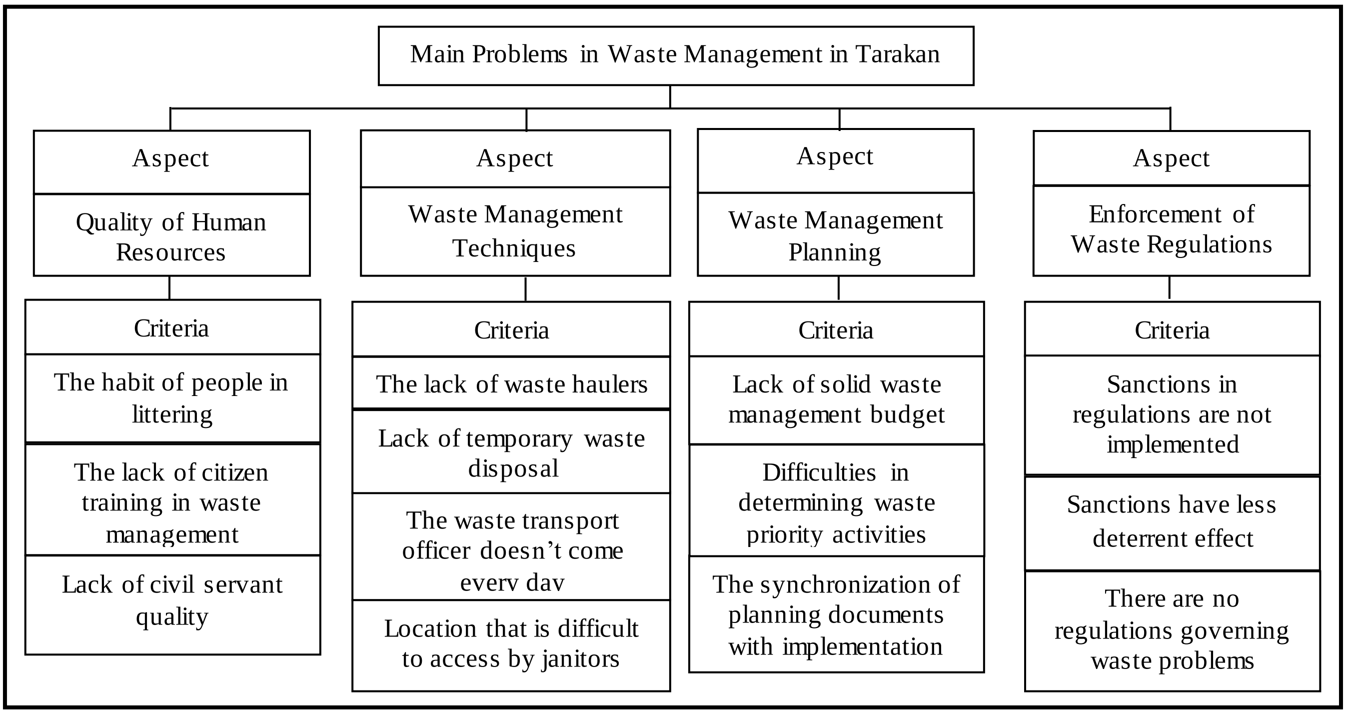

Fig 1. Hierarchy of waste management problems (Source: Primary survey results, 2019)

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3.2 Priority Aspect Analysis of Waste Management

Weighting on variables by conducting pair wise comparisons between aspects illustrates priority aspects that hinder waste management in Tarakan City.

In Table 1. shows the results of the calculation of the matrix, which is an assessment of each aspect.

Table 1. Weighting the Priority Aspects of Waste Management Problems

No.	Aspect	Weight
1.	Waste Management Techniques	0.387
2.	Enforcement of Waste Regulations	0.260
3.	Waste Planning and Funding	0.246
4.	Quality of Human Resources	0.107

(Source: Results of analysis, 2019)

In the aspects of Waste Management Technical, the respondents concluded that this aspect was considered very priority as a barrier factor for waste management in Tarakan City with a total weight of 0.387.

Furthermore, which occupies the second, third and fourth priority respectively as a problem aspect of waste management is the aspect of Enforcement of Waste Regulations with a weighting value of 0.260; aspect of Waste Planning and Financing with a weight value of 0.246; and aspect of the Quality of Human Resources with a total weight value of 0.107.

The level of inconsistency of this hierarchy was 0.02 or according to the expected inconsistency ratio of less than or equal to 0.1.[8]

The Technical Aspect of Waste Management is considered a top priority because it includes basic activities in the waste management from upstream in the form of waste existence at the source to end up in the downstream or final processing. The activities include waste placement, waste collection, waste transportation, waste management and waste final disposal.[9]

The next aspect is related to Enforcement of Waste Regulations. This is in line with research that states the lack of national policies and legal frameworks for municipal waste management. A legal framework for the field of waste that is well described is needed so that it can effectively deal with waste management.[10]

The implementation of Law No. 18 of 2008 concerning Waste Management is not yet running effectively, it can be proven that Indonesia has become the second largest domestic waste producing country in the world at 5.4 million tons per year.[11] Enforcement of regulations / laws in the waste sector is an act and/or process of coercion in the framework of complying with the law based on statutory provisions and/or environmental requirements.[12]

The Aspects of Waste Planning and Financing have been in line with Government Regulation Number 81 of 2012 concerning Management of Household Waste and the same type as Household Waste.[13]

Article 9 paragraph 1 and 2 states that the regency / city government in addition to establishing policies and strategies, also preparing waste management master plan documents containing restrictions on waste generation; recycling of waste; reuse of waste; sorting waste; waste collection; waste transportation; waste management; final waste processing and funding.

Furthermore, in the regulation in article 29 paragraph 1 and 2 it is stated that in the handling waste management, the regency/city government collects retribution to each person for the services provided which are set progressively based on the type, characteristics and volume of waste.

The last aspect in this study is related to the aspects of Quality of Human Resources (HR). The intended HR consists of the community as a component of waste producer (the source of waste generation) and also people who are given assignments and authority in managing waste, both by private institutions and government agencies.

Improving the quality of human resources will have a positive impact on waste management. The more the number of family members who have participated in solid waste management training, the smaller the weight of the waste produced or caused.[14] In relation to waste management institutions, in another study it was stated that the factor of partnership in an institution was not access to machinery and equipment, but the ability of managers to use effectively and efficiently.[15]

3.3 Analysis of the Main Problems in Waste Management

The analysis carried out to get the main problem that became a barrier in the waste management in Tarakan City was to do geometric mean.

Assessment was carried out simultaneously on 13 (thirteen) criteria that had been arranged in a problem hierarchy. The combination of the assessment of the five respondents can be seen in Table 2.

Table 2. Assessment of the Main Problems of Waste Management

No.	Aspect	Weig ht
1.	Lack of waste management budget	0.133
2.	The lack of waste transport equipment	0.130
3.	The habit of people in littering	0.118
4.	Sanctions in regulations are not	0.114

	implemented	
5.	Sanctions have less deterrent effect	0.107
6.	Lack of temporary waste disposal amount	0.076
7.	The waste transport officer does not come every day	0.072
8.	Location is difficult for janitors to access	0.070
9.	The lack of citizen training in waste management	0.053
10.	Difficulties in determining waste priority activities	0.044
11.	The synchronization of planning documents with implementation	0.040
12.	Lack of civil servant quality (Planning & Technical)	0.024
13.	There are no regulations governing waste problems	0.019

(Source: Results of analysis, 2019)

Based on the calculations in Table 2, it can be classified in four interval classes. This study will only discuss interval class 1 (0.105 - 0.133) which are the main problems of waste management.

From the calculation of geometric mean, it shows that the main problem of waste management in Tarakan City is in terms of the minimum waste management budget that gets an assessment of 0.133.

This is in line with the National Policy and Strategy study on the Acceleration of Waste Management which states that one of the problems in waste management in Indonesia is the low budget allocation for waste management, both from the Country's Budget (APBN), Provincial Budget (APBD Prov.), and Regency/City Budget (APBD Kab./Kota). Furthermore, it was stated that the comparison of the amount of waste management retribution revenue to the budget provided by the DKI Jakarta Province Sanitation Office was relatively small, only 1.3% of the total budget for the sanitation service.[16]

In a book published by the Ministry of Public Works and Public Housing it is also stated that there is a considerable difference between the ideal needs for waste management and what is stated in the 2015-2019 National Medium-Term Development Plan (RPJMN). The ideal need to achieve quality and waste service level of 100% from 2015 to 2019 is 66.33 Trillion while in the 2015-2019 RPJMN only allocates funding of 17.01 Trillion.[17]

Based on National Policy and Strategy Acceleration of Waste Management, the strategy in the aspect of funding is to allocate a waste management budget of at least 2% of the Regency/City Budget (APBD Kab./Kota).

In the last 2 (two) years, the City of Tarakan only allocated a budget of 0.5% annually for the management of waste. Waste management is intended to be used for the purpose of dissemination to the operations of the final processing site (TPA)

The further problem of waste management is due to the lack of waste transport equipment (carts, tricycles, dump trucks) with an assessment of 0.130. This is in accordance with the theory which states that the problems in operational technical of waste management include the inadequate capacity of transport equipment.[19]

In National Policy and Strategy the Acceleration of Waste Management also raises strategic problems related to the waste transportation, including:

a. The lack of quantity and quality of waste transport vehicles;
b. Waste transportation has not been done every day;
c. The schedule or period of waste transportation has not yet been coordinated with the collection schedule (by the cart/motorbike car /crossroad car) which causes the queue of carts /motorized carts at the waste transport points;
d. The specification of waste transport vehicles has not met the standard
e. The mode of waste transportation has not been separated

The lack of waste transport equipment in Tarakan City is in accordance with the data from The Environmental Department as shown in Table 3.

Table 3. The ideal need for waste transport equipment

No	Description	Existence	Ideal
1	Waste cart	154 units	220 unit
2	Three-wheeled waste motorcycle	71 units	200 unit
3	Open trucks	17 units	25 units
4	Compactor trucks	2 units	5 units
5	Arm Roll trucks	4 units	10 units

(Source: Environmental Department of Tarakan, 2018)

The ideal need for three-wheeled motorized vehicles in each Village is 10 units. With the number of Village in Tarakan City as many as 20 villages, there are still 129 three-wheeled motorized vehicles needed to be able to ideally service waste transportation in Tarakan City.

In addition to three-wheeled motorized vehicles, another urgent matter is related to the number of waste carts and also open trucks. This is because three-wheeled motorized vehicles and waste carts are used for waste collection from the source to the temporary waste disposal, if the amount is less then it will have an impact

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that the garbage will not be transported in people's homes. Whereas open trucks are used to transport waste from the temporary waste disposal to the final processing site which if the amount is minimal, there will be a buildup of waste in the temporary waste disposal.

With the results of the assessment of 0.118, the habit of residents in littering is a further problem in waste management.

The habit of residents in littering is in accordance with previous research which stated that the habit of littering was carried out in almost all communities, not only the poor, even those who are highly educated do so.[20]

In National Policy and Strategy the Acceleration of Waste Management was also mentioned about this, where there were problems with waste management in the socio-cultural field, namely:

a. The community has not done the sorting of waste
b. The community is still littering a lot
c. Program sustainability is difficult to maintain
d. Low knowledge of residents in managing waste

Thus, to get the right and good attitude towards waste disposal habits, it is necessary to provide information and counseling consistently about the dangers of waste to the environment and health. Increasing people understanding of waste management will give result to the right attitude towards waste and will form good habits so that a healthy, clean and free of waste environment is formed.

The habit of residents in treating this waste has been illustrated by a study conducted by Tarakan City government (Regional Development Planning Board/Bappeda). Of the total 20 villages in Tarakan City, only 10% or 2 (two) villages are categorized as not at risk of waste problems. The rest, as many as 30% or 6 (six) villages are in high risk category and as many as 12 villages or 60% are categorized as medium risk.[22]

The next problem is that sanctions in regulations that are not carried out with an assessment of 0.114. This is consistent with previous research conducted in Tarakan City, where it was stated that law enforcement was still considered weak, confirmation and repression of violators of the law had not been implemented optimally.[23]

In addition to Tarakan city, this law enforcement problem is also experienced by several cities in Indonesia. In Semarang city, the problem of waste management that occurs is the lack of law enforcement against the implementation of the Regional Regulation of Cleanliness and sanctions for violators of regulations.[21]

In Jayapura city, the problem of solid waste in the legal sector that arises is the weakness of the application of a legal framework that supports the implementation of solid waste services such as Regional Regulations and Decisions on provisions issued by the government.[22]

The weakness of law enforcement in the waste sector and unclear about the pattern of private government cooperation (build, operate and own) is the concern of the DKI Jakarta Province government in the management of waste.

In addition, in the National Strategy for the Acceleration of Waste Management, it was also stated that the strategic issues and problems of waste management in the legal sector include:

a. The application of legal sanctions for violators has not been a priority;
b. Limitation of Law Enforcement Devices (coordination and number)
c. Arrangement of sanctions in legislation related to waste management;
d. Synchronization of legal products related to waste management

The problem that is still related to the legal aspect and getting an assessment of 0.107 is related to sanctions that have less deterrent effect.

In law enforcement, basically it is influenced by several factors, in which these factors cannot stand alone and have a close correlation and influence each other. The factors include:[26]

a. The legal factor itself is the presence or absence of regulations and deterrent effects arising from the application of the law.
b. Factors in law enforcement, which includes the apparatus or institutions that form and implement the law.
c. Factors of law enforcement supports

In full, the hierarchical assessment of each aspect and criterion carried out by the five respondents can be seen in Figure 2.

From Figure 2, because character of the hierarchy that are interrelated with each other, the criteria for the minimum waste management budget which is the main problem with the highest score cannot stand alone.

This connection can be seen from almost all of the existing criteria, where the respondents considered the emergence of these criteria due to the lack of budget allocated for funding the solid waste sector.

To enforce sanctions in regulations, a large budget is needed. This budget starts from regulatory socialization, periodic monitoring, prosecution and trials.

Large budgets are also needed to change people's habits in managing their household waste. Waste Management Training, Waste Sorting and Composting are needed.

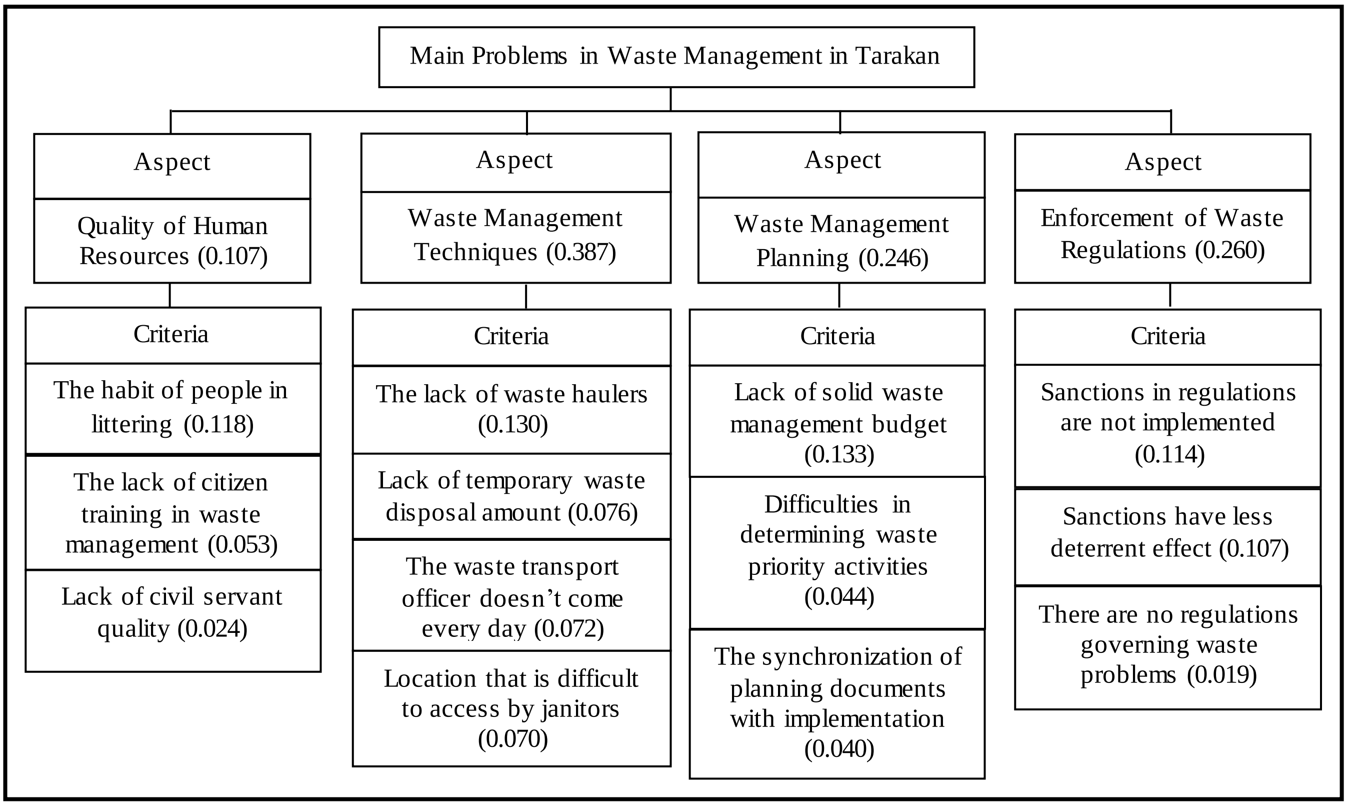

Fig 2. Hierarchy of the Main Problems in the Management of Waste in Tarakan City

The biggest budget issued by the City Government is related to the provision of garbage transport equipment. Increasing the amount of waste generation every year is not comparable with the increase in the number of garbage transport equipment. This is compounded by minor and major damage to the conveyance due to extra usage

The increase in the amount of waste per year is also not comparable with the increase in the budget in the waste sector. With limited budget in terms of handling or managing waste, the ability to determine priority programs / activities is needed.

The main problems of waste management that are at intervals of 1 (one) must be addressed in handling solutions. The solution is then used as a priority program/activity within a handling period of less than 2 (two) years.

IV. CONCLUSION

From the results of the assessment of the hierarchy that has been prepared, it can be concluded that the main problem in managing solid waste in Tarakan City is the lack of solid waste management budget. It cannot be denied, the course of a program/activity depends on the available budget allocation.

The community still believes that handling and managing waste are entirely the responsibility of the government. The results of waste collection are still far from enough to finance the operations of waste management. This is increasingly burdening the government budget, both central and regional governments, which has been minimal in this sector.

The waste sector is still considered less attractive to investors compared to other sectors such as the road, bridge, transportation and so on. The unclear pattern of cooperation between the government and the private sector adds another problem in finding other sources of funds outside the Country's Budget (APBN)/ Regional Budget (APBD).

The waste management budget in Tarakan City in the last 2 (two) years is still very minimal, only allocating 0.5% of the total Regional Budget (APBD) each year.

Further study/research is needed to obtain alternative solutions to the problems of waste management in Tarakan City. The solution is then used as a priority program/activity within a handling period of less than 2 (two) years.

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Correlation between oil Content and Yield of Some early Maturing Soybean (*GLYCINE MAX* (L.)MERILL) Genotypes in Keffi, Nasarawa State

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***Abstract*— *Soybean meals and oils are very essential for human and animal health. Six varieties of Soybeans were evaluated to determine their variability in oil content as well as the correlation between oil content and yield parameters. The study was carried out during the rainy season of 2015 at the Botanical garden of the Department of Biological Sciences, Plant science and Biotechnology unit farm of Nasarawa State University, Keffi. The experiment was laid out in Randomized complete block design (RCBD) with three replications. Data was collected on agronomic characters such as days to emergence, days to 50% flowering, plant height, number of leaves, stem diameter, grain yield, seed weight , number of flowers, leaf area and oil content per a hundred grams of seeds. The results from these data were analyzed using ANOVA and correlation analysis. All of the analyzed traits varied significantly ($P<0.05$) between varieties. The variety TG × 1989 – 45F had the highest weight per plot with a mean of 3733 while TG ×1990 – 21F had the lowest mean as 1000g. It was observed that Variety TG×1989 – 42F had the highest mean for oil content (17.0ml). Oil content showed a positive correlation with number of pods $r = 0.410$, weight with pods $r = 0.3406$, weight without pods $r = 1$, number of seeds per pod $r =0.9162^*$ and Grain yield $r = 0.215^*$. Based on this study soybean varieties TG ×1989 – 45F is recommended for farmers in Keffi area interested in high grain yield and oil content, respectively.*

***Keywords*— correlation, early maturing, oil content, soybean, yield**

I. INTRODUCTION

Soybean cultivation in Nigeria has expanded as a result of its nutritive and economic importance and diverse domestic usage. It is also a prime source of vegetable oil in the international market (Dashiel, 1993). The crop can be successfully grown in many states in Nigeria using low agricultural input. Annually, a good number of the world's total soybean production is processed .into different types of soybean meal and oil (Djekic*et al*., 2013). These meals and oils are very essential for child and adult health alike. Apart from human consumption, soybean is used for the production of nutritious animal feeds of different kinds in the market.

The importance of soybean in food security especially for the poor in Nigeria cannot be overemphasized. It is the best source of plant protein, substituting the animal-protein sources, which are usually inadequate in supply for poor households (Seyi, 2014).

Previous studies in different parts of the world suggest various plant traits which should be considered to be most important while selecting soybean genotypes for higher seed yield and oil content (Ashraf *etal*., 2012). Soybean breeders suspect that oil content is negatively correlated with yield (Babka*et al*., 2003).

An understanding of the relations among various characters with seed yield is essential so as to find appropriate selection criteria. Correlation studies is initiated with the objective of observing the mutual relationship of different morphological characters and also their contribution to yield parameters (Amsalu *et al*., 2014). Quite often, characters are correlated and selection for one character may lead to either positive or negative response in the other characters. This response can be predicted if the correlation and heritability of the characters are known (Morakinyo, 1996). It is a challenge to farmers and breeders to select specific varieties to use when planting for either seed yield, oil content or both. This serves as a problem and a foundation for undergoing this research. This study was aimed at evaluating the correlation between oil content and seed yield parameters of soybean (*Glycine max* (L.) Merill) in Keffi, Nasarawa State, Nigeria.

II. MATERIALS AND METHODS

2.1 Study Area

The field experiment was carried out during the rainy seasons of 2015 at the Botanical garden of the Department of Biological Sciences, Plant Science and Biotechnology unit farm of Nasarawa State University, Keffi. Nasarawa state is located 8°32′N 8°18′E in the Guinea Savannah Zone of Nigeria and annual rainfall figures range from 1100 mm to about 1600 mm.

2.2 Sample Collection

Six varieties comprising of three early maturing varieties with maturity dates ranging between 100 to 115days (TG × 1990 – 21F, TG × 1987 – 62F, TG × 1990 – 40F) and five late maturing varieties with maturity dates ranging between 115 – 130 days (TG × 1990 - 106FM, TG × 1989 – 45F, TG × 1448-2E) were used for the study. All of these varieties were sourced from International Institute of Tropical Agriculture, Ibadan (I.I.T.A).

2.3 Experimental Design

The experiment was laid out in a Randomized Complete Block Design (RCBD). (Gerald, 2012). The varieties were planted in three blocks and the treatments were the twelve different varieties. It was arranged such that each block had a different arrangement of treatments. This was to ensure no bias and to check the performance of varieties on different blocks (Gerald, 2012).

2.4 Site preparation

The land was prepared by manual tillage to ensure good germination and reduce weed infestation NPK 15:15:15 was incorporated into the soil at preparation. The spacing used was 50cm between rows and 10cm between stands. The plot size was 2.5m × 1.5m. Thinning was done two weeks after sowing to one plant per hill. At two weeks weeding was done manually and it was repeated at six weeks. At flowering a single spray of Cypermethrin + Dimethoate 10EC at the rate of 100ml in 15 liters of water was used the spraying was repeated after two weeks.

2.5 Data collection

Data was collected for the following characters.

1. **Days to 50% emergence**: The number of days from sowing to when half of the plant in each plot emerged was collected for all the plots studied in accordance with method adopted by Dashiel, 1993.

2. **Days to 50% flowering**: Number of days between sowing and 50% flowering was taken for the samples studied in accordance with method adopted by Dashiel, 1993.

3. **Plant height (cm)**: Average height of three plants measured from the ground to the point of attachment of the upper most (flag) leaf in centimeters was taken on a weekly basis until the plants attained 50% flowering. This measurement was taken for all the plot studied in accordance with method adopted by Dashiel and Osho, 1998

4. **Number of leaves**: Average number of leaves per plant in each plot was recorded on a weekly basis until the plants attained 50% flowering this was done by manual counting in accordance with method adopted by Dashiel and Osho, 1998

5. **Stem Diameter (cm)**: Thickness of the stem of three plants was measured with a tape the average was obtained and recorded this exercise was done on a weekly basis for each of the plots until 50% flowering was reached for the plants in accordance with method adopted by Dashiel and Osho, 1998

6. **Yield (g)**: The total weight for all the pods at maturity for each plot was taken using a weighing balance in accordance with method adopted by Dashiel and Osho, 1998

7. **Seed weight (g):** The total seed weight was taken per plot this was done in the following process according to the process adopted by Dashiel and Osho, 1998.

8. **Number of flowers**: three plants on each plot was selected and manually counted the average number of the flowers for each plot was then recorded.

9. **Leaf Area (cm^2)**: The process for determining leaf area was done in accordance to the method used by Dashiel and Osho, 1998.

To calculate for each leaf the formula used was:

λ_1 = length of leaf x breadth of leaf / Area of drawn leaf on graph sheet

Where $\lambda_1$is the calculated area for leaf 1

(vii) The average of all the calculated areas were obtained to get the leaf area index.

(viii)The leaf area index is the constant factor used to get the leaf areas.

(viii)The length and breadth previously recorded for each leaf was then multiplied by the leaf area index to get the correct leaf area for all the plots.

Length of leaf (cm) x Breadth of leaf (cm) x Leaf area index = Leaf area (cm^2)

2.5.2 Determination and measurement of oil content

The oil content in milliliters per 100 gram of seed was taken in accordance with the method by Kettle, 2013.

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The principle: The fat (oil) was extracted with ether (petroleum either was used instead of ether because of risk of explosion). The solution was distilled and the ether extract was derived and weighed in accordance with the method of Kettle, 2013.

2.6 Statistical analysis

The data collected was subjected to statistical analysis with the help of various standard statistical procedures.

2.7 Analysis of variance (ANOVA)

Analysis of Variance was conducted using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) version 9.0 Software to test pre-harvest and post-harvest traits. Duncan's multiple range test (DMRT) was used to separate the means of all yield parameters studied where significant differences exist.

The standard linear model for an RCBD is as follows:

$$y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$
$$i = 1........a,\ j = 1........b$$

Where: y_{ij} = An observation in treatment i and block j

μ = The overall mean

α_i = The effect of treatment i

β_j = The effect of block j

e_{ij} = Random error with mean 0 and variance σ^2

a = The number of treatments;

b = the number of blocks

2.8 Correlations

Correlation coefficients was worked out to determine the degree of association of characters with yield and also among the yield components, using statistical analysis system (SAS) programmer.

2.9 Estimate of correlations

Correlation coefficients were estimated using the Pearson correlation coefficient formula given by Singh and Chaudhary, (1985), Bozokalfa*et al* (2010)

$$r_{xy} = \frac{\operatorname{cov}(xy)}{\sqrt{\operatorname{var}(y)}\ \operatorname{var}(x)}$$

Where: $\operatorname{cov}(xy)$ = covariance of trait x and y

Var(y) =variance of y

Var(x) = variance of x

Among the six varieties studied, significant differences was observed for plant height, leaf area and stem diameter, except number of leaves among varieties. Significant differences (P <0.05) was also observed for pods weight per plot, 100 seed weight , number of seeds per pods and total number of pods per variety.This is so in this study as the different genetic properties of the varieties can be said to be responsible for the significant differences in all the parameters studied.

Ali *et al*. (2006) had a similar result when experimenting with different genotypes of soybean they reported that analysis of variance and mean performance for yield and its components revealed significant differences among all the genotypes for all the characters studied. These results were also in conformity with that of Khanghah*and Sohani* (which showed significant differences for days to maturity, plant height and seed yield.

III. RESULTS

3.1 Analysis of Variance for early maturing soybean varieties

Analysis of Variance was conducted for yield traits (plant height, leaf area, stem diameter number of pods,number of leaves, seed weight, weight of seeds without pods, grain yield number of seeds per pod, oil content) in 8 weeks for the seven varieties. The result (Table 3.1) showed the following results.

Table 3.1 Analysis of Variance for early maturing soybean varieties

Source	DF	NL	SD	LA	PH	NP	WT	WWT	NSP	Grain yield	OIL CONTENT
gen	6	90.603	0.190	5.670	4040.193**	18019.937**	0.857**	0.237**	3.429**	12876.432**	164.034**
rep	2	363.738	0.081	19.799	34.445	4162.667	20.621	6.047	0.381	330.321	1.977

No significant difference was observed for number of leaves, stem diameter and leaf area across varieties. (Table 3.1). Highly significant differences were observed in plant height (4040.19cm),between earlyvarieties of soybean observed at (P <0.05) (Table 3.1).

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There was high significant difference between number of podsacross varieties (18019.93) at (P <0.05). Significant differences were observedfor seed weight (0.857cm) and weight without pods at (P <0.05) (Table 3.1).Highly significant difference was observed inoil content for the seven varieties (164.034) at (P <0.05).

3.2. Analysis of Variance for some late maturing soybean varieties

Analysis of Variance was conducted for yield traits (plant height, leaf area, stem diameter number of pods,number of leaves, seed weight, weight of seeds without pods, grain yield, number of seeds per pod, oil content) in 8 weeks for the seven early maturing varieties. The result (Table 3.2) showed the following results.

Table 3.2. Analysis of Variance for late maturing soybean varieties

Source	DF	NL	SD	LA	PH	NP	WT	WWT	NSP	Grain yield	OIL CONENT
gen	4	3355.758	2612.614**	45.663*	574.760	12866.133**	14.844**	0.439**	5.333**	100344.88**	488.020**
rep	2	5756.275	7052.730	36.545	17046.110	330.533	5.239	0.035	0.533	450.643	0.078

No significant difference was observed for number of leaves andplant height varieties. (Table 3.2). Highly significant differences were observed in stem diameter, number of pods, seed weight, weight without pods and number of seeds in pods between latevarieties of soybean at (P <0.05) (Table 3.2).Also highlysignificant difference was observed foroil content across varieties (488.020) at (P <0.05).

Table 3.3 Correlation between oil content and yield parameters forsome early varieties

	OIL CONTENT	NSP	WWT	WT	NP	Grain yield
OIL CONTENT	1	-0.8317	-0.06329	-0.15336	0.36549	0.215**
NSP	-0.8317	1	-0.02207	-0.02993	-0.36055	0.459*
WWT	-0.06329	-0.022	1	0.91206	0.28266	0.462*
WT	-0.15336	-0.02993	-0.91206	1	0.31505	0.465*
NP	0.36549	-0.36055	0.28266	0.31505	1	0.552**
Grain yield	0.215	0.459	0.462	0.465	0.552	1

Correlation between oil content and yield traits for some early maturing varieties of soybeans

Correlation between weight with pods, weight without pods (r= 0.912) and number of pods(r= 0.315) was highly significant, but was not significant with number of seed per pod (r= -0.029) and oil content (r= 0.570)

Correlation between weight without pod, number of pods (r= 0.282) and weight with pods(r= 0.912) was highly significant. While oil content and number of seed per pods were not significant (r= -0.022).

Correlation between number of seed per pod, number of pod (r= -0.360) and oil content was highly significant (r= -0.832), while there was no significant difference between number of seed per pod and weight with pod (r= -0.029) and weight without pod (-0.022).

Correlation between number of pod with weight with pod (r=0.3150) and number of seed per pod (r= -0.360) and oil content (r= 0.365) was highly significant.

Correlation between grain yield, oil content(r= 0.215) and number of seed per pod (r= 0.459) was highly significant, while there was significant difference between grain yield, number of pods (0.552), weight with pod(r= 0.466) and weight without pod(r= 0.462).

Table 3.4 Correlation between oil content and yield parameters for some late varieties

	OIL CONTENT	NSP	WWT	WT	NP	Grain yield
OIL CONTENT	1	-0.59481	0.64714	0.02175	0.26273	0.1874**
NSP	-0.59481	1	-0.42406	0.02472	-0.61495	0.8432*

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WWT	0.64714	0.42603	1	0.19294	0.42603	0.6291*
WT	0.02175	0.56993	0.19294	1	0.56993	0.5102**
NP	0.26273	-0.61495	0.42603	0.56993	1	0.3362**
Grain yield	0.1874**	0.8432*	0.6291*	0.5102**	0.3362**	1

Correlation between oil content and yield traits for some late maturing varieties of soybeans

Correlation between weight with pods, and number of pods was highly significant (r= 0.569), but was not significant with number of seed per pod (r=0.569), oil content (r=0.022) weight without pod (r=.0.193)

Correlation between weight without pod, number of pods (r=0.42603) and oil content(r= 0.647) was highly significant. While weight with pods (r=0.193) and number of seed per pods were not significant (r=0.426).

Correlation between number of seed per pod, number of pod (r= -0.615) and oil content (r= -0.59) was highly significant, while there was no significant difference for weight with pod (r= 0.025)

Correlation between oil content, weight without pod (r=0.65) and number of seed per pod (r= -0.59) was highly significant, while there was no significant difference for number of pod (r=0.26)and weight with pod (0.022).

Correlation between grain yield, oil content (0.8174), weight without pods (0.510) and number of seed per pods (0.336) was highly significant, while there was significant difference between grain yield, number of pods (0.843) and weight with pods (0.629) was significant

IV. RESULT AND DISCUSSION

Varieties of testing were very important so we could evaluate which varieties were the best solutions for the specific growing region (Djekic*et al*., 2013). Adequate choice of soybean cultivar is of great importance for attaining high and stable yields to meet and surpass local needs.

Among the six early varieties studied, No significant difference was observed for number of leaves and plant height. (Table 3.2), indicating that there is no genetic variation for those traits. Highly significant differences were observed for stem diameter, number of pods, seed weight, weight without pods and number of seeds in pods between late varieties of soybean at (P <0.05) (Table 3.2). This indicates the existence of considerable amount of genetic variation among the traits. Also highly significant difference was observed for oil content across varieties (488.020) at (P <0.05), indicating the existence of genetic variability among varieties.

Ali *et al*. (2006) had a similar result when experimenting with different genotypes of soybean they reported that analysis of variance and mean performance for yield and its components revealed significant differences among all the genotypes for all the characters studied. These results were also in conformity with that of Khanghahand Sohani (1999) which showed significant differences for days to maturity, plant height and seed yield.

The positive correlation between all these parameters studied shows that an increase in each trait will also lead to an increase in the other trait it is correlated to that is if all required conditionsfor proper growth and environment are met. This findings was in line with that of Moradi and Salimi (2012) who observed significant variations in all the parameters studied and showed significantly positive correlation of plant height, pods per plant, dry matter, and branches per plant with grain yield.

Since all the varieties studied have been exposed to the same ecological and environmental factors they all showed the same positive significant correlation in spite of varying genetic components. Ali *et al*. (2003) reported interrelationships between yield and its components in different soybean genotypes and reported that seed yield per plant was positively and significantly associated with all parameters studied which is same as observed in this work. They reported that pods per plant has maximum positive direct effect on yield per plant followed by 100 seed weight and pods per plant, seeds per pod and 100 seed weight were the main yield components.

Leaf area showed a positive correlation with oil content and significant correlation with grain yield. This findings was in line with that of Ashraf *et al*. (2012) who reported significant and positive correlation of leaf area with pods per plant and oil content he suggested that the characters can be considered as selection criteria in improving the bean yield of soybean genotypes. Therefore from the obtained results varieties with larger amounts of grain yield would produce higher oil content. This can be explained that higher quantities of soybean would produce more oil. Similarly Rezaiand Siahsar(1999) also reported that the number of pod per plant had the greatest genotypic correlation with seed yield in soybean which is similar with results obtained from this study Jagdish*et al*. (2000)

reported that improvement can be done on the basis of pods per plant, 100 seed weight and seed quality. Ashraf *et al*. (2012) also lends support to this result as he reported that correlation coefficient of yield was significant and positive with leaf area, pods per plant and oil content. In soybean, seed yield, as in other crops, is a complex character, which is dependent on a number of variables. Thus, to make effective selection for high seed yield a thorough understanding of yield contributing characters and their interrelationships among themselves and with yield is necessary. Therefore, knowledge of relationship between yield and its components obtainable through correlation and regression analysis helps a great deal to formulate selection. Correlation coefficient (r), measures the degree (intensity) and nature (direction) of association between characters (Moradi and Salimi 2012).

V. DISCUSSION

Correlation between oil content and yield traits for some early maturing varieties of soybeans

A positive significant correlation between desirable traits is favorable because it helps in simultaneous improvement of both the characters. Negative correlation will hinder the simultaneous expression of both.

Weight with pods correlated positively with weight without pods and number of pods. While there was no correlation between weights with pods, number of seed per pod and oil content.

Weight without pod correlated positively with number of pods and weight with pods, while there is no correlation between weight with pod and oil content and number of seed per pods.

Number of seed per pod correlated positively with, number of pod and oil content, while there was no correlation between seed per pod, weight with pod and weight without pod.

Number of pod correlated positively with, weight with pods and oil content, while there was negative correlation between number of pod and number of seed per pod.

Grain yield correlated positively with, number of seeds per pods, number of pods, weight without pods, weight with pods, oil content, this result is in corroboration with the findings of Rajanna*et al*., (2000) who estimated significant and positive correlation of number of pods per plant, with grain yield and the findings of Chamundeswori and Aher (2003) for grain yield showed positive correlation with number of pods per plant.In such situation some economic compromise has to be made.This indicated that simultaneous selection for these traits might bring an improvement in seed yield.

VI. CONCLUSION

The results obtained in the present investigation clearly indicated that improvements in seed yield are simultaneously possible through indirect selection for number of pod per plant which is highly correlated with seed yield.

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Effect of Metal Ions and Enzyme Inhibitor on the Activity of Cellulase Enzyme of *Aspergillus flavus*

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***Abstract*— *Natural by- product of agricultural waste can be turned to products of commercial interests such as glucose, ethanol and single cell protein. Much effort from scientists and researches all around the world has been put to extend the full use of agricultural waste. Reports of cellulase enzyme production from the bioconversion of lignocellulosics materials has much been made. However there is still much space to find the most suitable condition by studying the effect of the various metal ions in the activity of the enzyme. In this research different metal ions were added to the enzyme reaction mixture in 1-5 mM amounts, incubated at room temperature and then used to carry out enzyme assay using CMC, Filter paper and cotton wool. The purpose of this research was to investigate the effect of various metal ions on the activity of the cellulase enzyme. Result shows that only Fe^{2+} had a pronounced stimulating effect ($P<0.05$) on the enzyme activity in all the substrates at 1mM concentration respectively. This was followed by Cu^{2+} in CMC but which was also found to be inhibitory when cotton wool and filter paper were used as substrates. However, other divalent metals were found to have either slight or appreciable inhibitory effects on the enzyme activity. According to these results, these ions must be avoided in future cultivations for a high cellulase production.*

Keywords*— *Metal Ions, Enzyme Inhibitor, Cellulase Enzyme, Enzyme Activity, Aspergillus flavus.

I. INTRODUCTION

Currently, most commercial cellulases (including β-glucosidase) are produced by Trichoderma species and Aspergillus species (Cherry and Fidantsef, 2003; Esterbauer *et al*., 1991; Kirk *et al*., 2002). Cellulases are used in the detergent market for color care and cleaning, in the food industry for mashing; in the paper and pulp industries, textile industry, drainage improvement, and fiber modification (Cherry and Fidantsef, 2003; Kirk *et al*., 2002). The cellulase market will enlarge when cellulases are used to hydrolyze pretreated cellulosic materials to glucose, which can be fermented to products including bio-based products on a large scale (Cherry and Fidantsef, 2003; Himmel *et al*., 1999; van Beilen and Li, 2002). The large potential and the importance of cellulases in bio-based product industries will stimulate interest for the development of better cellulase preparations for cell wall cellulose hydrolysis. These improved cellulases are expected to have properties necessary for bio-refineries, such as higher catalytic efficiency on insoluble cellulosic substrates, increased stability at elevated temperature and at an optimal pH, and improved tolerance to end-product inhibition.

II. MATERIALS AND METHODS

2.1 Study area

The work is carried out at the Department of Microbiology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. Awka is the capital city of Anambra state, which lies within the southern part of Nigeria. The geographical coordinates of Awka corresponds to 6.22 North and 7.07 East and falls within the humid tropics of Nigeria. The town Awka was made after clearing much of the tropical grassland, and outskirts of the city are still covered with grassland. It has a moderate climate with a very high temperature during the dry season and average rainfall during the rainy season. Awka has the mean annual temperature and precipitation of 35°C and 1117mm, respectively (NIMET, 2006).

Materials and Methods

The materials used in this research include reagents, salts, solvents, resins, substrates media among others. Most of the materials were kindly provided by Professor F. J. C. Odibo of Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, while others were either obtained from the Research Laboratory or purchased from FinLab. Ltd., Enugu.

Experimental Design

Generally, to ensure accuracy, most parameters were measured three times and the mean taken as the value of the parameter. Indices that were measured on graded levels were statistically analyzed using one way analysis of variance (ANOVA) and the differences between treatment means were separated using Duncan's New Multiple Range Test (DNMRT).

Also, data collected were presented in graphs and histograms to increase clarity. Other descriptive statistics such as range, intervals and the like were employed were necessary.

Methods

Samples of rotten wood and compost were collected from Botanical garden of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The samples were pulverized and shaken in distilled water, and filtered using white cloth. A drop of each of the filtrate was placed on Czapex Dox medium to which 1 % carboxymethylcellulose (CMC) of low viscosity (BDH) was incorporated and spread. It was then incubated for 48 h at room temperature (28-30 °C.)

A total of seven dominant colonies were isolated and purified by successive subculture on fresh Czapex Dox medium. The cellulolytic activities of the colonies were determined by point inoculation of each fungal isolate on Czapex Dox-carboxymethyl cellulose medium and incubated for 72 h. After the incubation, zone of clearing which is an indication of cellulolysis was detected by flooding the cultures with 0.5 % Congo- red solution for 15 min. and distaining with 1M sodium chloride for 10 min (Teather and Wood, 1982). The zones were measured and result recorded.

Identification of the Fungal Isolates

The colonies with the highest zone of clearance were observed by slide culture technique under the microscope with the aid of methylene cotton blue stain, for the characteristic morphological features using standard reference manuals (Ellis, 1976; Raper and Fennel, 1965).

Screening for Cellulase Production

The selected isolates were cultivated in a Mandel and Weber (1969) medium containing the following in g/l:

$(NH_4)_2S0_4$	1.4
KH_2P0_4	2.0
Urea	0.3
$MgS0_47H_20$	0.3
$CaCl_2$	0.3
$FeS0_47H_20$	0.005
$ZnS0_47H_20$	0.0014
$MnS0_4H_20$	0.0016
$CoCl_2$	0.002
Tween 80	2.0 ml
Carboxymethylcellulose	10.0
pH	6.8

A loopful of conidia was inoculated into 100 ml of the sterilized medium in a 500 ml flask and incubated at 35 ^{0}C on a Stuart orbital shaker model S150 for 7 days at 200 rpm. After the incubation, the broth culture was subjected to centrifugation at 4000 rpm for 20 min using Centurion Centrifuge to remove the mycelia and other insoluble materials. The supernatant was recovered and used for the enzyme assays. The isolate with the highest enzyme activity was then selected and used for further studies.

Enzyme Purification

Concentration of the Supernatant by Dialysis

A 5 M sucrose solution was prepared and 700 ml of the enzyme supernatant contained in dialysis bag were suspended in the solution and left overnight at 4 ^{0}C until a considerable reduction in the volume of the supernatant was achieved.

The concentrated enzyme solution (100 ml) was recovered and the enzyme assay repeated using CMC, filter paper, cotton wool and crystalline cellulose. The protein content of the concentrated crude enzyme was also determined using Bradford's method.

Ion-exchange Chromatography

A column (18x1.5 cm), packed with Q-Sepharose fast flow was set up and washed with 150 ml of 0.2 M phosphate buffer (pH 6.8) to equilibrate the resin to the buffer pH. Then, 20 ml of the enzyme concentrate was applied and allowed to permeate the column and then washed down with the same buffer. Fractions were then collected with test tubes calibrated to 10 ml volume at the flow rate of 0.5 ml/ min at room temperature. After collecting 17 fractions, a sodium chloride gradient (0-0.5M) was applied and further fractions collection continued until a total of 40 fractions were collected.

The fractions were subjected to protein estimation by measuring absorbance at 280 nm using an Eppendorf biophotometer and the values plotted against the fraction numbers. The fractions were assayed for enzyme activity using CMC, filter paper and cotton wool. The fractions with pronounced activity were pooled and concentrated as earlier described. The enzyme concentrate was assayed again and protein content determined by Bradford (1976) method.

Gel filtration using Sephadex G-200

The enzyme concentrate was subjected to gel filtration using Sephadex G-200 resin packed in a column (1.8 x 44 cm). The column was equilibrated with 50 ml of phosphate buffer pH 6.8. Fractions were then collected in 5 ml volume at the flow rate of 0.5 ml/ min and a total of 43 fractions were collected. These were subjected to protein estimation by measuring absorbance at 280 nm using an Eppendorf biophotometer.

The fractions with observable protein peaks were used for enzyme activity on CMC, filter paper and cotton wool.

Metal ions and Enzyme Inhibitor EDTA

The effect of metal ions of the following salts; Magnisum sulphate ($MgS0_4.7H_20$), calcium chloride ($CaCl_2$), zinc sulphate ($ZnS0_47H_20$), manganese sulphate ($MnS0_4H_20$), barium chloride (BaCl), ferrous sulphate ($FeS0_47H_20$), copper sulphate ($CuS0_4$), mercuric sulphate ($HgS0_4$), cobalt chloride ($CoCl_2$) and lead sulphate ($PbS0_4$) on the enzyme activity was investigated.

Equal volumes of the purified enzyme and 1mM solutions of the above salts and EDTA in 0.2M phosphate buffer (pH 6.8), were pre incubated at room temperature for 10 min. To assay for enzyme activity 0.1 ml of various substrates which include Cmc, Cotton wool and Filter paper in 0.2 M phosphate buffer (pH 6.8) was added to pre incubated mixture and then incubated at 40 ^{0}C for 30 min, 1h and 24 h, respectively and the reducing sugars quantitated using DNS. The reducing sugar released was estimated by 3, 5-dinitrosalicylic acid method (Miller, 1959) as follows; at the end of incubation, the enzyme reaction was stopped by adding 0.5 ml of 3, 5- dinitrosalicylic acid reagent (BDH). The mixture was placed in boiling water for 10 min, after which it was cooled, and 5 ml distilled water added. The absorbance was then read at 540 nm using the substrate solution treated in the same way as blank to zero the spectrophotometer (JENWAY), model 6405. One unit (IU) of CMCase activity was defined as the amount of enzyme required to liberate 1 µmol of glucose from the substrate under the assay condition.

The control tube having no metal ion or inhibitor was taken as 100 %.

III. RESULTS AND DISCUSSION

Results of cellulase activity in the presence of metal ions revealed that only Fe^{2+} had a pronounced stimulating effect ($P<0.05$) on the enzyme activity in all the substrates (Figures1-3). This was followed by Cu^{2+} in CMC (Figure 1) but which was also found to be inhibitory when cotton wool (Figure 2) and filter paper (Figure 3) were used as substrates. However, other divalent metals were found to have either slight or appreciable inhibitory effects on the enzyme activity. All ions tested had significant effect on cellulase activity. A considerable decrease (>80% inhibition) in activity was observed in the presence of Cu^{2+}, Pb+, Hg+, Ba^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+},Co^{2+} and Zn^{2+}. These ions are commonly cited in the literature as inhibitors for several microbial cellulases [25–27]. Activity is probably inhibited through the attack of certain groups at the active site of the enzyme, for example, the thiol groups, leading to inactivation [25]. According to these results, these ions must be avoided in future cultivations for a high cellulase production. The effect of balance between different metal ion concentrations could be more.

Metal cations such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Co^{2+} and Zn^{2+} were more important than their individual effects. For example, iron is needed for cellulase production, but it is also inhibitory at higher concentration. It has been hypothesized that the metals may prevent some components necessary for induction from leaking out of the cells.

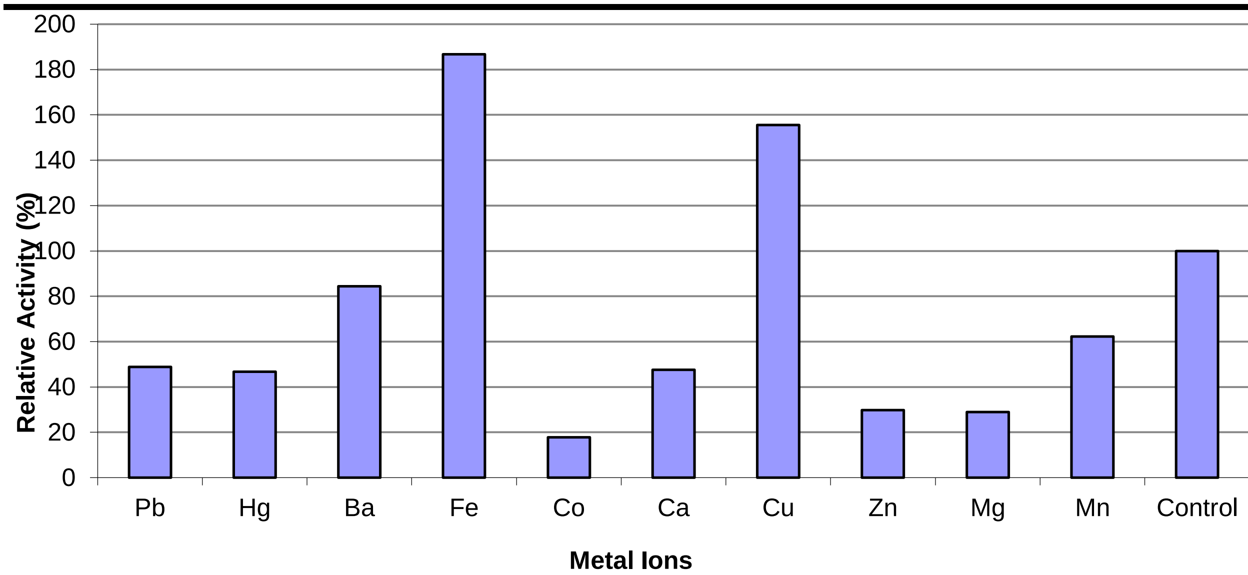

Fig.1: Effect of Metal Ions on the CMC activity of the Cellulase

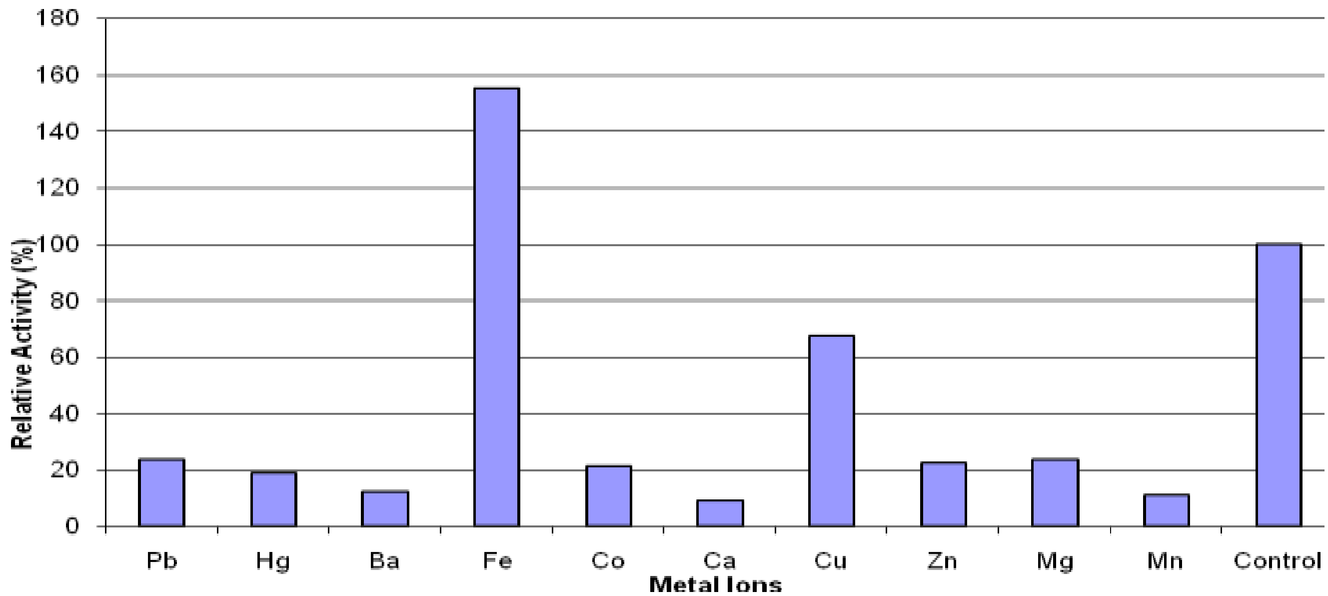

Fig.2: Effect of Metal Ions on Cotton Wool Activity of the Cellulase

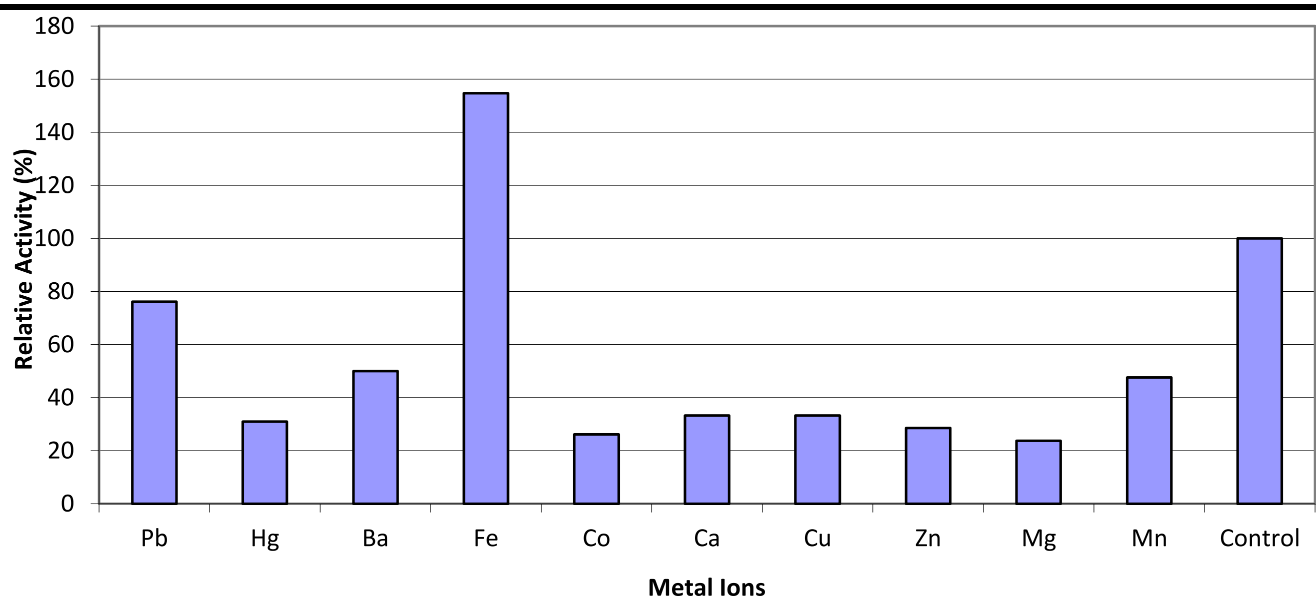

Fig.3: Effect of Metal Ion on Filter Paper Activity of the Cellulase

Effect of different Concentrations of Fe^{2+} on the stability of the enzyme using various Substrates

Varied concentrations of Fe^{2+} exhibited pronounced effects ($P<0.05$) on thermal stability of the *Aspergillus flavus* cellulase both within and across substrates. That is, statistical differences were observed in cellulase activity between various concentrations of Fe^{2+} in a given substrate (Figure 4), and between different substrates (Figure 5). The highest thermal stability of the enzyme was observed at 1 mM concentration of Fe^{2+} for the three substrates studied (CMC, filter paper and cotton wool), and the least thermal stability obtained at 5 mM concentration. In fact, proportionate decline on the stability of the enzyme was recorded as the Fe^{2+} concentration increased, irrespective of the substrate (Figure 4-6).

Again, there was a proportionate increase in thermal stability of the enzyme on filter paper, followed by CMC ($P<0.05$).

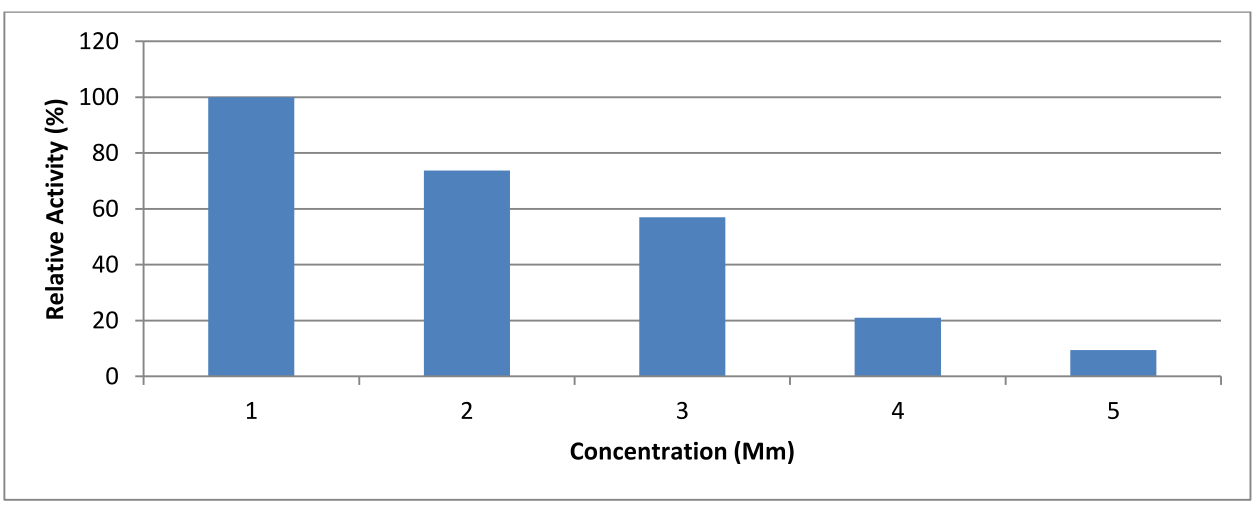

Fig.4: Effect of different Concentration of Fe^{2+} on CMC Activity of the enzyme (Histogram)

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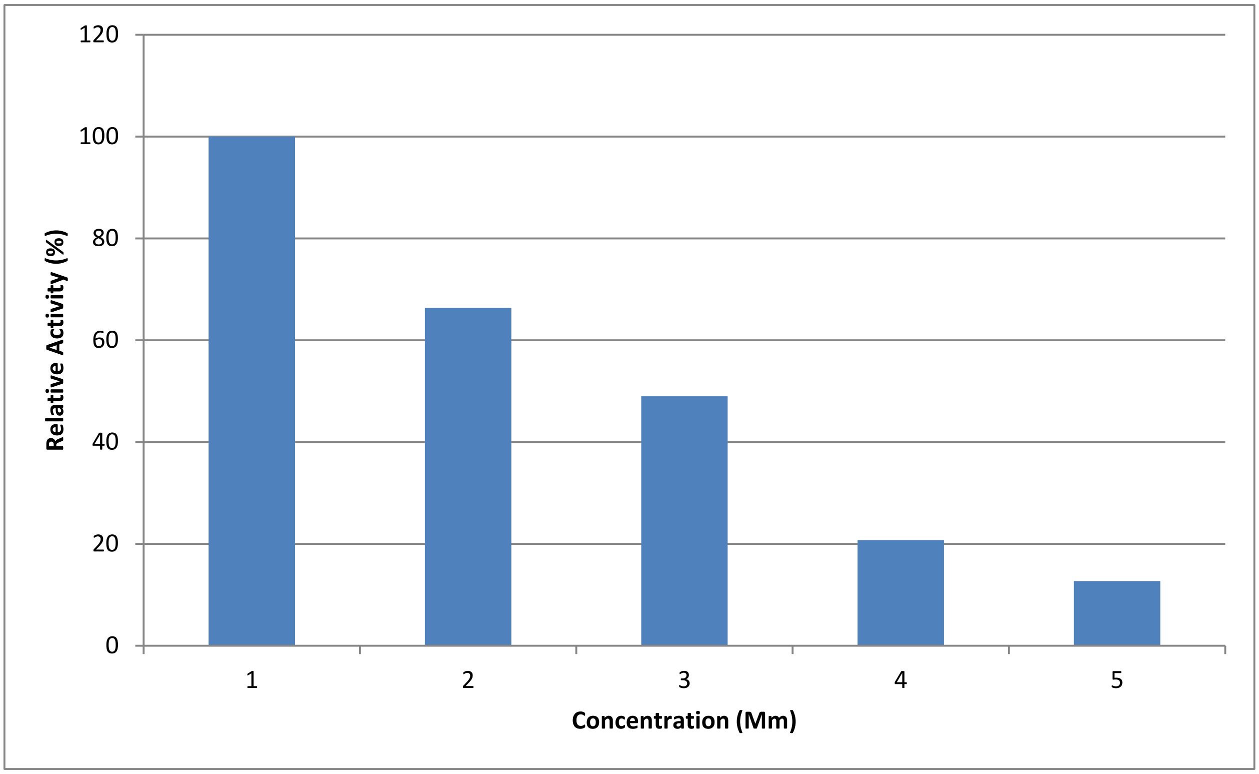

Fig.5: Effect of different concentration of Fe^{2+} on Filter Paper Activity of the enzyme (Histogram)

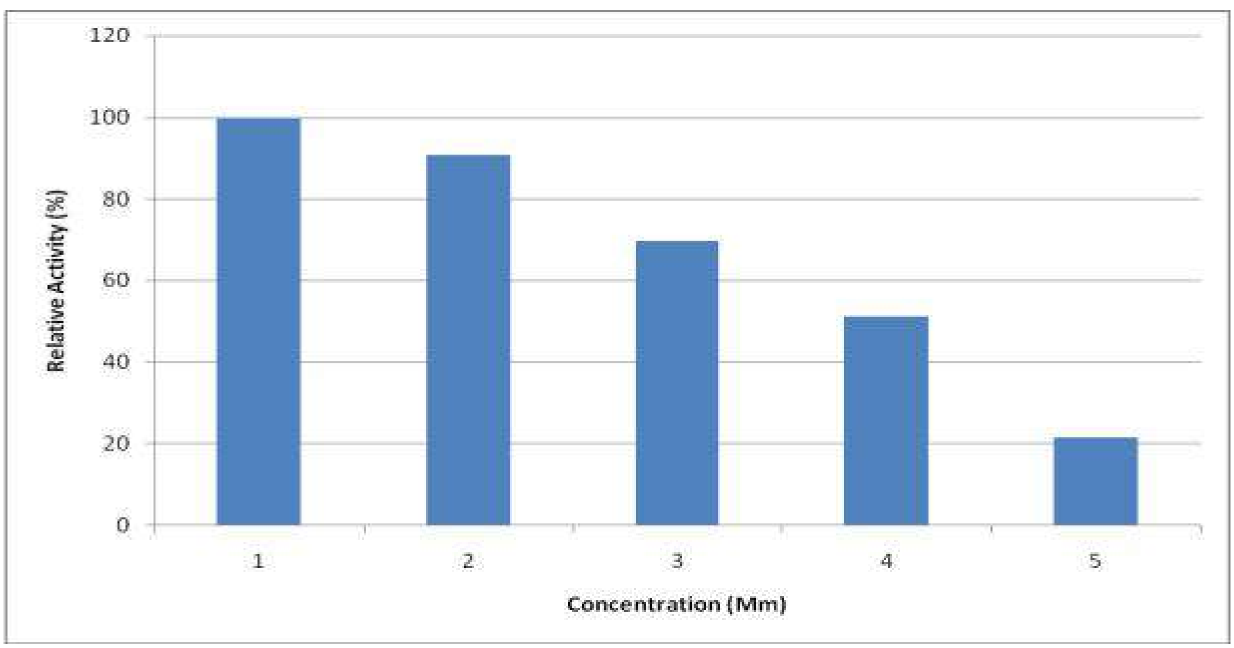

Fig.6: Effect of different concentration of Fe^{2+} on Cotton Wool Activity of the enzyme (Histogram)

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Effect of EDTA on enzyme activity

EDTA had slight inhibitory effect on the cellulase activity (12.4 % in CMC, 38.1 % in filter paper and appreciable effect, 80.9 % in cotton wool) as shown in Figure 7.

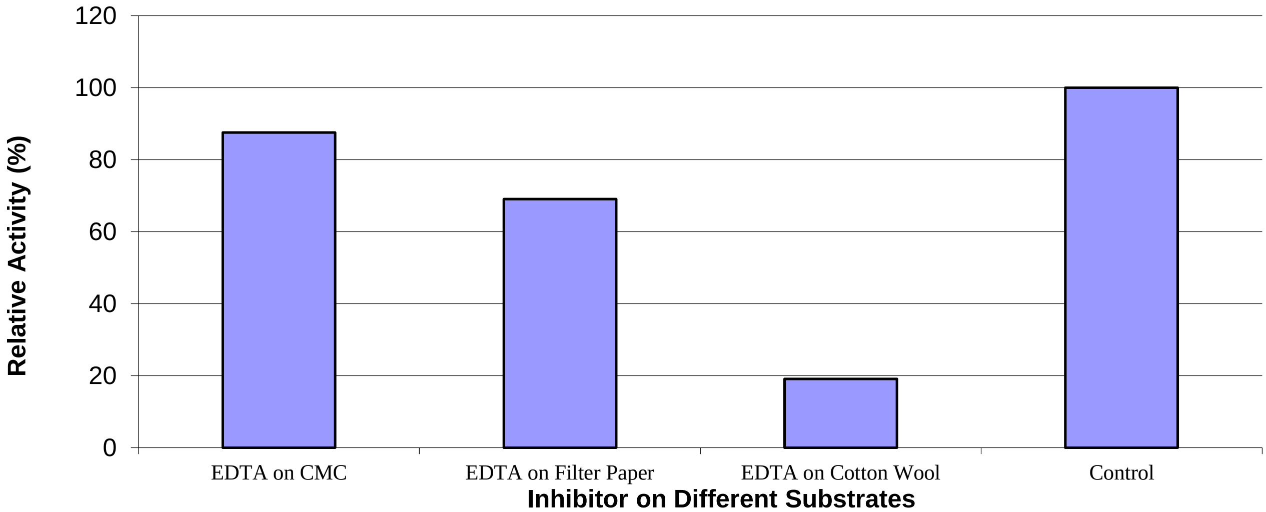

Fig.7: Effect of EDTA on enzyme activity

The role of divalent metals in the stability and activity of this enzyme is confirmed by the reduction of activity in enzyme treated with EDTA which is said to chelate metal ions necessary for enzyme activity and stability. While the exact mechanism of action is still unclear, EDTA is known as an ionic chelator and its inhibition ability indicate that specific ions might be actively involved in the catalytic reaction of the enzyme (Kotchoni *et al*., 2006). It is also deduced that the enzyme sub unit has a structural and catalytic binding sites. The low level activity in EDTA treated samples; probably indicate the important role these metal ions play in the structural integrity and activity of the enzyme. According to Hartley *et al*. (2000) metal ion binding to both sites is essential for the activity of the enzyme.

All the metal ions tested had slight or appreciable inhibitory effects on the purified enzyme which agreed with Liu and Xia (2006) and Petrova *et al*. (2009), except for Fe^{2+} which has the highest stimulatory effect. The implication of Fe^{2+} as a good stimulator of cellulase of *Aspergillus flavus* is in line with the reports of Adams *et al.* (1976) and Shahriarinour1 *et al.* (2011), but disagreed with the finding of El-zawahry *et al.* (2010) who recorded inhibition by Fe^{2+}.

Results suggest that Fe^{2+} apparently protected the enzyme against thermal denaturation and played vital role in maintaining the active form of the enzyme at high temperature (100 ^{0}C). Hence, Fe^{2+} enhances the stability of the enzyme by 55, 86.7 and 54.8 % at 1 mM concentration over values obtained without Fe^{2+}. However, a better understanding of the mechanism of activation of enzyme by divalent ion is needed for clearer understanding of the observation of Fe^{2+} with the cellulase enzyme

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Effect of Sun Dried, Dehulled and Boiled Kidney beans on Hematological and Serum Biochemistry of Broiler Chickens

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***Abstract*— *A four-week feeding trial was conducted to assess the effect of sun dried (raw) (SD), dehulled (D) and boiled kidney beans (BKB)on the haematological and serum biochemistry of broiler birds. One hundred and twenty unsexed broiler birds were used for the study. The birds were randomly assigned to four dietary treatments replicated three (3) times with twelve (12) birds per replicate in a completely randomized design (CRD). The treatment groups were control diet (CD), sun dried kidney bean (SDKB), dehulled kidney bean (DKB) and boiled kidney bean (BKB), designated as T1, T2, T3 and T4, respectively. The feeding trial lasted for four weeks.The proximate composition of the kidney beans used was also determined. The proximate composition obtained showed that kidney beans is a good protein source for birds (crude protein content of 20.98%). At the end of the feeding trial, blood samples were collected and the haematological and serum biochemical parameters of the birds were determined using standard methods. Generally, the diets used exhibited significant effects on both the haematological and serum biochemical parameters of the birds studied. Diet without kidney bean (Control) and diet containing sun dried kidney bean gave the best result, though all the haematological and biochemical values obtained in the studyfall within the referral range indicating that processing method had no negative effect on the haematological and serum biochemical parameters of the birds. Based on the findings in this study, it was recommended that kidney bean is a good source of plant protein in animal diet and does not necessarily need to be processed prior to its incorporation in animal feed.*

***Keywords*— Sun dried, dehulled, boiled kidney beans, haematology, serum biochemistry, broiler birds.**

I. INTRODUCTION

Feedstuff such as fishmeal and soybean are valuable components of poultry diets because of their high protein content and amino acid profile. However, current trends in the diversified use of these known protein sources both in industry and as human food have increased their market values. Therefore, identification, development, and utilization of potential alternatives are imperative for the sustainability of poultry industry. One of such alternatives is the kidney bean which is a legume plant protein source. Kidney bean has not gained widespread industrial, economic and nutritional importance because its acceptability and utilization has been limited (Ofongo *et al*., 2007).

Kidney bean (*Phaseolus vulgaris*) is one of the neglected beans among the tropical legumes. It is a herbaceous annual plant. It is an excellent source of vegetable protein, starch, soluble and insoluble fiber, vitamins (especially the B group) and minerals (particularly potassium, Iron, Zinc, Magnesium and Manganese), but very low in fat content (Enneking, 2011). It can be used to fortify cereal-based diets especially in developing countries because of its high protein content (Hussein *et al*., 2015).It is also a rich source of vitamin, minerals and relatively high in crude fibre. Kidney bean is one such protein source which when used in the fortification or enrichment of cereal based diets could go a long way in improving their nutritional status. An important aim of research in animal production is to enhance livestock production while providing adequate animal protein and livestock by-products for human consumption. Kidney beans are mainly composed of carbs and fibre but are good and rich source of protein. Although the nutritional quality of bean plant protein is lower than animal protein, beans are an affordable alternative for many people in developing countries. In virtue of its amino acid constituent, kidney bean plays a significant role in the growth, egg production, immunity, adaptation to the environment and in many other biological functions. Optimization of its protein supply requires a thorough understanding of the protein requirement of the birds and manipulation of the protein supply to suit various environmental conditions and health status of birds. Research reports showed that it possess excellent nutritional profile with 22.7% protein, 3.5 % mineral 1% fat and 57.7% carbohydrates, out of which, total carbohydrate have 38.6% starch and 18.8 % dietary fibre(60% insoluble and 40% soluble). Its protein has high lysine content of about 5% (Marzo*et al*., 2002; Mustapha*et al.,* 2016).

Proteins mostly found in these beans are storage proteins, that is, 75-80. Because kidney bean is an excellent source of lysine, it can be used for the fortification of cereal based diets (Loggerenberg, 2007). He maintained that it is the best source of vitamin B seriesand essential minerals like K, Ca, Mg, P and Fe.

II. MATERIALS AND METHODS

Study site

The experiment was carried out at the poultry unit of the Department of Animal Science Teaching and Research Farm, Nnamdi Azikiwe University, Awka, Anambra State. The farm is located behind the works Department off the road connecting to the East gate of the University, with an annual mean temperature and rainfall of about 34^0C and 1500mm, respectively. It lies within the latitude of $6^0$15'10''N and $7^0$08'31.9''E, according toObikaonu *et a*l.(2011)

Procurement of the Kidney bean and experimental birds

The kidney beans that were used for the experiment were sourced from the Eke Awka market in Awka, Anambra State. One hundred and twenty day old broiler birds of the Arbo acre strain were procured from Fidan hatchery in Ibadan, Oyo state.

Processing of the experimental material

The kidney beans that were used were sorted out to remove dirt (including stones, dust, etc.), weighed and then subjected to different processing methods (Treatments):

a) ***Sun dried kidney beans***: After removing the dirt and weighing, the seeds were ground.
b) ***Dehulled kidney beans***: The sorted and weighed seeds were soaked in cold water for 18-24 hours. The seed coat was then removed with the aid of a grinding machine (decorticated).The beans were separated from the coat manually after the dehulling operation. They were oven dried at 85^{oc} and later on sun dried for four days before been ground.
c) ***Boiled kidney beans***: The sorted and weighed seeds were poured into a cooking pot containing 100^cboiling water and heated for an hour after which the water was drained out and the seeds obtained were oven dried at 85^oC and later on sun dried for four days before grinding.

Formulation of the experimental diet.

Four (4) experimental diets were formulated as shown in Table 1. Treatment 1 (T1) contained no Kidney beans and as such, served as the control diet. T2, T3, and T4 contained 10% each of sun dried (raw) kidney bean, dehulled kidney bean and boiled kidney beans, respectively. The feed ingredients used for the formulation of the various experimental diets were all procured from Palmark Agro Ventures and Bonitas Agro Ventures, both of which are located at Afor Nnobi market in Nnobi, Idemili South Local Government Area of Anambra State.Calculated chemical composition of the broiler starter diet of the experimental diets is shown in Table 2, while Table 3 showed the Proximate composition of the sun dried, dehulled, and boiled kidney beans.

Table.1.Composition of the starter diet fed to the birds

Ingredients	TI (0%)	T2 (10%)	T3 (10%)	T4 (10%)
Maize	50.50	47.00	47.00	47.00
Soybean	21.00	21.00	21.00	21.00
Fishmeal	3.50	4.00	4.00	4.00
KBM	------	10.00	10.00	10.00
Wheal offal	4.00	4.00	4.00	4.00
PKC	6.75	4.00	3.75	4.00
GNC	10.00	5.75	6.00	5.75
Bone meal	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.50	0.50	0.50	0.50
Vitamin premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total (kg)	100.00	100.00	100.00	100.00

Table.2.Calculated chemical composition of the broiler starter diet

CP (%)	22.02	21.90	21.94	21.90
CF (%)	4.30	4.36	4.34	4.36
Cal Energy	2979.70	2949.07	2948.63	2949.07

Table.3.Proximate composition of the sun dried, dehulled, and boiled kidney beans.

Proximate Fractions	Sun dried(raw) kidney bean	Dehulled kidney bean	Boiled kidney bean
Moisture content (%)	8.69	13.47	9.99
Ash (%)	3.78	3.47	3.40
Crude protein (%)	20.98	21.00	19.21
Ether extract (%)	1.60	1.80	1.85
Nitrogen free extract (%)	58.45	56.79	58.38
Dry matter (%)	91.31	86.53	90.01
Crude fiber (%)	6.50	3.00	7.20
ME kcal/g	3553.54	3252.01	3515.56

Design of the experiment and housing of the experimental birds

The experiment was conducted using a Completely Randomized Design (CRD) with four dietary treatments. The model employed was:

Xij= µ + Ti + £ij

Where:

Xij= Observation made on i^{th} treatment (haematological or biochemical indices) arising as a result of:

µ = Population mean

Ti = Treatment effect

£ij = Experimental error

Each treatment had 30 birds which were assigned into three different groups of 10 each (replicates). The experiment lasted for four (4) weeks.

Management of the experimental animal

120 unsexed four weeks old commercial broiler birds of the Arbor acre strain were procured from Fidan hatchery located at Ibadan and used for the experiment. The chicks were given a solution of sugar and milk in water on arrival as an anti-stress. The birds were acclimatized for one week; thereafter they were randomly allocated to the four dietary treatments. During the period of the experiment, feed and water were given *ad libitum*. Vaccination programs were carried out as required. Other routine poultry management activities such as washing of drinkers, cleaning of the experimental house and changing of the litters were strictly observed.

Collection and preparation of blood samples for haematological and serum biochemical tests

At the end of the fourth week, the haematological and serum biochemical indices of the birds were determined. Blood samples were collected by bleeding the wing with the use of 2ml syringe and needle set. For haematological test, the blood samples were collected into universal bottles containing EDTA (Ethylene diamine tetra-acetic acid). Samples for biochemical test were collected into another set of universal bottles without EDTA. All blood samples collected were immediately placed in a flask containing ice cubes to prevent haemolysis. The samples for serum were centrifuged at 300rpm for I0 minutes to obtain the serum and kept frozen (-4^{oc}) until required for analysis.

Determination of haematological and serum biochemical indices of the birds.

The haemoglobin content (Hb) was determined using Sahil's method, white blood cell (WBC) by Neubauers method and red blood cell (RBC) by the use of formal citrate solution. The values obtained for RBC,Hb and PCV were used to calculate the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration(MCHC).

Serum cholesterol, AST and ALT were determined using the Randox diagnostic kit. Creatinine (Calorimetric method),Urea (Berthelot method), Total protein (Biuret's method), Globulin (Kjeldahl's method), Glucose (GOD POD method) and Albumin (Photometric method).

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) using statistical software (SPSS 20) and the differences between treatment means were separated using Duncan Multiple range test (DNMRT) at5% α-level.

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III. RESULTS AND DISCUSSION

Haematology indices

There were significant ($p<0.05$) variations in the blood cellular constituents of birds fed control diet (CD), sun dried kidney bean (SDKB), dehulled kidney bean (DKB) and boiled kidney bean (BKB) based diets (Table 4). Birds maintained in SDKB based diet (T2) had the highest Hb value, followed by those in the control diet (CD), while those in boiled KB (BKB) had the least. RBC was highest in birds raised with control diet, followed by those in SD based diet and least in those BKB. PCV, MCV, MCH and MCHC followed the same trend. They were highest for the birds in control diet and least in those in BKB based diet.

Virtually all the haematological values obtained in this study fall within the referral range (Adeyemi *et al*., 2000; Aeangwanich *et al*.,2004;Akande*et al*., 2013;Antyev *et al*., 2017)Since haematological values are important indicators of health status of animals, and indispensable tools in the diagnosis, treatment and prognosis of many diseases, all the birds were sound health wise, notwithstanding the method of processing the kidney beans. However, based on haematological profile, kidney beans are best processed by sun drying, and boiling of kidney beans is the least option.

Again, the higher values of RBC and Hb found in birds in CD and SDKB based diets is an indication of increase in the rate of the oxygen carrying capacity of the blood. It also indicates that the nutrients were more adequately utilized by the birds. The lower values obtained in other birds could be ascribed to less preference of the feeds(Remi-Adewum *et al*.,2004). PCVvalues which were high in birds fed control and SDKB diets was as a result of the birds trying to meet up with the increase in metabolic actions taking place in their bodies (Ofongo and Ologhobo, 2007; Piotr Minias, 2015).There was a significant ($p<0.05$) difference in the mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean cell volume (MCV) of the birds fed the different diets containing the various test material. The values obtained for MCV, MCHC and MCH in all the test diets falls within the normal reference range for broiler birds as given by Aeangwanich *et al*. (2004) andAntyev *et al*. (2017), although the values obtained were significantly different, it indicates that there was no negative interaction between the energy and protein levels in the diets.There was also a significant ($p<0.05$) difference in the white blood cell count obtained from the birds when fed the different dietary treatments .The values of white blood cell count obtained in all the diet was more than that given in the reference range for broilers by Aeanwanich *et al*. (2004) and Akande*et al*. (2013).The elevated white blood cell in all the diets could be as a result of physiological adjustment against antigenic effects associated with the diets. The white blood cell is known to play an important role in antibody.

Table.4.Haematological parameters of finisher broilers fed control diet, sun dried (raw), dehulled and boiled kidney beans based diet

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	p-val.
Hb (g/dl)	10.42 ± 0.19^{b}	11.06 ± 0.12^{a}	10.05 ± 0.04^{c}	8.51 ± 0.09^{d}	0.00
RBC ($x10^6/mm^3$)	7.46 ± 0.09^{a}	7.10 ± 0.02^{b}	5.40 ± 0.08^{c}	3.00 ± 0.05^{d}	0.00
WBC ($x\ 10^3/mm^3$)	5.63 ± 0.26^{a}	5.39 ± 0.17^{ab}	5.10 ± 0.03^{bc}	4.82 ± 0.07^{c}	0.00
PCV (%)	35.56 ± 0.28^{a}	31.99 ± 0.04^{b}	0.68 ± 0.25^{c}	26.32 ± 0.07^{d}	0.00
MCV (fl)	32.43 ± 0.24^{a}	31.42 ± 0.11^{b}	30.30 ± 0.10^{c}	29.78 ± 0.09^{d}	0.00
MCH (pg)	31.45 ± 0.13^{a}	28.06 ± 0.03^{b}	26.52 ± 0.08^{c}	22.07 ± 0.03^{d}	0.00
MCHC (%)	31.45 ± 0.15^{a}	30.08 ± 0.05^{b}	25.16 ± 0.15^{c}	23.25 ± 0.05^{d}	0.00

Means bearing different superscripts in the same row are significantly different ($p< 0.05$)

Serum biochemistry

Serum enzymes AST and ALT were significantly ($p<0.05$) influenced by the dietary treatment as shown in Table 5. These serum biochemical components of animals have been reported to be positively correlated with the quality of the diet which animals were fed (Adeyemi *et al*., 2000).Control diet and SDKB based diet had the highest serum enzyme content.These enzymes are key enzymes needed in the biotransformation and detoxification of various toxicants (Akande *et al*., 2013). The increased activities of the hepatic transferase are indicative of increased catabolism of amino acids (Obikaonu *et al*., 2011).The serum urea can be used as a test of renal function and protein breakdown.

Birds fed boiled kidney beans had the highest urea content ($p<0.05$). This implies that there was better digestion, utilization and absorption of protein in the diet containing the boiled kidney beans. . This agrees with the report by Obikaonu*et al*. (2011) andAntyev *et al*. (2017).The creatinine level was high in birds fed BKD based diet than in other birds, with birds fed control and SD diets having the lowest. High level of creatinine in the blood serum can be diagnosed as the possible increase in the tear and wear of muscles as the birds grow and carry out their metabolic activities. It could also imply that there is possible occurrence of renal damage since the amount of urea to be excreted by the bird has increased (Loggerenberg, 2007; Sadeghi and Pourreza, 2007; Antyev *et al*., 2017).

The serum total protein, globulin and albumin of the birds were significantly ($p<0.05$) affected by the diets. The birds fed control and SDKD based diets had high values of the serum protein, globulin and albumin.Serum albumin and globulin content of blood depends on the availability of dietary protein. This means that the proteins of the Control and SDKB based diets were similarly available to the birds confirming the observation by Hoffenberg *et al*. (1966). Similarly, the cholesterol contents were higher in birds fed control and SDKB based diets than those in the other diets. However, this does not agree with the work of Sadeghi and Pourreza (2007) who said that the cholesterol level in the serum has a negative correlation with the fiber content of the animal's diet. The discrepancies might be as a result of the nature/ source of the fibre fed.

Table 5.Serum biochemical parameters of broiler birds fed sun dried (raw), dehulled and boiled kidney bean based diet

Serum parameter	Treatment 1 CD	Treatment 2 SDKB	Treatment 3 DKB	Treatment 4 BKB	P- Value
Total protein (g/dl)	73.39 ± 0.37^{a}	68.33 ± 0.38^{b}	66.37 ± 0.42^{c}	60.51 ± 0.30^{d}	0.00
Albumin (g/dl)	24.74 ± 0.22^{a}	24.17 ± 0.26^{b}	23.22 ± 0.22^{c}	22.72 ± 0.11^{d}	0.00
Globulin (g/dl)	48.62 ± 0.30^{a}	43.45 ± 0.15^{b}	42.16 ± 0.23^{c}	41.56 ± 0.28^{d}	0.00
Urea (mg/dl)	3.05 ± 0.42^{d}	3.49 ± 0.15^{c}	3.65 ± 0.19^{b}	4.09 ± 0.11^{a}	0.00
Cholesterol (mg/dl)	35.40 ± 0.30^{a}	34.52 ± 0.21^{b}	33.09 ± 0.19^{c}	28.53 ± 0.18^{d}	0.00
Creatinine (mg/dl)	3.21 ± 0.16^{d}	3.61 ± 0.09^{c}	6.00 ± 0.05^{b}	6.53 ± 0.20^{a}	0.00
Glucose (mg/dl)	38.33 ± 0.15^{a}	35.39 ± 0.27^{b}	32.29 ± 0.28^{c}	28.48 ± 0.17^{d}	.00
AST (IU/L)	125.52 ± 0.18^{a}	123.66 ± 0.21^{b}	116.51 ± 0.30^{c}	117.31 ± 0.13^{d}	0.00
ALT (IU/L)	29.66 ± 0.21^{c}	34.35 ± 0.18^{a}	29.53 ± 0.31^{d}	30.17 ± 0.19^{b}	0.00

Means bearing different superscripts in the same row are significantly different ($p< 0.05$)

IV. CONCLUSION

Based on the findings of this study, kidney beans can be used as an alternative source of protein in poultry diet by eithersun drying or dehulling or boiling the beans without any serious deleterious effect on the haematological and serum biochemical indices of the birds. However, sun drying the kidney bean is the best processing method followed by dehulling the beans.

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Distribution and Damage of African Citrus Psyllids (*Trioza erytreae*) in *Casimiroa edulis* Producing Areas of the Eastern Zone of Ethiopia.

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***Abstract*— *The common white sapote, Casimiroa edulis occurs both wild and cultivated in central Mexico and produced in a different part of Ethiopia as home garden fruit crop for consumption as food. People also consider the fruits as one of the stimulant fruit crop. Currently, African citrus psyllids (Trioza erytreae) became one of the important pests of C. edulis in Ethiopia. The distribution and severity of this pest were recorded with an irregular pattern in the eastern part of Ethiopia. From all surveyed area (except some districts of Dire Dawa), very high infestation with high population density were recorded. The pest is currently in Ethiopia. This is first record of Trioza erytreae as devastating pest of casimir trees in Ethiopia. It is worth reporting to promote coordinated efforts amongst stakeholders, research specialists and extension officers to create awareness for proper management of the pest.*

***Keywords*— African citrus psyllids, White sapote, Casimir, distribution, severity.**

I. INTRODUCTION

White sapote *(Casimiroa edulis* L1ave & Lex), is native to Mexico and Central America. It can be found in central and southern Mexico as a cultivated and wild species and is also grown in Guatemala, EI Salvador, and Costa Rica. Commercially, it is grown in New Zealand, Australia, and Israel. The fruit has recently been introduced in Japan (Yamamoto *et al*., 2007) and on a small scale in South Africa, Egypt and different part of Ethiopia (Mathewos *et al*., 2013; Reta, 2013; Emelda, 2012; Haileab *et al*., 2011; Mesele *et al*., 2012).

Few pests affect white sapote crop, however, the fruit is highly infested by *Anastrepha ludens* (Aluja *et al*., 1987). Some volatile compounds in white sapote have been found to attract *A. ludens* to baited traps (Gonzalez *et al*., 2006). Although not a target, white sapote has been attacked by the African citrus psylla *(Trioza erytrea)* as one of its a host (Fernandes and Franquinho A., 2001).

African citrus psyllid is native to Africa. It has spread to islands off the coast of Africa and to Saudi Arabia and Yemen (Van den Berg, 1990). Del Guercio, originally described the species in 1918 from samples collected from Citrus limon (L.) Burm (Rutaceae) in Eritrea. The species currently is present mostly throughout the Afrotropic ecozone, including Sub- Saharan Africa and the islands of St. Helena, Mauritius, Reunion and Madagascar, and in Saudi Arabia and Yemen (EPPO 2005; CABI 2015). The species recently has invaded Macaronesia (West Palaearctic), where it was found in Madeira in 1994 (Carvalho and Aguiar, 1997) and the Canary Islands in 2002 (Gonza´lez-Herna´ndez, 2003). *T. erytreae* apparently remained confined to these non-continental areas of Europe, until it was initially recovered, in August 2014, in northwestern Spain (Pe´rez-Otero *et al*., 2015). The psyllid was found in subsequent months at two other locations in the province, at six locations in the province of Pontevedra (Galicia) and in the district of Oporto in Portugal (Pe´rez-Otero *et al*. , 2015), where it has been detected at eight locations (Anonymus, 2015). This psyllid lives from about 100 to 1300 m a.s.l. in the various geographical areas where it has been recorded (Gonza´lez-Herna´ndez 2003; Ekesi, 2015). The distribution of the psyllid in Africa, Saudi Arabia, and Yemen show that it has been able to adapt and settle under a variety of environmental conditions such as in equatorial, arid, and warm temperate climates with different temperatures and rainfall.

The invasion of *T. erytreae* into northern Spain is very similar to that of *T. citricidus*, which, despite its tropical origin, was found first in 2002 on the coast of Asturias and later became adapted to the climatic conditions of northwestern Iberian Peninsula (Ilharco *et al*., 2005). The adaptability of *T. erytreae* and its optimal development according to the climatic factors of the country indicates that, if it finds suitable host plants, it might disperse within Portugal, northern Spain, and into the interior of the peninsula, although, with greater difficulty because of the more extreme climatic conditions. Whatever the path, *T. erytreae* could reach the Mediterranean coast of Spain, the major citrus-producing area of the country, and possibly other citrus growing areas of Europe and North Africa.

T. erytreae was considered a pest of secondary importance for citrus in native regions for many years because of the negligible direct damage caused to adult trees (Catling, 1973; van den Berg & Deacon, 1988). The damage consists of the evident open gall-like structures on leaves, which are diagnostic for the presence of the insect. Leaves can become chlorotic and slightly curled, especially when heavily infested. Van der Mewre (1923) reported that infested leaves normally could perform their vital functions without dropping. Tamesse and Messi (2002), however, reported that *T. erytreae* could be an important pest for nurseries, causing strong deformations on leaves, which can cause 90 % of young plants to die in the absence of insecticidal control. An additional direct damage may also be due to the abundant honeydew excreted by nymphs as soft, white, sticky granules that, in severe infestations, give a dusty appearance to the plants (van den Berg *et al*. 1991), facilitate the development of fungi such as Capnodium sp. and attractants that collect it and disrupt the protective action of natural enemies.

Van den Berg *et al*. (1987) monitored indigenous plants near a citrus orchard and found citrus Psylla, *Trioza erytreae,* on fifty *C. anisata*,; twenty *Z. capense* and ten *V. lanceolata* plants. Adult citrus psyllas were also found, in a feeding position, on *Casimiroa edulis*, however, it was uncertain as to whether the psylla could feed on the plant (van den Berg & Deacon, 1989*T. erytreae* was observed to be feeding on the prevalent *C. anisata* trees in the highlands of Cameroon and Ethiopia (Aubert *et al*., 1988).

Currently, in the different parts of Ethiopia such as East Harerge, *T. erytreae* severely distorted leaves of White sapote (*Casimiroa edulis*), which stunted and galled, and appeared dusted with faecal pellets. Young leaves turned yellow when severely damaged. The presence of small pit galls on young leaves can indicate *T. erytreae* damage. *T. erytreae* is one of the major problems of White sapote producing farmers in Eastern Ethiopia. The infestations were so severe and sometimes caused complete devastation of the white sapote plants. However, there is no detail data concerning *T. erytreae* in Ethiopia. Therefore, this survey was aimed at studying the distribution and severity of *T. erytreae* to create awareness among stakeholders in Eastern Ethiopia and beyond.

II. METHODS AND MATERIALS

The survey was conducted in 2018 cropping season (3/05/2018 - 12/05/2018) to determine African citrus psyllid (AfCP) distribution and severity in *C. edulis* growing area of West Harege, East Harereghe, Harari regional state, and Dire Dawa as shown in Table-4. The survey was carried out in four (4) Zones, twenty one (21) districts/kebeles and one hundred forty three sites. From each zone, survey fields were selected after categorizing the producers based on the size of the plantation. From each surveyed area five to ten small-scale farmers were selected. Accordingly the following number of small scale farmers were selected, namely: Gumbi Bordode (5), Miheso (4), Chiro (6), Tulo (6), Doba (2), Goro Gutu (8), Meta (5), Kurfa Chele (4), Gurawa (4), Kombolcha (6), Haramaya district including Haramaya University (56), Kersa or Adele (5), Dire Dawa town (8), Erere Woldiya (3), Sofi (7) and Harari regional state town (7). Five plants were taken from each small scale farmer's site to assess distribution and severity of damage due to AfCP. Twenty leaves sample was collected near to middle canopy of each Casimir tree purposely from four cardinal directions (North, South, West, and East).

Data collection included field visit at Haramaya University and its surrounding for physical observation on *C. edulis* farms, discussion with men, women, children (boys and girls), and key informant interview with relevant government officials and staff and other knowledge rich individuals. Accordingly, a total of 40 participants (12 males and 10 females), 10 children (5 boys and 5 girls) and eight agricultural officers participated in key informant interview in the area.

The identification was done based on macro and micro growth and morphological characters of the insect using identification guideline for an insect. Relative frequencies were computed as follows:

Relative frequencies of AfCP **occurrence = Number of** Af CP **recorded per Casimir site/Total number of** AfCP **recorded from the survey site**.

Table 1 Severity status scale developed by Kataria and Kumar, 2012

Relative frequency of AfCP occurrence	Severity	Grades of severity status
0	0	No infestation

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1-5	1	low infestation
6-10	2	High infestation
≥ 11	3	Very high infestation

Source: Kataria and Kumar. 2012.

Geographic data (Longitude, Latitude, and Altitude) of each sampling site were recorded by the use of GPS. Sampled leaves were transported to Haramaya University plant protection laboratory for further quantification of the insect life stage and each leaf was examined under a stereomicroscope for counting the immature (egg, larvae/nymphs, and adult). ArcGIS 10.3 was used for spatial data management and Mapping of AfCP distribution. Relative frequencies of African citrus psyllids occurrence at each site were calculated by the use of formula adopted from Kataria and Kumar (2012). The value obtained was used to define severity index from which severity status at each site was determined, as follows. Microsoft office excel were used for the data organization. SAS 9.0 software package was used for population variation between surveyed districts.

Identification

After laboratory diagnosis and using internet resource it was confirmed that the observed pest is the African citrus psyllids, Trioza erytreae, which is currently devastating Casimir trees in Ethiopia (Figure 5&6).

III. RESULT AND DISCUSSION

5.1. Distribution and Severity of AfCP in south western part of Ethiopia

The present survey revealed that there was an irregular pattern of African citrus psyllids distribution and severity in the eastern part of Ethiopia. A total of 3 regions (Oromiya, Harari and Dire Dawa) were surveyed and a total of 143 plantations of Casimir were assessed for the presence or absence of African citrus psyllids during the survey. This pest was recorded causing various degree of severity in all surveyed area (Table 2, 4 and Fig-1). Except for Dire Dawa, all surveyed areas showed low to very high AfCP infestation (Table 1 and 4). During the survey AfCP free casimir trees were also recorded at Dire Dawa (Table 3). There is significant difference in AfCP population density among the surveyed areas. The high population density of AfCP in these Zones resulted in high to very high damage severity on Casimir trees in these particular areas (Table. 3). Microscopic observation of leaves collected from surveyed area showed significant difference in egg, nymph, and adult of African citrus psyllids (Fig 2, 3 and 4).

The severely damaged Casimir by psyllid adults and nymphs feeding caused the newly forming leaves to twist and curl which was similar to the feeding damage by green aphid (Fig. 2). Psyllid feeding also results in the reduction of shoot length giving a witches' broom effect (Fig. 3). Population density variation recorded in these areas were probably caused by varietal difference, ecological variation, farmer cultural practice, elevation, Casimir trees population scattered over surveyed area and time of arrival of AfCP to the area (Fig.2, 3 & 4). During the survey, it was also observed that citrus and Ficus spp. (*Yeshola Zaf*) infested with AfCP at Harari regional state and Haramaya district.

These findings are in line with that of Halbert & Manjunath., (2004); Flores *et al*. (2009) who indicated that Asian citrus psyllid, *Diaphorina citri* and is an important pest of citrus because it transmits phloem-limited bacteria (citrus greening disease), currently considered the world's most serious disease of citrus were found at Florida citrus plantation fields.

2.2. Agricultural experts, Farmers' and youngster perceptions about the pest

As far as the current distribution of the insect is concerned, all the Casimir growing kebeles in the surveyed areas were already infested, according to the discussants participated in the assessment. The discussants in Haramaya disclosed that they observed the infestation of their Casimir trees for the first time in 2015 and 2016. They also said that they had never ever seen such kind of problem in their Casimir farm and considered it as a new phenomenon for the people in the area. But they did not know whether the problem was caused by insect pest or disease. They commonly called it "*Besheta*" because of its fast expansion and deadly effect on Casimir trees. As per the discussant farmers, the insect was less in number during the rainy period. It limited itself to some part of the tree like leaves and lower part of the stem. However, the pest started to increase in population and found on every leaf of Casimir trees at the end of the rainy period. Following the vegetative/leaves regenerated the insect gradually distributed to the top of the trees during the flowering stage and present in almost every part of the tree. This helped the insect to easily infest and attack the fruit crop at maturity.

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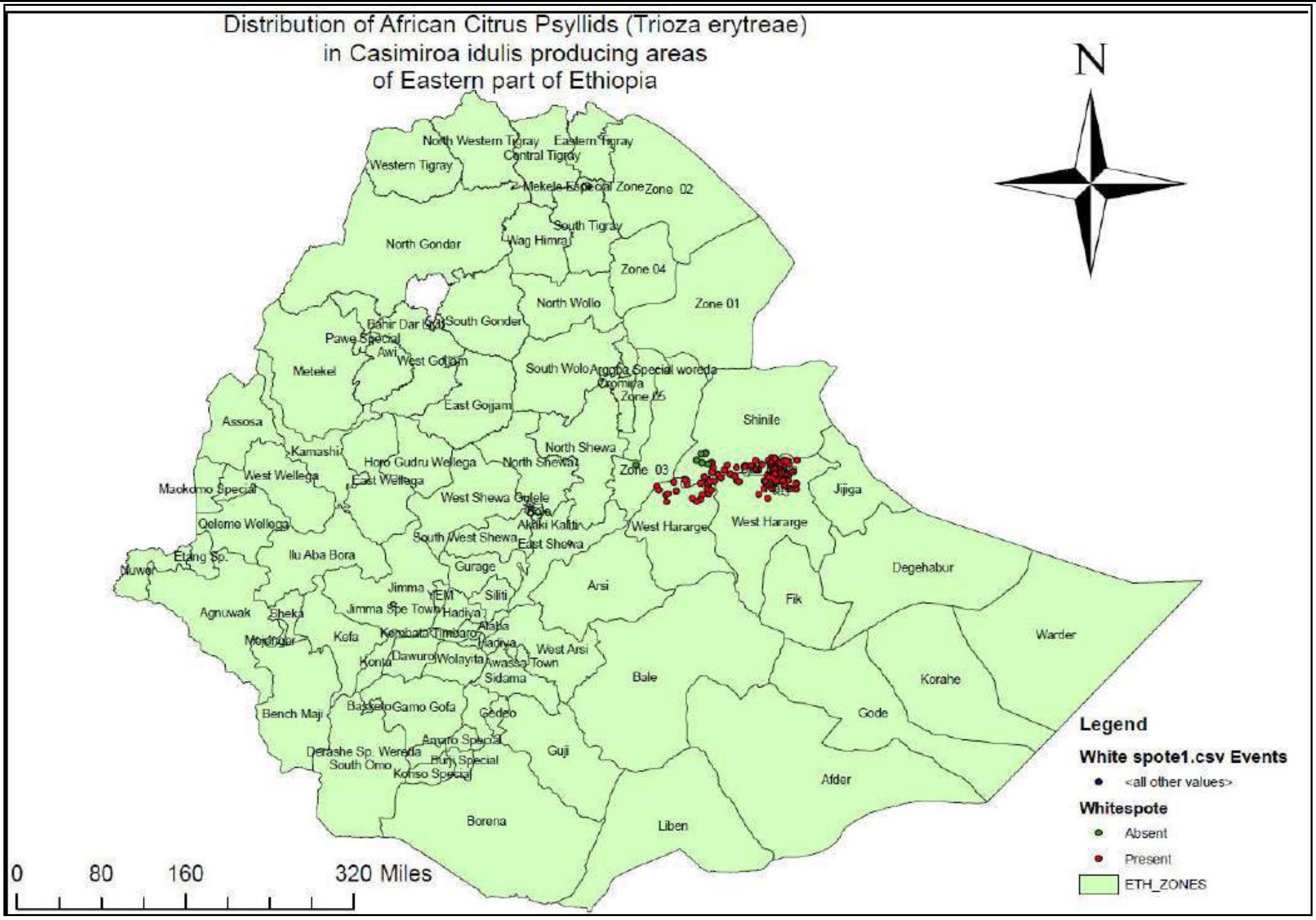

Fig.1: Map shows distribution of African citrus psyllids on Casimir producing areas in Eastern part of Ethiopia during 2018 cropping season

Region	Zones	District	Eggs	Nymphs	Adult Females & Males
Oromia	Eastern Hararghe	Haramaya University	276.8CD	599.8BAC	7.523DBC
„	Eastern Hararghe	Gend Boyi	424.3CB	968.8 A	11.195 B
„	Eastern Hararghe	Finkile	138.3FED	265.3BC	0.612 E
„	Eastern Hararghe	Tinike	236.8CED	492.0BAC	0.612 E
„	Eastern Hararghe	Bate	531.0 B	1051.3 A	10.590 B
„	Eastern Hararghe	Haramaya Town	343.0CBD	805.3BA	8.825CB
„	Eastern Hararghe	Keresa/Adele	414.0CB	1002.0 A	8.613CB
Dire Dawa	Dire Dawa	Dire Dawa	0.0 F	0.0 C	0.030 E
Harari	Harari reginal state	Yerer Weldiya	62.5FE	52.8 C	4.765CED
Harari	Harari reginal state	Sofi	402.8CB	464.5BAC	8.363CBD
Harari	Harari reginal state	Harar Town	1047.5 A	1053.3 A	16.508 A
		CV (%)	40.92023	71.96	46.16
		LSD (0.05)	28.27	38.21	4.887
		F Value	12.33	2.47	6.48
		P Value	<.0001	0.0201	<.0001

Means followed by the same letters within columns are not significantly different at P<.0.05 level of probability

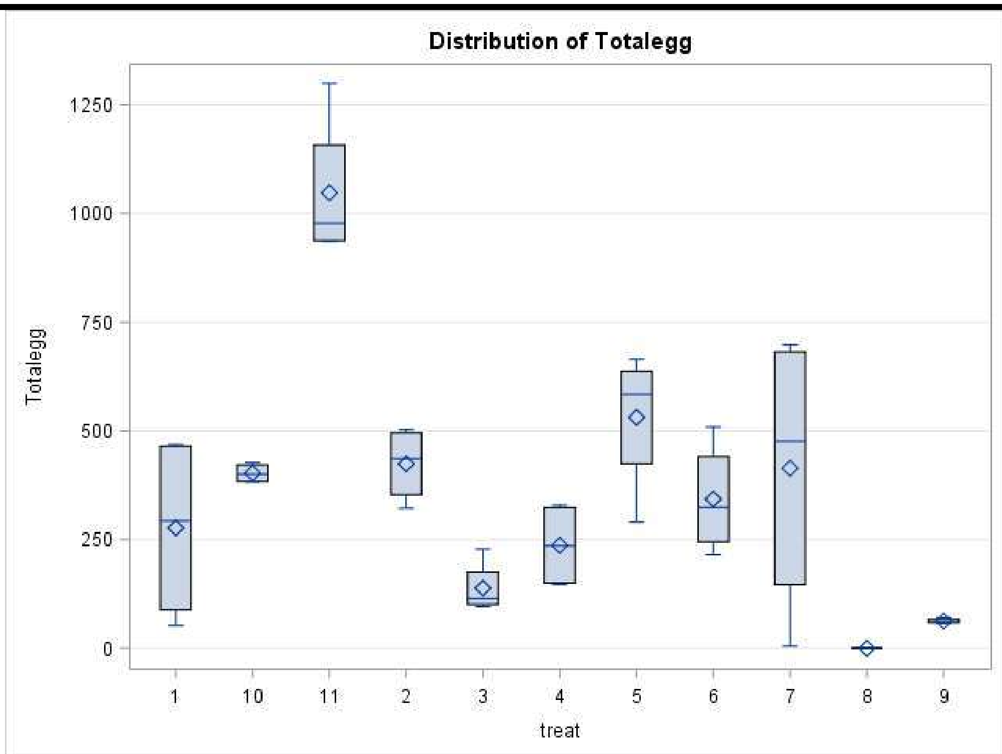

Fig.2: Distribution of total egg of African citrus psyllids, Trioza erytreae over surveyed areas.

NB: Treat represents districts or place of the survey and 1- refer Haramaya University, 2 - Gend Boyi, 3 - Finkile, 4 - Tinike, 5- Bate, 6- Haramaya Town, 7- Keresa, 8- Dire Dawa, 9- Yerer Weldiya, 10 - Sofi, 11- Harar Town

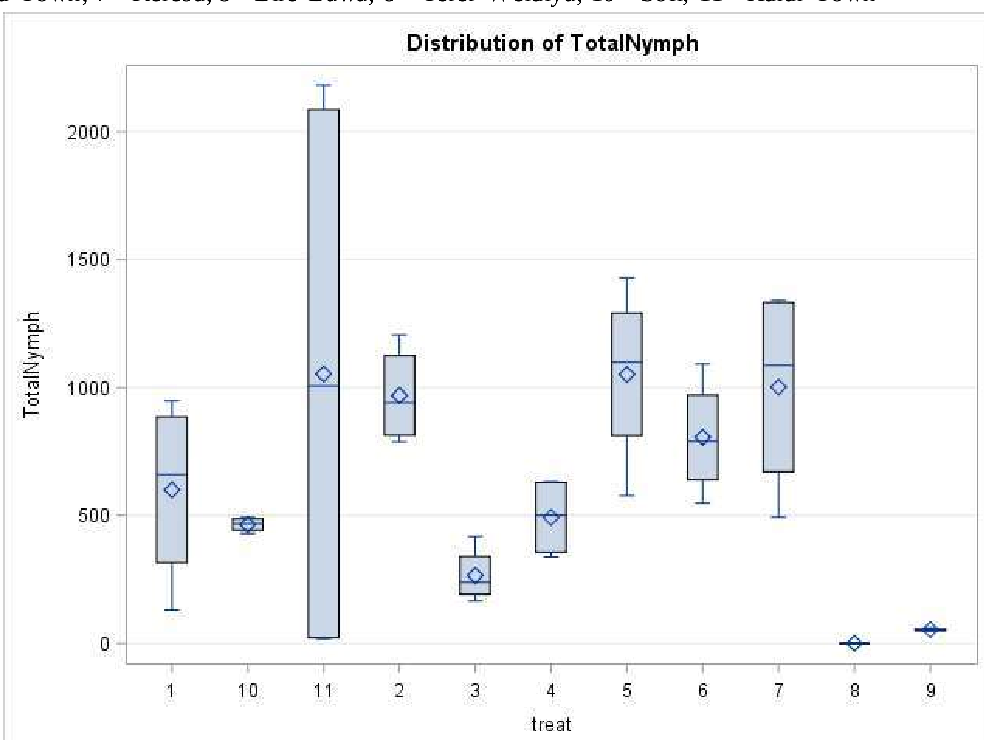

Fig.3: Distribution of total Nymph of African citrus psyllids, Trioza erytreae over surveyed areas.

NB: Treat represents districts or place of the survey and 1 refer Haramaya University, 2 Gend Boyi, 3 Finkile, 4 Tinike, 5 Bate, 6 Haramaya Town, 7 Keresa, 8 Dire Dawa, 9 Yerer Weldiya, 10 Sofi, 11 Harar Town

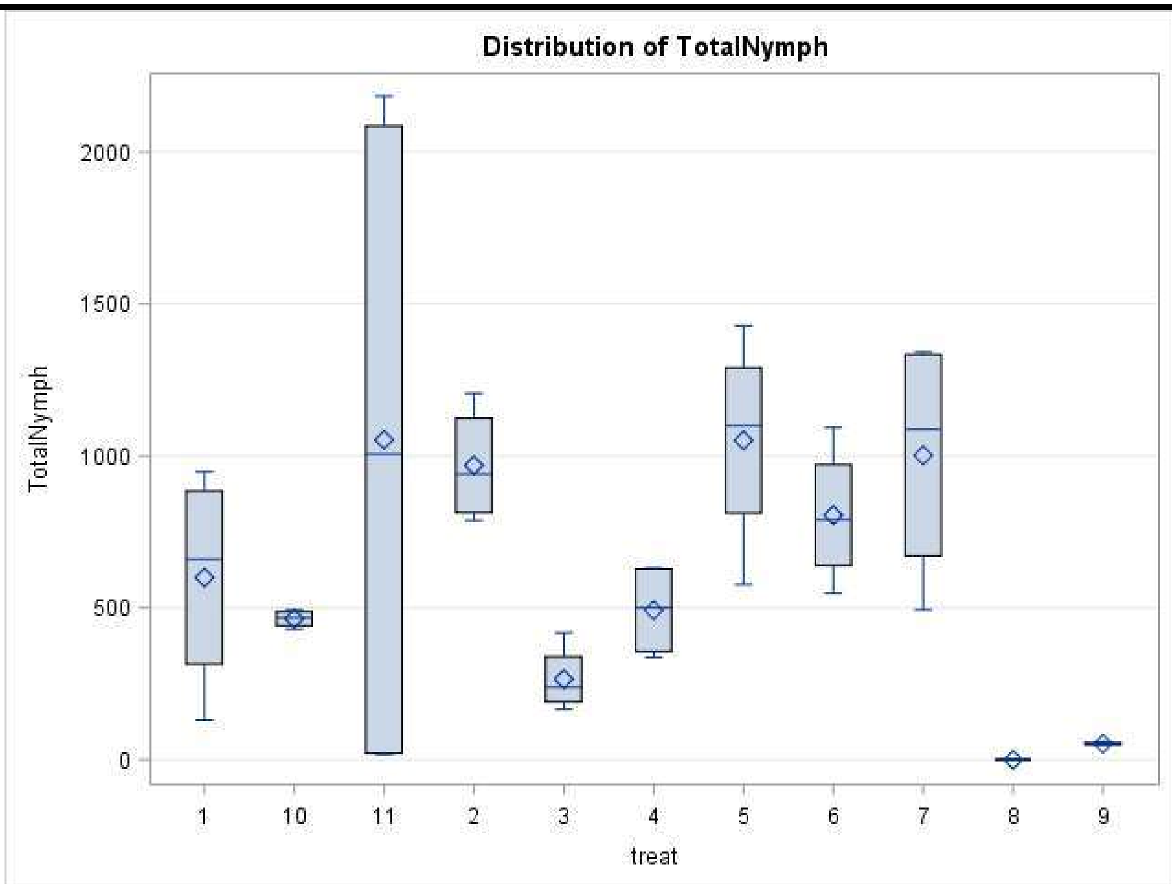

Fig.4: Distribution of total Adult of African citrus psyllids, Trioza erytreae over surveyed areas.

NB: Treat represents districts or place of the survey and 1 - refer Haramaya University, 2- Gend Boyi, 3- Finkile, 4 - Tinike, 5- Bate, 6- Haramaya Town, 7- Keresa, 8- Dire Dawa, 9- Yerer Weldiya, 10- Sofi, 11- Harar Town

Table 2 Severity status of African citrus psyllids in eastern part of Ethiopia during 2018 cropping seasons

Region	Zone	District or Kebele	Severity index	Severity status	Number of field observed
Oromia	East Harerege	Gumbi Bordede	3	Very high infestation	5
,,	East Harerege	Miheso	3	Very high infestation	4
,,	East Harerege	Chiro	3	Very high infestation	6
,,	East Harerege	Tulo	2	High infestation	6
,,	East Harerege	Doba	2	High infestation	2
,,	East Harerege	Goro Gutu	2	High infestation	8
,,	East Harerege	Meta	3	Very high infestation	5
,,	East Harerege	Kurfa Chele	1	Low infestation	4
,,	East Harerege	Gurawa	1	Low infestation	4
,,	East Harerege	Kombolcha	3	Very high infestation	6
,,	East Harerege	Haramaya University	3	Very high infestation	15
,,	East Harerege	Gand Boyi	3	Very high infestation	10
,,	East Harerege	Finkle	3	Very high infestation	5
,,	East Harerege	Tinike	3	Very high infestation	4
,,	East Harerege	Bate	3	Very high infestation	5
,,	East Harerege	Haramaya	3	Very high infestation	15

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,,	East Harerege	Kersa	3	Very high infestation	9
Dire Dawa	Dire Dawa	Dire Dawa Town	0	No infestation	8
Harari	Harari reginal state	Erere Weldiya	1	Low infestation	3
,,	Harari reginal state	Sofi	1	Low infestation	7
,,	Harari reginal state	Harari reginal state Town	2	High infestation	7

Fig.5: African citrus psyllids infesting casimir leaves in Eastern part of Ethiopia

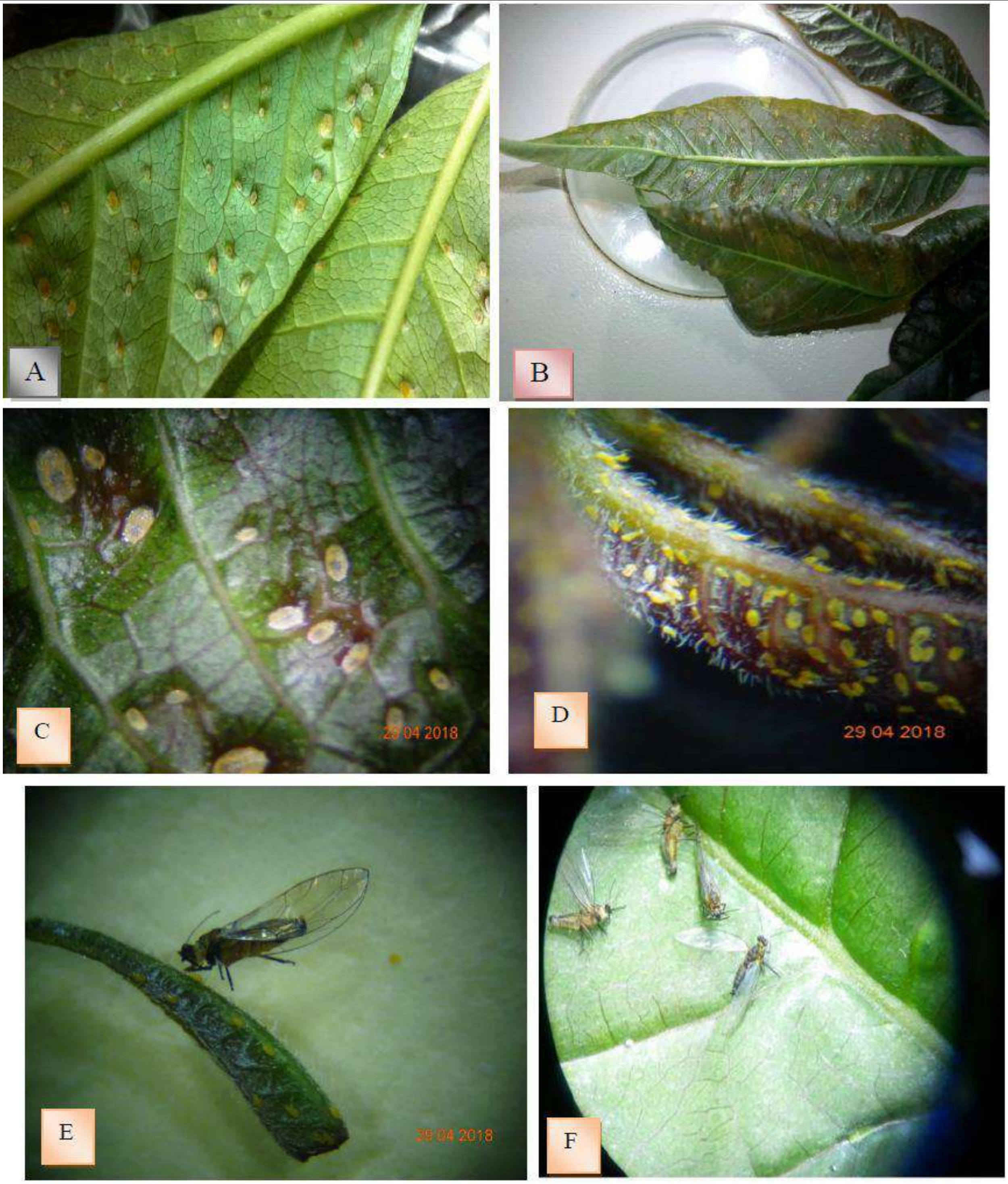

Fig.6: The microscopic features of T. erytreae on casimir tree leaves: A and B lower face of an infested leaf, with nymphs settled at each gall, C, fourth and fifth instars nymph, D, eggs, E and D, adult male and female.

IV. SUMMARY AND CONCLUSION

The current survey showed the distribution of AfCP in eastern part of Ethiopia and considerably threatened casimir tree production and productivity. No AfCP was observed on Casimir at 8 different areas of Dire Dawa zone. On other areas West Hararghe (Gumbi Bordede, Miheso, Chiro, Tulo and Doba), *Eastern Hararghe (Haramaya University, Gand Boyi, Finkile, Tinike, Bate, Haramaya Town, and Keresa,*

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Meta, Kurfa Chele, Gurawa and Kombolcha) and Harari regional state zone (Erere Wolediya, Sofi, Harar town), mild to very high infestation of AfCP was observed.

Studies conducted in different countries on this insect showed that in addition to direct damage, the insect is the vector for citrus greening pathogen but was not given attention in Ethiopia. Research centers, Agricultural experts and plant health clinics, require awareness on this newly attaining pest status in Ethiopia. There is no adequate information regarding this important pest in Ethiopia and requires studies on further distribution, pest status, and management options. Awareness creation and provision of training for Casimir growers and development agents is critical to overcome the negative effects of the pest. Different techniques including natural enemies of the pest require research for the effective management of *T. erytreae*.

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Pollution Caused by Humans: A Curse on Animals

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***Abstract*— *The study explores the pervasiveness and causes of Pollution causing detrimental impact on health conditions of animals finally leading to increase in the death ratio of animals in the country. Pollution can be of various types such as Air pollution, Noise Pollution, Water Pollution and so on. These types of Pollution are caused due to the release of Hazardous Chemicals in our environment and by the improper activities done by the human beings in the society. On the contrary a complete on Pollution within the country would not be possible, but a conscious effort needs to be there to save our Country and its human beings and animal species. Already there has been a huge increase in the death Ratio of animals in the country from past decades. There are various ways in which Pollution within the Country can be managed and reduced such as walking smaller distances than using vehicles causing pollution, not throwing garbage particles in the water bodies, eating plant based diet and not slaughtering animals(which is major cause of pollution in environment). This research paper tends to strike the drastic damage caused to life of animals due to pollution by taking some universal essentials and also show their preclusion.*

Keywords*— *Hazardous Chemicals, Increase Death Rate, Animal Slaughtering, Damage.

RESEARCH QUESTIONS

1. How far is the pollution causing detrimental impact on health of Animals?
2. What is the effect on animals in present and what will it be in the future due to pollution?

RESEARCH METHODOLOGY

The researcher in the following research paper has adopted the Doctrinal Research Method. The researchers have collected the primary sources from the various policies and acts prevailing in the country and the secondary sources are from the various books, articles, documentaries and decided case laws using library and net sources.

INTRODUCTION

The protection of environment is a global issue which is not an isolated problem of a particular area or a nation, rather is a problem that prevails everywhere. The problem of pollution in the environment is one the increasing concerns among all the countries irrespective of their size or ideology. This problem doesn't depend on the level of development in the country and also this problem is not new in its origin.

Environment in the world is one of the most important and the beautiful aspect. As per Section 2(a) of the Environment Protection act, 1986 our Environment is comprising of interrelationship and the interdependency between land, air and water with the human beings, plants and Animal Creatures (land and aquatic animals).

Our Environment is a complex system as it is the combination of both physical as well as chemical factors. The physical factors comprise of land, air and water that provide the basic means for sustainability in the country and on the other hand chemical factors are the gases that are being released in the environment.

Unfortunately in this beautiful aspect of the world a lot of pollution is being caused by the human beings. Pollution is being defined in Section 2(c) of the Environment Protection Act, 1986. According to this section environment pollution means the presence in the environment of various environmental pollutants.

This diagram (figure 1) denotes various types of environmental pollutants as per Section 2(b) of the Environment Protection Act, 1986 that tend to be injurious to the environment. Pollution in the environment is causing a lot of damages and imbalance in the society which is ultimately causing a detrimental effect on the living conditions of animals in the society.

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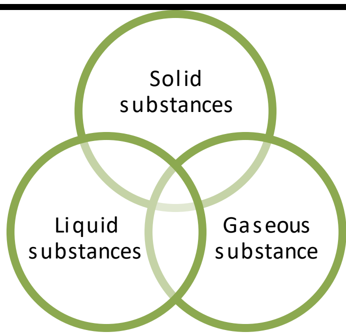

Fig.1: Various forms of Pollutants in the environment as per Environment Protection Act,1986.

Pollution in the environment is caused due to various factors and also by the day to day activities of human beings in the country. On contrary these day to day activities of the human beings are important as there these activities are helping them to earn their livelihood and is also causing an increase in the GDP of the Economy, so a complete on these activities would not be possible. Due to these activities in the economy there is a lot of contradiction in the environment which is further reminding the authors about the conflicting approach being discussed under the Stockholm declaration.

According to the Stockholm declaration approach there must be proper harmony between the sustainability and the development in the environment. In the modern era there is no proper harmony being done to the environment. People are only being concerned about benefiting their own lives and are least bothered about the conditions of others. People in today's world are only looking towards the merits and the opportunities they can prevail for themselves and fail to look towards the harm they have been causing towards the Animals.

This negligence in the society is causing a detrimental impact on the living conditions of the Animals in the present and if continues will also cause impact in future.

CAUSES OF POLLUTION

Some of the major causes of environmental pollution are as follows:

- Vehicles used to ply from one place to another.
- Industrial activities being conducted by the humans for the welfare of the humans in the locality.
- Slaughtering of animals because when the animals are being slaughtered then there is huge amount of methane gas being released in the environment causing pollution and due to this pollution health condition of animals are getting spoiled and animals becoming endangered. According to the documentary film by *Louie Psihoyos (Oscar Wining Director)* methane gas released in the environment by slaughtering is 86 times more destructive than the Carbon Dioxide and Nitrogen gas being released from the vehicles.

EFFECTS OF POLLUTION ON ANIMALS – AN OVERVIEW

Pollution in the environment has been causing a great effect on the living as well as the health conditions of the animals. Authors herein have tried to bring a light on the same through various reports and submissions.

- According to the report in 2017 by *Sciencing*, it has been accessed that many species have been experiencing huge pollution events till date which eventually has been causing them death or a threat towards their habitat.

  This report also shows that there are huge oil spills in the water bodies by the human beings which has been causing death toll to the animals and species residing in the oceans. According to the report more than 1,00,000 sea birds have died till date and it has not stopped. Also there have been more than 144 bald eagles that have also died till date.

Fig.2: Animals beings effected because of chemicals and oil spills in water bodies

- According to the report by *WWF*, the increased amount of pesticide spray being done on the crops by the farmers to control the pesticides has been causing mutation and fertility problems in the animals. This report also shows that these chemicals can also be found in the blood of the native animals residing in the nearby areas. Also there has been a report of *Sciencing* in 2017 which shows that the synthetic chemicals being used to control pesticides

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are highly destructive to the animals. These chemicals are causing a reproductive system failure and also neurological effects on the animals in the environment.

- Not only is this but there are times when we ourselves have witnessed that the animals are dying due to the waste we humans have been throwing around in the locality. There are various plastic bottles and jars thrown around which gets attached to the animals causing deep cut on the body of animals finally leading to the death of the animals in the environment. It is mainly because of the uncompleted food being packed in the plastic bottles thrown up randomly attracting these animals towards those food bottles and containers causing them injuries.

Fig.3: Shows animal getting trapped in the garbage thrown by humans

- According to the WWF report there are a lot of waste particles that are thrown into the water bodies by humans that it has become so much polluted that living conditions for animals have become difficult. According to the report around 2.4 billion people along with the aquatic animals have been suffering.

Fig.4: Depicts most of the garbage in water bodies is by plastic waste Locality

- Not only this rather there is a report by *One Green Planet*, which shows that 90% of the garbage in the water bodies is by the plastic being thrown in the locality. This report says the plastic does not decompose entirely and the particles break into small microscopic plastic this has been causing a detrimental impact on the marine wildlife. Plastic in the water gets stuck around the animal's body causing them suffocation, dehydration, starvation eventually leading towards death.

These reports tend to bring to the notice need and importance of reducing pollution in the environment in order to safeguard our society and the animals residing. It is a high time that we showcase our responsibilities towards the protection of our environment. Without animals in the environment it would be difficult for us also to stay alive as we are all dependent on each other. If not for the sake of animals then at least for our own sake we should try to avoid polluting the environment.

LEGAL REMEDIES AVAILABLE TO ANIMALS IN INDIA

India is having amazing laws and principles which are legally bound to be followed. These laws and principles are to be followed in order to prevent our environment and animals living in the environment. Some of these laws and principle are as follows:

- First and the foremost remedy available for the protection is Article 51A (g) of the Constitution of India. According to this article we the people of India should be compassionate towards the animals living in the Environment. This remedy has been interpreted by the authors because as human beings we should not indulge in the activities that use plastics because usage of plastic is causing a detrimental impact on animals. If we human beings are using plastic then we are not being compassionate towards the animals and are also causing violation of our fundamental duties towards the animals.
- Secondly as per Section 428 and section 429 of the IPC 1860, it is a criminal offence to kill or to cause injury to any of the animals residing in the environment (includes stray animals).
- Thirdly as per the PCA 1960, if there is any form of sudden abandoning of any animal then as per Section 11j (i) of the act there can be an imprisonment for a period of three Months.

- There are principles as well in the society that acts as a form of remedy to prevent animals and the environment. One such principle is Polluter's Pay Principle. According to the above mentioned principle polluters have to bear up the entire cost of causing pollution in the environment. This principle helps to collect the funds in order to protect the environment also this principle in same way or the other helps to reduce pollution which is leading to a form of protection for the animals in the country. The concept of this principle was best laid down in Shri Ram Gas Leak Case in 1987.
- Also if there is no sufficiency of food being provided to the animals we are keeping as pets in our homes then also as per the PCA 1960 there can be an imprisonment for three months.

The authors have figured out that there is a gap in the Legal System of the country. There are remedies that are being available for the protection of animals in the country, but since there is inadequacy of laws thus human activities that are leading to direct/ indirect death of animals are not getting penalized.

Thus there should be petitions filed for the protection of Animals by proper monitoring of the activities also various other governmental activities should take place in order to maintain proper harmony between the development and environmental sustainability.

RECOMMENDATIONS

The authors have discussed how various kinds of pollution in the Environment have become a curse of the lives of animals. If this form of pollution in the environment continues to prevail then that day is not far when the existence of animals would be rare. Already many of the animals have extinct from the country and many are on the verge of getting extinct from the country. Thus in order to avoid such an incidence in the country authors has tried to mention few of the recommendations to protect the animals in the environment. Some of the recommendations are as follows:

- We all should try to be self- conscious and should try to prevent ourselves from using harmful substances in the environment that are causing effect on the living conditions.
- Try using eco friendly products in the environment.
- Plastic recycle bins should be placed in the locality so that plastic bottles and cans are not littered here and there rather those plastic products are recycled immediately. This action would prevent animals from getting injuries from plastic cans and bottles.
- There should be replacement of plastic bags with the handmade paper bags and these bags should also be reused and recycled.
- Try to promote sustainable living by educating and conducting workshops and seminars in schools and also in other locality areas.
- There should be appointment of staff members by the municipality and the government for the regular cleaning up of litter from land as well as from the water bodies. There should also be an appointment of other staff members to look after the activities of the current staff members.
- Save energy when not in use as it will reduce the amount of particle emission in the environment and animals could breathe in a better air.
- Try to make sure that the gas stoves are well ventilated so that there is no much smoke in the environment and the throat of the animals would also not be chocked.
- Proper waste management system should be there in the locality in order to manage the waste production in the locality. Also monitoring of waste in industrial sector to be done to avoid causing harm to aquatic animals.
- There should be proper and effective replacements for the harmful chemicals and substances in the environment.

CONCLUSION

Pollution in the environment is causing a detrimental impact on the living condition of the animals in the country. Though pollution in the country is having an immense impact on the environment but a complete on this pollution in the country is also not possible. There are human activities in the locality which even after various preventive measures are causing an indirect impact on the environment. There are various principles and laws in the country that are working to bring peace, sustainability and development in the country, but these principles and laws alone are not sufficient to overcome the prevailing situation of the country until and unless the huge mass doesn't join hands together.

Even in the modern era of today where there is proper well developed and well organized technological sector there still prevails a sector where people are not aware of the fact that their day to day activities are causing a degradation in the environment and instead of understanding their responsibilities towards the environment they are more concerned about their own living conditions and their own welfare. People in today's era fail to understand that environment in which they are living is not their birth property rather they are sharing it with many others as well so it becomes their duty to protect them as well. People's activities in order to gain more comfort is not only causing an impact on living conditions of animals but is somewhere or the other also causing an impact on their own living conditions.

It's never too late; hence we should all try to find a permanent replacement to such a problem, Thus in order to fix the prevailing condition of the country and to bring peace, harmony and sustainability strong preventive measures to be taken up by the government and also by the people themselves.

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Risk Assessment of Heavy Metals Level in soil and Jute Leaves (*Corchorus olitorius*) Treated with Azadirachtin Neem seed Solution and Organochlorine Pesticides

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Abstract*—Synthetic agrochemicals are increasingly being relied upon as the easiest of way eliminating pests on our farm. However, synthetic chemical increases heavy metals in the soil, which is then likely transferred to plants that grow on such soils, with the associated risks of long term toxicity to humans that consume them and other biota in the ecosystem. Nonetheless, some plants like the neem plant have been reported to contain components that are natural pesticides. This study was therefore to determine the comparative presence and concentration of some heavy metals in Jute leaves (Corchorus olitorius) treated with Azadirachtin Neem Seed Solution (ANSS) and Organochlorine Dichloro-diphenyl-trichloroethane (DDT) pesticides and the human health risk associated with their consumption. Jute plant treated with ANSS and DDT and their corresponding soils were collected in triplicate from 6 pots and a control without treatment using same soil and seed. Physicochemical properties of the soil samples were determined using a standard methods. The concentrations of Pb, As, Cd, Cr and Cu in the soil before and after planting and in the leaves were determined using Atomic Absorption Spectrophotometry. The potential health risk from the consumption of these vegetables was assessed using standard methods. Results obtained showed the presence of heavy metals (Pb, As, Cd, Cr and Cu) in Corchorus olitorius leaves and soils treated with each pesticide. Treatment with DDT pesticide elicited higher ($P<0.05$) heavy metals concentrations in the soil and vegetable compare to ANSS biopesticide treatment. The concentrations of Pb, As, Cd, Cr and Cu were 1.41, 2.06, 1.04, 1.85 and 3.78mg/kg respectively in Corchorus olitorius treated with DDT exceeded the WHO/FAO permissible limit of (0.3, 0.5, 0.2, 0.3, and 3.0mg/kg respectively) for edible vegetable. The Hazard Index (HI) of heavy metal contamination in the vegetables treated with ANSS and DDT was less than 1 suggesting it is safe for consumption, however the result shows that children are at greater risk from continuous consumption of Corchorus olitorius treated with DDT pesticide. The study concludes that the concentration of heavy metals in Corchorus olitorius treated with DDT pesticide exceeded the WHO/FAO permissible limits in vegetable. This showed that consumption of these vegetables treated with synthetic pesticide could pose health risk from heavy metal contamination.*

***Keywords*— Azadirachtin, Corchorus olitorius, Dichlorodiphenyltrichloroethane, Health risk, Heavy metal.**

I. INTRODUCTION

In Nigeria, the loss of crops due to pest, plant disease and competition from weeds is quite very high. To avert these losses, farmers now increasingly deploy pesticides in their agronomic practices. Pesticides are chemical substances used in agricultural practices to aid the production and yieldby repelling, preventing, and destroying pests. Agrochemicals especially herbicides, insecticides and fungicide have resulted in the increase in crop production to a large extent, but their residual concentrations in the soil and potential environmental hazards in the ecosystem is of great concern. Pesticides produced to kill these pests in order to prevent these damages, especially those produced from synthetic materials also tend to have adverse effects on humans and environment in various ways (Yuguda *et al*., 2015).The continuous application of synthetic pesticides in agriculture over the years has caused accumulation of pesticidal residues such as heavy metals in the environment leading to various chronic illnesses and toxicity to non-target organisms.

Thus heavy metals from agrochemicals particularly pesticides not only contaminate soil and affect food quality butare subsequently transferred to human through the food chain (Olowoyo *et al*., 2011; Mutune *et al*., 2012) and could be responsible for series of health ailments in humans. Ninety percent of the applied synthetic pesticides like Dichlorodiphenyltrichloroethane (DDT) enter the various environmental resources as a result of run-off, exposing the farmers as well as consumers of the agricultural produce to severe health issues and sometimes take decades for some of these chemicals to break down. DDT affects the nervous system by interfering with normal nerve impulses. DDT causes the nerve cells to repeatedly generate an impulse which accounts for the repetitive body tremors seen in exposed animals (Rayindran *et al*., 2016). Therefore, increase attention has been given toward the development of alternate environmentally friendly pesticides that would aid an efficient pest management system and also prevent chronic health challenges.

One of the significant alternative strategies is, the use of neem plants (Binomial name: *Azadirachta indica*) (Chaudhary *et al*., 2017).The neem plant is a bitter tonic herb with anti-cancer properties, reduces inflammation and remove toxins, while promoting healing and improving all body functions. Apart from this, it has parasitic, insecticidal spermicidal properties and hence destroys a wide range of organisms such as pest (Kumar *et al*., 2015). It has been observed that the various medicinal values of neem are its constituent phytochemicals Present (Kwasi *et al*., 2011).The most active complex secondary metabolite present in the neem seed is Azadirachtin, which has been established as a pivotal insecticidal ingredient. It acts as an antifeedant, repellent, and repugnant agent and induces sterility in insects by preventing oviposition and interrupting sperm production in males. Azadirachtin has two profound effects on insects. At the physiological level, azadirachtin blocks the synthesis and release of molting hormones (ecdysteroids) from the prothoracic gland, leading to incomplete ecdysis in immature insects. In adult female insects, a similar mechanism of action leads to sterility. In addition, azadirachtin is a potent antifeedant to many insects. Neem as a repellent, prevents insects from initiating feeding. As a feeding deterrent, it causes insects to stop feeding (Rhoda *et al*., 2006).

Jute leaves (*Corchorus olitorius*) also known as ayoyo in Hausa, ewedu in Yoruba and kerenkeren in Igbo is a leafy vegetables mostly consumed by the Yoruba's in the western region of Nigeria where it is an important components of daily diets. Leafy-vegetables contain protein, essential minerals, fiber, vitamins, carotene and some essential amino acids required for normal metabolic activities of the body (Mavengahama, 2013; Conrad *et al*., 2018). These nutrients help to repair worn out tissues, reduce cancer risks, lower cholesterol levels, normalize digestion time, improve vision, fight free radicals, and boost immune system activity. The vegetables also act as antioxidants that help to protect human body from oxidant stress, cardiovascular diseases and cancers (Santhakumar *et al*., 2018).

Vegetables in developing countries are contaminated with high doses of pesticides. This can pose serious health risk to millions of individuals that consume them. These pesticides have heavy metals such Zinc (Zn), Cadmium (Cd), Lead (Pb), Copper (Cu), Cromium (Cr), Arsenic (As) etc. as their constituents. A lot of new pesticides whose actual chemistry are not known are continually infiltrating the markets and from all indication, no screening of these agrochemicals was conducted to permit entry into the country and the potential hazards should be of great concern. The result of this is the high accumulation residues of these agrochemicals in the environment with their consequent risk hazards (Liu *et al*., 2013) on human. Soil is the main reservoir of heavy metal and is the main source of pollution to the ecosystem at large. There are several pathways by which humans could be exposed to heavy metals contaminations. These could be through direct ingestion of the vegetables (food chain), direct ingestion of soil particles, dermal contact with soil particles, inhalation of soil dust and other particles from the air, oral and or dermal intake from groundwater.

Recent studies have shown that heavy metals contamination of food crops and its resultant health consequences is becoming a global issue (Shah *et al*., 2013; Nazir *et al*., 2015; Fonge *et al*., 2017). There is paucity of information on the heavy metals contents of vegetables from agrochemicals and their health risks especially in Nigeria that produce them. Risk assessment is an effective scientific tool which enables decision makers to manage and control sites so contaminated in a cost-effective manner while

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preserving public and ecosystem health. Hence, this study was designed with the aim of determining heavy metals (As, Cd, Cr, Cu and Pb) contents in soil and *Corchorus olitorius* vegetable treated with two pesticides Azadirachtin neem seed solution and Dichloro-diphenyl-trichlororthane and the health risk associated with them.

II. MATERIALS AND METHODS

2.1 Sample collection

The research was conducted in the University of Nigeria, Nsukka. The top soil sample used was collected from Agricultural garden, University of Nigeria, Nsukka, using disinfected hand shovel from 6 inches (i.e. 15cm) below the surface. The soil was bulked, mixed thoroughly, air-dried at room temperature (20^{o}C) for seven (7) days. This was done to halt all the microbial activities in the soil. The air-dried samples were sieved using a 2mm sieve mesh size and also handpicked to remove debris and stones. The soil air-dried and sieved samples were analyzed for various soil physicochemical parameters before and after planting.

2.2 Experimental Design

The soil was divided into three groups and each group had triplicate bags. Group one were not treated with any pesticide, and served as control, group two were treated with ANSS biopesticide and group three were treated with DDT pesticides. Each pot contains 2kg of soil in a perforated polythene bag. Seven *Corchorus oliforius* (CO) seeds were planted in each bag. After two weeks of planting, the pesticides DDT and ANSS were sprayed on the test vegetables once every week for another five weeks to control pest except on the control groups. And the number of spot on *Corchorus olitorius* leaves damage by pest was observed by circling the damaged spot with a permanent marker

2.3 Preparation of ANSS and DDT

The seeds of neem plant Azadirachtin kernels (the seed of which the seed coat has been removed) were collected from agricultural garden and dried at 27^{o}C room temperature, then pulverized with a blender to obtain a homogeneous mixture. The pulverized seed (30g) was mixed in 1 litre of distilled water and left overnight in 2 litre cornical flask. The next morning, the solution was filtered through a fine cloth and used immediately for spraying. The same process was repeated for other week's application. Following the DDT pesticide label 2g of powdered chemical was added to 2liter of water. All necessary precaution was taken while mixing.

2.4 Determination of the Physico-Chemical Parameters

The soil pH samples were measured by potentiometric meter using a digital pH meter (Systronics type LI-101 ELICO). Soil samples (10 g) were stirred with 100ml of distilled water with a glass rod and the pH of the suspension was recorded. Physicochemical parameters of the soil before and after treatment were determined according to (Nimyel *et al*., 2015).The physicochemical parameters measuredin all the four groups were soil texture, pH, total organic carbon, organic matter, total nitrogen, total phosphorus, exchangeable cation (sodium ion, magnessium and potassium ion). The Physico-chemical properties of the soil were analysed in order to check the biodegradable process.

2.5 Determination of Heavy metal

Samples of both soils and vegetables (1.00 ± 0.1g each) were placed into 100ml beakers separately, to which 15ml of tri-acid mixture (70% high purity HNO_3, 65%, $HClO_4$ and 70% H_2SO_4 in 5:1:1 ratio) were added. The mixture was digested at 80^{0}C till the solution became transparent. The resulting solution were filtered and diluted to 50ml using deionized water and analyzed for As, Pb, Cr, Cd, and Cu, by atomic absorption spectrophotometry (Barau *et al*., 2018).

2.6 HUMAN HEALTH RISK ASSESSMENT

2.6.1 Estimation of Bioaccumulation Factor (BAF)

The transfer coefficient was calculated by dividing the concentration of heavy metals in vegetables by the total heavy metals concentration in the soil. This index of soil – plant transfer or intake of metals from soil through vegetables was calculated using the following relationship described by (Olowoyo *et al*., 2010).

BAF = C_{veg}/C_{soil}----------------------1

Where; BAF represent the transfer factor of vegetable

C_{veg} = metal concentration in vegetable tissue, mg/kg fresh weight

C_{soil} = metal concentration in soil, mg/kg dry weight.

BAF > 1 indicates that the vegetable are en-riched in elements from the soil (Bio-accumulation) BAF < 1 means that the vegetables excluded the element from soil (excluder)

2.6.2 Estimation of the Daily Intake of Metal (DIM)

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The Daily intake of metal was calculated using the following formula used by (Olowoyo and Lion, 2013).

$ADDM = DI \times MF_{veg}/WB$-------------------------2

Where; ADDM = represents the average daily dose (mg,kg/d) of the metal

DI = is the daily intake of leafy vegetable (0.182 kg/d for adults and 0.118kg/d for children.

MF_{veg} = is the trace metal concentration in the vegetables tissues (mg/kg)

WB = represent the body weight of investigated individuals (55.7kg for adults and 14.2kg for children).

2.6.3 Estimation of the Potential Hazard of Metal to Human (Hazard Quotient HQ)

The Hazard Quotient (HQ) was used to calculate the possible human health risks associated with the consumption of vegetables harvested from the contaminated soils from Sewage areas. The following equation (Nabulo *et al.*, 2010) for calculating human health risk from consumption of leafy vegetables used to calculate the Hazard Quotient of vegetables.

HQ is the ratio between exposure and the reference oral dose (RFD)

If the ratio is lower than one 1, there will be no obvious risk.

$HQ = ADDM/RFDM$-----------------------------------3

Where; ADDM = represents the average daily dose (mg,kg/d) of the metal

RFDM = is the reference dose (mg,kg/d)

RFDM = is define as the maximum tolerable daily intake of metal with no adverse effect

2.6.4 Estimation of Hazard Index (HI)

The hazard index (HI) was calculated to determine the overall risk of exposure to all the heavy metals via the ingestion of a particular vegetable crop (USEPA, 2002). The hazard index (HI) was calculated as the summation of the hazard quotient (HQ) arising from all the metals examined. The value of the hazard index is proportional to the magnitude of the toxicity of the vegetables consumed. HI > 1 indicates that the predicted exposure is likely to pose potential health risks. However, a hazard index >1 does not necessarily indicate that a potential adverse health effects will result, but only indicates a high probability of posing health risks.

$HI = \sum HQ_{As} + HQ_{Cu} + HQ_{Pb} + HQ_{Cd} + HQ_{Cr}$----------------4

STATISTICAL ANALYSIS

The data obtained were analysed using IBM Statistical Product and Service Solution (SPSS) version 20 and Microsoft excel 2013. The results were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was carried out as $p<0.05$ considered statistically significant. Duncan's multiple range test (DMRT) was used to compare mean values of test groups and control as well as differences within group means of the various test groups.

III. RESULTS AND DISCUSSION

3.1 Physicochemical properties of soil samples before and after planting

Table 1presents the summary of the physicochemical properties of soil samples. The pH of the soil before planting (control soil with no pesticide), soil with no pesticide after planting, soil with Azarachtin neem seed solution (ANSS), and soil with dichloro-diphenyl-trichloroethane (DDT) were 6.89, 6.82, 6.28 and 5.25 respectively. Thelow pH (5.25) at soil with DDT might probably be due to the synthetic organochlorine pesticide use on the soil. The total nitrogen, phosphorus, organic matter, and organic carbon content in the soil before planting (control soil with no pesticide), soil with no pesticide after planting, soil with ANSS, and soil with DDT were (1.94, 20.74, 2.70 and 2.52), (1.90, 20.60, 2.81, 2.59), (1.84, 18.98, 2.74, 2.26), and (1.59, 18.12, 2.31, 2.77 %) respectively. They was significantly ($p < 0.05$) difference between DDT soil and ANSS soil.The soil with DDT has the least total Nitrogen content of 1.59%. Similarly, soil with DDT have the least total phosphorus content with 18.12%. The total Organic Carbon content of the soils ranged from 2.52% at control to 2.77% at DDT. The result was similar to (Alex, 2012) which organic carbon ranged from 1.03% to 2.11% and decreased with depth from Gongulon Agricultural Site, Maiduguri, Borno State, Nigeria. The exchangeable Cations K^+, Mg^{2+} and Na^+in control soil, ANSS soil and DDT soil were (1.89, 13.19, 8.18), (1.66, 12.22, 7.85) and (1.32, 11.27, 7.18Meq/100g) respectively. This result changes the soil physicochemical parameters, especially the soils treated with DDT pesticides. This increases the heavy metals in the soil, which is then likely transferred to plants that grow on such soils, with the associated risks of long term toxicity to humans that consume them and other biota in the ecosystem (Table 1).

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Table 1: Physicochemical properties of soil samples before and after planting

Soil Properties	Control(Before) No pesticide	No pesticide after planting	Soil with ANSS after planting	Soil with DDT after planting
Texture	loamy	loamy	loamy	loamy
pH	6.89 ± 0.03^{a}	6.82 ± 0.04^{a}	6.28 ± 0.04^{b}	5.25 ± 0.03^{c}
Total nitrogen %	1.94 ± 0.03^{a}	1.90 ± 0.03^{a}	1.84 ± 0.02^{b}	1.59 ± 0.08^{c}
Total phosphorus %	20.74 ± 0.19^{a}	20.60 ± 0.1^{a}	18.98 ± 0.07^{b}	18.12 ± 0.15^{c}
Organic matter %	2.70 ± 0.10^{a}	2.81 ± 0.01^{a}	2.74 ± 0.11^{a}	2.31 ± 0.06^{b}
Organic carbon %	2.52 ± 0.01^{b}	2.59 ± 0.02^{b}	2.26 ± 0.03^{c}	2.77 ± 0.06^{a}
K^{+} meq/100g	1.89 ± 0.08^{a}	1.87 ± 0.02^{a}	1.66 ± 0.04^{b}	1.32 ± 0.06^{c}
Mg^{2+} meq/100g	13.19 ± 0.02^{a}	13.15 ± 0.03^{a}	12.22 ± 0.08^{b}	11.27 ± 0.04^{c}
Na^{+} meq/100g	8.18 ± 0.06^{a}	8.11 ± 0.04^{a}	7.85 ± 0.03^{b}	7.18 ± 0.06^{c}

Results expressed as Mean ± SD. Mean values with same superscript letters on the rows are considered not significant (P>0.05). n=3

3.2 Physical observation on the number of spot on *Corchorus olitorius* damage by pest

Table 2 represent thetotal numbers of damaged leaves treated with ANSS and DDT pesticide also a control without treatment. The observations was started after two weeks of planting to the six weeks. The group with no treatment recorded the highest damage with 26 damage spot on the leaves, while *Corchorus olitorius* treated with ANSS and DDT recorded 8 and 5 respectively. The both pesticides reduces the effect of pest on the leaves. DDT pesticides recorded the least damage due to its effectiveness. Despite the effectiveness of DDT it can increase toxic substances in the leaves and soil which pose risk to human. The counted spots were marked to avoid repetition of already counted spot (Table 2).

Table.2: Physical observation on the number of spot on CO damage by pest

Number of weeks	**Physical observation on number of damage spot**		
	No treatment	CO treated with ANSS	CO treated with DDT
2	2	2	2
3	5	1	-
4	10	2	2
5	5	2	1
6	4	1	-
Total spot in six weeks	26	8	5

CO= *Corchorus olitorius*

3.3 Heavy metal Concentration in soils treated with ANSS and DDT pesticide

Table 3presents the summary of the mean concentrations (mg kg-1) of heavy metals Lead (Pb), Copper (Cu), Chromium (Cr), Cadmium (Cd), and Arsenic (As) analysed in the soil samples at Soil with ANSS, DDT and soil without pesticide. The concentration of As, Pb, Cu, Cd, and Cr in soil with ANSS, DDT and without pesticide were (2.05, 2.14, 0.71, 0.32, 3.35), (3.74, 5.83, 1.58, 0.78, 5.46), and (2.05, 2.44, 0.73, 0.31, 3.64) respectively. The level of metals obtained in this study were all below the FAO/WHO, permissible limit in soil. Analysis of variance (ANOVA) revealed a significant ($p < 0.05$) variation in the concentrations of the five elements in the soil, which is an indication of the extent of metal pollution in the soils. The mean Pb content in DDT pesticide recorded the highest values compared to all other metals. It ranged between 0.76 to 5.83 mg kg-1 in Cd and Pb respectively. Generally, the soil heavy metal concentrations decreased in the order Pb >Cr> As > Cu > Cd. The control soils had significantly lower heavy metals than all the soil with pesticide. The lower Cd in the DDT pesticides soil could be attributed to very low Cd levels in the Pesticides. The soil heavy metals were all below the WHO/FAO, permissible limits. The

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result was similar to the findings of Nimyel *et al*., 2015 that the control soils had significantly lower heavy metals than all the farm soils except Cr. The lower Cr in the pesticides contaminated farm soil could be attributed to very low Cr levels in the Pesticides. The soil heavy metals in the vegetable farm soils were below the WHO/FAO permissible limits. The study revealed that the concentrations of most of the elements were significantly ($p < 0.05$) higher at the soil with DDT pesticide compared to the other soil samples (Table 3). Soil with ANSS biopesticide generally had the lowest concentrations of most of the metals analyzed. Contrary to this finding, (Barau *et al*., 2018) reported high concentration of heavy metals in soil contaminated with synthetic pesticide (Table 3).

Table.3: Heavy metal concentration in soils treated with ANSS and DDT pesticide

Heavy Metals (mg/kg)	Treatments			
	Soil with ANSS	Soil with DDT	Control Soil with out pesticide	PL(mg/kg) in soil FAO/WHO, 2001)
As	2.05 ± 0.02^{b}	3.74 ± 0.12^{a}	2.05 ± 0.03^{b}	20
Pb	2.14 ± 0.14^{c}	5.83 ± 0.04^{a}	2.44 ± 0.13^{b}	50
Cu	0.71 ± 0.03^{b}	1.58 ± 0.10^{a}	0.73 ± 0.18^{b}	100
Cd	0.32 ± 0.03^{b}	0.78 ± 0.04^{a}	0.31 ± 0.03^{b}	3.0
Cr	3.35 ± 0.06^{b}	5.46 ± 0.07^{a}	3.64 ± 0.37^{b}	100

Results expressed as Mean ± SD. Mean values with same superscript letters on the rows are considered not significant ($P>0.05$). PL= Permissible limit. n=3

3.4 Heavy Metal Concentration in *Corchorus olitorius* treated with ANSS and DDT pesticide

Table 4presents the summary of the mean concentrations (mg kg) of heavy metals Lead (Pb), Copper (Cu), Chromium (Cr), Cadmium (Cd), and Arsenic (As) analysed in *Corchorus olitorius*treated with ANSS, DDT and a control without pesticide.The mean concentrations of heavy metals in the vegetables are shown in Table 3. The concentration of As, Pb, Cu, Cd, and Cr in vegetable with ANSS, DDT and without pesticide were (0.35, 0.05, 0.05, 0.09, 0.35), (2.06, 1.41, 3.78, 1.04, 1.86), and (0.35, 0.03, 0.03, 0.07, 0.31). The level of metals obtained in vegetable with DDT were all above the FAO/WHO permissible limit in vegetable. The permissible limit for As, Pb, Cu, Cd, and Cr in vegetables are 0.5, 0.3, 3.0, 0.2 and 0.3 mg/kg.In the vegetable analyzed for heavy metals, *Corchorus olitorius* treated with DDT pesticide generally had higher heavy metals concentrations than the other treated with ANSS.Vegetable treated with DDT exceed the WHO/FAO permissible limit of metal in vegetable. These differences were significant ($P<0.05$). Heavy metals and nutrients absorbed by the roots are usually translocated and allocated to different parts of the plants which could limit the concentrations in the leaves. However, availability of metals in the soil and continuous absorption by the roots could lead to higher concentration in the leaves.

The mean concentrations of the heavy metals in *Corchorus olitorius* treated with DDT pesticide decreased in the order Cu > As > Cr > Pb > Cd in table 4. Cu had the highest concentration (3.78 mg/kg) in the vegetable which exceed the permissible limit (3.0mg/kg).The range was from 1.04 mg/kg at Cd to 3.78 mgkg-1 at Cu. The increase in Cu could be attributed to continuous application of DDT pesticide used. In a similar study, (Pradhan and Kumar, 2014) reported a range of Cu values from 11.08 mg/kg to 23.07 mg/kg in a study conducted in China. According to (Maobe *et al*., 2012) high levels of copper can cause metalfumes fever with flu-like symptoms, hair and skin decolouration, dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and nausea. Also (Dixit *et al*., 2015) reported that copper is indeed essential, but in high doses it can cause anaemia, diarrhea, headache, metabolic disorders, vomiting, liver and kidney damage, stomach and intestinal irritation on human health. All the metals analyse on *Corchorus olitorius* treated with DDT pesticide exceed FAO/WHO, permissible limit compared with the treatment of ANSS. Jabeen *et al*., 2010 reported that cadmium causes both acute and chronic poisoning, adverse effect on kidney, liver, vascular and the immune system.(Barakat, 2011) reported that high dose of chromium is observed to cause Bronchopneumonia, chronic bronchitis, diarrhea, emphysema, headache, irritation of the skin, itching of respiratory tract, liver diseases, lung cancer, nausea, renal failure, reproductive toxicity, and vomiting. Nagajyoti *et al*., 2010 reported that Lead can cause serious injury to the brain, nervous system, red blood cells, low IQ,

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impaired development, shortened attention span, hyperactivity, mental deterioration, decreased reaction time, loss of memory, reduced fertility, renal system damage, nausea, insomnia, anorexia, and weakness of the joints when exposed to high lead. The presence of heavy metals in varying concentrations in vegetables under investigation could be attributed to the presence of these trace metals in the pesticides in discriminately used on the vegetable. Previous studies have reported the presence of these metals in pesticides (Shah *et al*., 2013; Nazir *et al*., 2015; Fonge *et al*., 2017).

The mean concentrations of the heavy metals in *Corchorus olitorius* treated with ANSS bio pesticide decreased in the order As > Cr > Cd > Pb > Cu. As had the highest concentration (0.35 mg/kg) in the vegetable which is below the permissible limit (0.5mg/kg).The range was from 0.05 mg/kg at Pb and Cu to 0.35 mgkg-1 at As. The presence of As in the vegetation samples could be attributed to the atmospheric condition and human activities in the area. The concentration of Arsenic is in line with the findings of (Barau *et al*., 2018) on vegetables treated with pesticide. According to (Luo *et al*., 2011) atmospheric deposition is a major factor for high metal accumulation in plantsamples, and this could therefore be the cause of the Arsenic in the samples analysed. All the metals analyse on *Corchorus olitorius* treated with ANSS bio pesticide were below FAO/WHO, permissible limit. ANSS bio pesticide do not add metals in the soil and on the leaves, they cause less or no toxic effect on vegetables and are biodegradable. The mean concentrations of the heavy metals in *Corchorus olitorius* treated with no pesticide were all below the FAO/WHO, permissible limit. The range was from 0.03 mg/kg at Pb and Cu to 0.35 mg/kg at As. The differences in the accumulation of these metals in the vegetables under study could be attributed and not limited to the varying physiological phenomenon such as absorption rate of different metals viz a viz soil physicochemical properties, choice of plants in selecting which mineral is allocated andstored in its parts among other factors (Alloway, 1990) (Table 4).

Table.4: Heavy Metal Concentration in CO treated with ANSS and DDT pesticide

Heavy Metals (mg/kg)	Treatments			
	CO treated with ANSS	CO treated with DDT	Control CO with no pesticide	PL (mg/kg) in vegetable FAO/WHO, (2016)*,(2001)**
As	0.35 ± 0.04^b	2.06 ± 0.04^a	0.35 ± 0.02^b	0.5*
Pb	0.05 ± 0.02^b	1.41 ± 0.02^a	0.03 ± 0.13^b	0.3*
Cu	0.05 ± 0.03^b	3.78 ± 0.11^a	0.03 ± 0.02^b	3.0**
Cd	0.09 ± 0.01^b	1.04 ± 0.02^a	0.07 ± 0.01^b	0.2*
Cr	0.35 ± 0.05^b	1.86 ± 0.03^a	0.31 ± 0.02^b	0.3*

Results expressed as Mean ± SD. Mean values with same superscript letters on the rows are considered not significant (P>0.05).
PL= Permissible limit. CO = *Corchorus olitorius* n=3

3.5 Estimation of Bioaccumulation Factor (BAF)

Table 5 shows the Bioaccumulation factor (BAF) of heavy metals from the soil to plants, which is the ratio of the concentration of metals in plants to the total concentration in the soil. The bioaccumulation factors of metals As, Pb, Cu, Cd, and Cr obtained in CO treated with ANSS, CO treated with DDT and Control CO with no pesticide were (0.07, 0.28, 0.07, 0.28, 0.07), (0.55, 0.24, 2.39, 1.36, 0.33), and (0.17, 0.01, 0.04, 0.22, 0.08) respectively. The BAF for the same metal in the farm lands were significantly different from those for control and according to the type of plants. The highest BAF value obtained were in *Corchorus olitorius* (CO) treated with DDT Cu(2.39) and Cd (1.36).Plants are known to take up and accumulate trace metals from contaminated soil (36). The BAF value for the same metal in the CO treated with DDT pesticide were significantly different from those treated with ANSS. The BAF of all other elements in the treatment are within normal range less than 1. Any value of BF >1 indicates the plants as hyper accumulator and could be used in bioremediation of heavy polluted soil.The soil-plant BAF of different heavy metals showed the following order- BAF_{Cu}>BAF_{Cd}>BAF_{As}>BAF_{Cr}>BAF_{Pb}. Where TF > 1 indicates the vegetable are en-riched in elements from the soil (Bio-accumulation). TF < 1 means that the vegetables exclude the element from soil (Excluder). The result of (Barau *et al*., 2018) BAF suggest that all the vegetables under study viz, pepper, tomato and onion were hyper accumulators of Cr and Cd with the highest accumulation of Cr in pepper (10.20).

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BAF > 1 indicates that the vegetable are en-riched in elements from the soil (Bio-accumulation).

BAF < 1 means that the vegetables exclude the element from soil (Excluder) (Table 5).

Table.5: Estimation of Bioaccumulation Factor (BAF)

Heavy Metals (mg/kg)	**BAF**		
	CO treated With ANSS	CO treated with DDT	Control CO with no pesticide
As	0.07	0.55	0.17
Pb	0.28	0.24	0.01
Cu	0.07	2.39	0.04
Cd	0.28	1.36	0.22
Cr	0.07	0.33	0.08

CO = *Corchorus olitorius*

3.6 Daily Intake, Potential Hazard of Metal (Hazard Quotient) individual

Table 6 shows the hazard quotient and Daily intake was calculated for both adults and children from trace metals in leaves of *Corchorus olitorius*. The daily intake of heavy metals (DIM) was estimated according to the average vegetable consumption. The estimated DIM through the food chain is given in Table 6, for both adults and children. The DIM values for heavy metals were significantly little high in the vegetables grown with DDT pesticide. The values obtained were from *Corchorus olitorius* treated with DDT pesticide for both adults and children.

The HQ of metals through the consumption of vegetables for both adults and children were given in Table 6. The HQ of heavy metal in *Corchorus olitorius* treatment with DDT pesticide were Pb(0.01 and 0.03), Cd(0.01 and 0.04), Cu(0.00 and 0.01), As(0.01 and 0.03) and Cr(0.02 and 0.05) in both adult and children respectively. The HQ values for heavy metals were significantly high in *Corchorus olitorius* treated with DDT pesticide than the treatment with ANSS. The values obtain in HQ in *Corchorus olitorius* treated with DDT pesticide and *Corchorus olitorius* treated with ANSS pesticide were less than 1 (<1) as shown on table 6, which indicated that consumption may not lead to accumulation of these metals in the body. The results of (Barau *et al.*, 2018) of HQ obtained suggest high health risk from Pb, As and Cd contamination in both adults and children and only a slight health risk of Zn (1.058) contamination in children consuming onion. The HQ for Pb ranged between 1.29 to 2.22 in adults and 2.63 to 15.30 in children. In the adult's category, the HQ for As was 1.29 to 2.44 while 0.52 to 2.89 was recorded for children (Table 6).

3.7 Estimation of hazard index (HI) of metal for individuals

The calculated HI for both Adult and children in both *Corchorus olitorius* treated with ANSS, DDT and control were all less than 1. The result showed that children are more likely to be affected with continuous consumption of*Corchorus olitorius* treated with DDT pesticide in table 7. The result of this study regarding the HI revealed that *Corchorus olitorius* vegetable grown with ANSS and DDT are safe for consumption. (Akande and Ajayi, 2017) also observed high values of HI greater than 1 on vegetables grown on peri-urban farm. Similarly (Barau *et al.*, 2018) also indicate that Children had the greatest health risk than adults from heavy metal contaminated vegetables fumigated by pesticides (Table 7).

Table 7: Estimation of hazard index (HI) of metal for individuals

	HI for Individuals			
	Individuals	CO with ANSS	CO with DDT	CO with no pesticide
	Adult	0.00	0.05	0.00
HI=∑HQ (HM)	Children	0.02	0.16	0.02

HI = Hazard index. ∑ = Summation of the Hazard Quotient (HQ) arising from all the heavy metals (HM) examined. CO = *Corchorus olitorius*

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Table 6: Daily Intake, Potential Hazard of Metal (Hazard Quotient) individual

Heavy metals	DIM and HQ for individuals				
	Individuals	Hazard	CO with ANSS	CO with DDT	CO with no pesticide
Pb	Adult	DIM	0.00	0.00	0.00
		HQ	0.00	0.01	0.00
	Children	DIM	0.00	0.01	0.00
		HQ	0.00	0.03	0.00
Cd	Adult	DIM	0.00	0.00	0.00
		HQ	0.00	0.01	0.00
	Children	DIM	0.00	0.00	0.00
		HQ	0.00	0.04	0.00
Cu	Adult	DIM	0.00	0.01	0.00
		HQ	0.00	0.00	0.00
	Children	DIM	0.00	0.03	0.00
		HQ	0.00	0.01	0.00
As	Adult	DIM	0.00	0.01	0.00
		HQ	0.00	0.01	0.00
	Children	DIM	0.00	0.02	0.00
		HQ	0.01	0.03	0.01
Cr	Adult	DIM	0.00	0.01	0.00
		HQ	0.00	0.02	0.00
	Children	DIM	0.00	0.02	0.00
		HQ	0.01	0.05	0.01

CO = *Corchorus olitorius*, DIM = Daily intake of metal, HQ = Hazard quotient

IV. CONCLUSION

The presence of heavy metals (Pb, Cd, Cr, As and Cu) from application of synthetic pesticide and bio pesticide in*Corchorus olitorius* plant was detected and the corresponding farm soil. The bioaccumulation factor for the vegetable showed that they exclude the element from soil (Excluder).The concentration of heavy metals in *Corchorus olitorius* treated with DDT pesticide exceeded the WHO/FAO permissible limits in vegetable. This showed that consumption of these vegetables treated with synthetic pesticide pose health risk from heavy metal contamination because of pesticides applications. People need to break the habit of using harmful inorganic pesticides such as DDT and switch to rising organic ones such as ANSS that break down quickly in the sunlight and in the soil. The faster a chemical breaks down, the sooner the soil can return to a healthy state. Most organic pesticides are also safe to use around people and pets. They can easily be washed from vegetables making them healthier for use.

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Assessment of agricultural practices for improving quality of cocoa beans: South-West Cameroon

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Abstract*— *Purpose: *Cocoa is the main cash crop in Cameroon, with about 30% of Gross Domestic Product of agricultural produce for export and processing. Many studies depict that, despite the efforts of Cameroonian producers, the quality of their production still needs improvement. This research work was carried out in the MEME district south-west region of Cameroon, with the aim to assess the different agricultural practices used by producers to improve the quality of their cocoa beans.*

Research method: *Seventy-eight cocoa farmers were chosen in that district and interviewed using structured questionnaires and personal observations if possible.*

Findings: *Our survey shows that the difficulties farmers face in providing better cocoa beans quality reside in the financial credits; diseases and pests; the cost of inputs including spraying cost and lack of access to energy. These constituted 83.89% of the difficulties related to the quality of cocoa beans in that area. Also, our findings depicted that to ensure the quality of cocoa beans, most farmers apply normal spraying, proper fermentation and proper drying.*

Research limitation: *The lack of technical know-how in good agricultural management practices, lack of input subsidies, credit facilities and insufficient electricity grid are the obstacles that reduce the quality of cocoa beans.*

Original value*: We recommend that cocoa mass spraying be extended to all farmers, to prevent pests and diseases effects; In addition, facilitate access to credit and increase the electricity grid; which will enable easy access to new technologies to dry cocoa beans.*

Keywords*— *Cameroon; Cocoa beans; Quality; Agricultural Practices.

I. INTRODUCTION

Agriculture is the main source of growth and foreign exchange in Cameroon (here in after known as CMR), the contribution of agriculture to the economic growth of Cameroon is about 76.38% (CMR, 2019). In 2015, agriculture accounted for 22.3% of GDP of the economy of Cameroon. According to the World Record in an article published in June 2013, agriculture was the main occupation for nearly 60% of the active population. In Cameroon, agriculture is rich and varied and cocoa is one of the main cash crops amongst coffee, rubber, banana, cotton, tea etc. According to government statistics, cocoa beans accounts for about half the country's exports earnings, with an average annual production up than 300 000 tons (Business in Cameroon, 2018). Cocoa beans sales contribute about 250 billion CFA francs ($426 million). The cocoa sector accounts for about 2% of the national GDP and 6% of the primary GDP and about 30% of the GDP for the export and processing of agricultural products (*Pierre E.A, 2006*). The cocoa sector is largely dominated by exporters of unfinished products, namely raw cocoa beans. However, producers are still struggling on how to improve its quality.

The cocoa sector has drawn the attention of government over the past two decades to increase production levels, quality and foreign exchange earnings. Achieving these objectives has called for measures to maintain and improve the quality of cocoa beans which is directly related to cocoa beans value (*KWESSEU P.J.M*, 2008. The national goal is to produce 600,000 tons of cocoa by the year 2020 (Yasmine B.D, 2018). A significant harvest of more than two hundred thousand tons (200 000t): 382, 000t in the 2016/2017 season (Yasmine B.D, 2018) suggests that the country is likely to meet or exceed its target. Among the reasons for the good performance of Cameroon's cocoa sector over the last decade are, the prudential policy reforms of the cocoa sector, including strategic reforms, various incentives, strengthening of producer supervision; furthermore, efforts to improve the quality of cocoa beans are equally important, for example, there is strong government involvement in export marketing and quality

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control through Licenses Buying Companies (LBCs) (*IRAM. 2012*), the construction of equipment to better treat the cocoa beans; rigorous quality controls at village-level buying centers; and pick-up points for the onward shipment of cocoa beans for export and processing. All these through strict recommendations to LBCs by the government for them to pay more attention in order not to adulterate cocoa beans sold to the cocoa board for shipping.

When perceived for its quality, it appears that the quality of cocoa beans has endured a shot/hit since the progression of the segment in 1991 (Pierre E.A, 2006). As per the United Nations Conference on Trade and Development (UNCTAD), market progression has made new challenges, including controlling general production and keeping up the nature of production in an aggressive market condition. They remarked that the debasement of yield quality was one of the primary difficulties of the progression of export harvests, for instance, cocoa bean. Numerous specialists have upheld this theory by showing that the advancement of this sector has highlighted and quickened the debasement of the cocoa beans quality, because of competition from purchasers to combine or enlarge their piece of the overall industry; it was additionally discovered that the pressure applied by LBCs in Cameroon to acquire cocoa from farmers was behind the clearance of high-moisture cocoa beans. This has favored the improvement of shape, a standout amongst the most significant quality deformities of cocoa beans and for which purchasers will limit intensely in the global market. For example, in 2013, 1,300 kg of low-quality cocoa beans were bend in the area of Bafia in Cameroon.

Market-decided premiums in product markets are based, in addition to other things, on view of value. It was noticed that, in perspective on the global prerequisites for the quality of cocoa beans, Cameroon still needs to attempt to improve the quality of its cocoa beans, as market requests keep on developing (CMR, 2019). The purchaser, the cocoa processor and the chocolate maker must be happy with the quality of the products conveyed on the worldwide market. A low-quality cocoa bean can prompt attribution cases, loss of share of the overall industry and salary, and stain the notoriety of Cameroon's cocoa beans. Basic quality issues in the cocoa and related items include incorporate form, slate, fat substance, dampness content, taste, presence of foreign material, and different imperfections in cocoa beans (Nlend A.L et al., 2017). Quality is assessed dependent on flavor, smell, consistency, yield, bean estimate, hull percentage and fat substance, all these influence the decision of cocoa beans. (Barel, M. 2013). The physical attributes of cocoa beans and the flavor of cocoa beans are unequivocal factors in the quality of these cocoa beans.

Cameroon can build the quality of its export cocoa beans by setting up and spreading great agricultural practices and by giving sponsorships to farmers. Cocoa farmers in Cameroon apply practices that don't generally ensure the quality of cocoa beans, a label that to be pleased with. To plan cocoa beans available to be purchased, the farmer must be mindful so as to deal with great and bad seeds of the cocoa beans, regularly discarding the greater part of the bad ones. The production of value cocoa beans relies upon a few components and it has been contended that 80% of the quality of cocoa beans relies upon the proper cultivation, drying and fermentation techniques embraced by farmers (C. Amani, 2014). Issues that obstruct the accomplishment of international quality measures for cocoa beans are commonly arranged into natural and structural / financial factors components. Characteristic elements incorporate the impacts of climate, daylight and drying of cocoa beans while, structural / financial components include: absence of direct extension services; subsidizing of research activities; elevated amounts of chemical residues; presence of heavy material; utilization of jute sacks for the export of cocoa beans; and delivering technique. The underlying driver of anomalies in cocoa beans could be credited to poor agricultural practices, pest and disease invasion, mishandling, poor fermentation, deficient drying, high moisture that makes the product defenseless against form and bacterial development. Different reasons for low quality of cocoa beans are poor and long timeframe bringing about fat debasement and nuisance invasion in the absenceof fumigation and different measures in order to maintain quality (Nlend A.L et al., 2017). As supported by the government (CMR, 2019), the principal challenge to keep up the certainty of cocoa bean purchasers in Cameroon, is to guarantee consistency and quality improvement of the cocoa beans given by the nation. As indicated by the Government, "this infers that, cocoa farmers must proceed not exclusively to follow and receive great agronomic practices, yet in addition to improve the great agricultural practices they presently apply" (CMR, 2019).

The goal of this survey article is to look at agricultural practices that will assist farmers with ensuring great quality of their cocoa beans, relieve difficulties farmers face in keeping up cocoa bean quality, and furthermore assess measures to improve the quality of cocoa beans in the MEME region of Cameroon. Moreover, this investigation will likewise assist us with diagnosing and feature the shortcomings of value confirmation measures in the Cameroonian cocoa division, so the nation can keep on improving its quality status on the international market.

II. MATERIALS AND METHODS

Sample area of the survey study

MEME district is in the south-west region of Cameroon, which is the country's largest producing area with a 45.45% (table4) share of production at the national level (CMR, 2019). The region is in the forest zone and the principal occupation of the occupants is farming, exchange/business and little scale mining. The climate of the region is characterized by average annual rainfall of about 3 000 mm and a long rainy season from March to November, attenuated by a small dry season (in June), and interrupted by a long dry season of 3 months (December to February). Two forest formations predominate in South-West Cameroon; (1) the Biafrane evergreen forest which is very diversified, has a high abundance of Caesalpiniaceae, (2) the Atlantic littoral forest, which is poor in Caesalpiniaceae and characterized by the abundance of Loflhiraalata (Ochnaceae), Sacogottisgabonensis (Humiriaceae), Coula edulis (Olacaceae) and Cynometrahankei (Caesalpiniaceae). Furthermore, Lake Ossa, in areas not degraded by the development of food crops, the Atlantic coastal forest dominates (Isabelle et al., 1996). The district is suitable for cocoa production, therefore, three communities in that district were purposively selected for data collection. These communities are part of the main cocoa producing communities in the district. Seventy-eight cocoa producers were randomly selected from them, namely KUMBA I, KUMBA II and KUMBA III (Fig.1). The total population of MEME is about 384, 286 people (FOLEFACK D.P, 2003) which account for about 7.1% of the national population; the land area of MEME is 3105 km^2 (CMR, 2019). About 26 farmers from each community were approached and interviewed using well-structured questionnaires and personal observations where possible. The information was coded and analyzed down utilizing the Statistical Package for the Social Sciences (SPSS, 2007) version16. The results were displayed in tables, figures, rates.

III. RESULTS AND DISCUSSION

Producers' Socio-Economic Characteristics:

The results of the respondents revealed that 97.43% were men and 2.56% were ladies (fig 2). Likewise, as indicated by the results acquired from the study, 37.17% of the respondents were aged between 55 to 75 years of age, 48.71% were aged between 35 to 54 years of age, and 14.10% were aged somewhere in the range of 18 and 34 years of age (fig 3). The age structure demonstrates that most farmers are moderately old. Additionally, cocoa development in the examination zone was for the most part done by generally older male farmers. Cocoa cultivating is a dreary activity that requires a great deal of strength [ASEAN Free Trade Area (AFTA), 2005]; the generally propelled time of cocoa producers can influence their productivity and basic decision making. Men's dominance in cocoa production may likewise be clarified by the way that, men generally claim land and have simpler access to land in most rural communities. About 16.53% of farmers had casual training; near 57.69% has essential/middle school education, 21.45% has secondary school education and 4.33% had tertiary education (fig 4). The level of education of the respondents was accordingly low, which could influence their speedy adoption of agricultural technologies. Almost 89.74% of farmers have been growing cocoa for around 20 years, while 10.26% have been growing it for around 10 years (fig 5). This delineated that most respondents have impressive involvement in cocoa production. We likewise discovered that about 95% of the respondents were experienced cocoa farmers with over 10 years of involvement in cocoa production (fig 5). The generally long periods of cultivating are probably going to improve their insight into cocoa production; pre and post-harvest agricultural practices.

Issues encountered by farmers in ensuring quality of cocoa beans:

As indicated by the survey study, the fundamental issues influencing the nature of cocoa beans in that region were absence of access to credit, pest and disease invasion, surprising high expense of inputs (spraying, fertilizer and so forth.) and energy (Table 1). These represented 84.6% of the issues influencing the nature/quality of cocoa beans (Table 1).

As indicated by farmers, they face financial difficulties in actualizing the prescribed practices to guarantee high quality of their cocoa beans and they credited this to the high expense of agrochemicals and spraying machines to protect their farms against pest and disease (Table 1). As indicated by Denis et al., (2002), the rate of pest and disease invasions of cocoa in Cameroon (south-west) endured and added to the weakness of quality yields. The presence of pest, for example, Tapinanthusglobiferus compete with the cocoa tree for water and supplements, which diminishes yield, while diseases, for instance, black pod additionally cause deformity of the cocoa pods, bringing about a decrease in the size of the beans. As revealed by (Assonwa F.E, 2015), most farmers did not control pests and diseases, due to the high cost of pesticides, spraying equipment and labor. Respondents showed that not all farmers profited from the government mass cocoa spraying exercise, and the individuals who needed to spray their own cocoa farms found the cost exorbitant. Farmers anyway noticed that when farms are appropriately sprayed, pods are sheltered from disease, bringing about quality beans. (Kokou E, 2014) revealed that government spraying efforts to spray cocoa farms have been called attention to by respondents as not covering the majority of the producer farms and furthermore not being continuous. They likewise guarantee that the spraying of cocoa farms to control pests and diseases has not achieved its maximum capacity as it has not had the option to satisfy its order to guarantee that cocoa farms are sprayed two times every year. Then again, the increasing expense of work represents a test to the capacity of farmers to keep their farms free of weeds, in this way trading off the quality of the cocoa beans produced. Moreover, the issue of energy thwarted the entrance to new advances in drying cocoa beans; along these lines, influencing the quality, since the drying phase in cocoa beans is significant (Denis et al., 2002).

Producers' Practices to Ensure the Quality of Cocoa Beans

Producers sell their cocoa beans legitimately to license buying companies (LBCs) which decide the quality of cocoa beans preceding buy. They arbitrarily select a portion of the cocoa beans that they have split-up to decide the brownness of the cocoa beans, a certification of value. According to the United States Department for Agriculture (USDA), 2005, poor quality cocoa beans have a purple coloration, heterogeneous bean size, displaced taste and aroma, sick beans, moisture and the presence of foreign materials. These problems result from irregular rainfall, pests and diseases, improper fermentation and poor drying of cocoa beans. Moisture, for example, results from inadequate and / or incomplete drying.

From Table 2, it has been found that the primary strategies utilized by cocoa producers to ensure astounding cocoa beans are normal spraying, appropriate fermentation and legitimate drying. These practices represented about 58% of farmers' reaction to measures taken to guarantee high quality of cocoa beans. As confirmed by C. Amani (2014), low quality cocoa beans are the after effect of unpredictable rainfall, pest and diseases, inappropriate fermentation and poor drying of cocoa beans. Likewise, ordinary and satisfactory farms upkeep results in uniform measured cocoa beans while normal weeding control prevents weeds that harbor pests and diseases. Farmers likewise permit the pods to ripen appropriately before gather as they need to ensure the quality of their cocoa beans. Likewise, the breaking of the pods is completed following reaping to dodge the loss of quality of the product. Pruning, application of fertilizers, prevention action of over-torn seeds and choice of ailing seeds are different practices received by farmers to guarantee their cocoa bean quality. National Cocoa and Coffee Board (NCCB), 1991, based on international recommendations, has distinguished the cocoa quality control measures to be embraced by cocoa farmers in Cameroon, including appropriate mamagement practices, convenient harvesting, and appropriate fermentation, legitimate drying and good storage of the dried cocoa beans.

Producers' proposals on measures to guarantee high quality of cocoa beans

As portrayed in Table 3, it can be seen that, 57.69% of farmers proposed loaning to farmers through microfinance plans to enable farmers to purchase the necessary inputs, for instance, spraying guns as this will enable them to spray their farms two times per year as prescribed to guarantee great control of pests and diseases. Twenty-five-point sixty four percent (25.64%) of cocoa producers have likewise recommended subsidies on inputs, particularly spraying guns (Table 1) in order to decrease the expense of renting guns and guaranteeing regular farm spraying. Eleven-point fifty three percent (11.53%) of respondents likewise proposed post-harvest management expansion preparing to empower farmers to deliver high quality of cocoa beans (Table 1) and 5.12% respondents also mentioned the creation of farmers' cooperatives as a means of training in extension and information dissemination **(Table 1)**.

IV. CONCLUSION AND RECOMMENDATIONS

Getting great quality cocoa beans requires joint effort between various on-screen characters in the cocoa business, to be specific farmers, authorized purchasing organizations, quality government establishments (CICC/NCCB), extension service providers, cocoa processors and worldwide purchasers. However, farmers play an urgent and focal job in guaranteeing the quality of cocoa beans through

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their management practices and the treatment of cocoa beans pre and post-harvest. Cocoa farmers in Cameroon are quality cognizant and attempt incredible endeavors to guarantee better quality of their cocoa beans. There are, however, a few reasons against the insurance of the quality of cocoa beans, yet these difficulties have not prevented farmers from attempting to improve the quality of their cocoa beans. The liberalization of the internal marketing of cocoa has led to a reduction in the quality of cocoa beans in Cameroon. Farmers' capacity to keep up quality in a changed framework infers that they fuse certain production costs, which will eventually diminish the makers' gains of cocoa farmers from cocoa production. It is very prescribed that all on-screen characters in the cocoa business in Cameroon keep on working eagerly to keep up and improve the quality of cocoa beans with the goal that the nation can trade high grade of cocoa beans to keep up its notoriety in the global market and to guarantee a better status. In addition, it is suggested that farmers be given credit facilities to control pests and diseases to protect thequality of cocoa beans. Building up a component for farmers to acquire credit so as to rapidly adjust their management practices will improve the quality of cocoa beans. Also, the cocoa mass spraying activity ought to be appropriately checked to guarantee that all farms have access under the suggested routine. At long last, the expansion of the electricity grid should be extended to cocoa growing communities, so as to allow producers to have access to new drying systems, thus insuring better quality of cocoa beans.

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Table 1: Issues encountered by farmers in ensuring quality of cocoa beans:

Problems	Response (% score)	Rank
Financial constraints	42,3	1
Pests and diseases	23,1	2
High cost of spraying	11,5	3
Problem of energy	7,7	4
High cost of labor	5,1	5
Unavailability of good variety	3,8	6
Irregular rainfall	3,8	6
Long distance of cocoa farm	1,3	7
Poor health of farmer	1,3	7

Table 2: Producers' Practices to Ensure the Quality of Cocoa Beans

Problems	Response (%score)	Rank
Proper drying	26,9	1
Regular spraying	16,7	2
Proper fermentation	14,1	3
Planting good variety	10,3	4
Quick breaking of cocoa pods	7,7	5
Allowed pods to mature well	6,4	6
Pruning	6,4	6
Application of fertilizer	5,1	7
Avoiding over-ripped pods	3,8	8
Selection of diseased pods	2,6	9

Table 3: Producers' proposals on measures to guarantee high quality of cocoa beans

Problems	Response (%score)	Rank
Access to credit	57,7	1
Subsidies	25,6	2
Education on post-harvest management	11,5	3
Formation of farmers' co-operatives	5,1	4

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Table 4: Cocoa bean production 2016 in Cameroon

Regions	Production (tons)	Percentage (%)
South-West	132 492.204	45.45
Centrral-region	107 859.44	37
Littoral	20 347.53	6.98
South	15 546.44	4.99
East	9 853.10	3.38
West	4 256.07	1.46
North-west	3 760.50	1.29

Table 5: Quantity production, harvested area, production/yields, average price to farmers

Years	**Production quantity (tons)**	**exported**	**Production yields (hg/ha)**	**Average price to farmers/kg in XAF**
2000	120 619	371 742	3 298	580
2001	120 877	364 753	3 347	700
2002	112 281	365 938	3 416	700
2003	128 646	450 000	3 444	500
2004	126 752	490 000	3 403	600
2005	130 490	400 000	3 500	690
2006	164 523	440 000	3 740	800
2007	212 619	131 127	3 866	939
2008	229 203	178 101	3 885	1 152
2009	230 032	193 973	3 925	1 853
2010	244 077	193 881	3 941	1 491
2011	246 120	190 214	3 582	1 695
2012	250 000	173 794	4 014	1 356
2013	268 941	192 836	4 104	1 095
2014	232 530	198,129	4 021	1 358
2015	269 495	181 893	4 012	1 500
2016	231 642	169 530	4 027	1 050
2017	382 000	170 981	-	1400

Source: Faostat/Oncc, 2017. INS, 2015, Pierre E.A, 2006

Table 6: Quality of cocoa from 2000/2001 to 2012/2013 (Kg).

	GI/GF	%	GII/FF	%	HS	%	NC	Residues	Total
2000	2 104,618	2	103 126,305	98,00	0		0	0	105 230,923
2001	2 624,738	3	84 866,541	97,00					87 491,279
2002	1 377,170	1,29	105 151,276	98,67	43 080	0,04			106 571,526
2003	1 289, 210	1,11	114 488,589	98,89					115 777,799
2004	1 756,740	1,41	123 275,370	98,59					125 032,110
2005	6 069,360	5,07	113 409,659	94,72	250 800	0,21			119 729,819
2006	10 796,280	7,55	131 853,802	92,26	234 240	0,16	25 080		142 909,402
2007	1 805,760	1,11	160 004,220	97,94	1 407,938	0,86	144 706		163 362,624
2008	2 377,508	1,28	178 838,608	96,53	3 994,082	2,16	52 080		185 262,278
2009	175,560	0,10	171 285,323	98,57	2 185,415	1,26	100 320	21 285	173 767,903
2010	2 936,521	1,44	197 828,701	97,28	3 014,237	1,25		48 000	203 355,822
2011	5 783,540		173 219,958		3 014,237		25 080	84 577	182 127,312
2012	727,320		196 380,485		8 120,209		99 940	361 346	205 689,300

Source: ONCC, 2015. Note: GF = Good Fermented; FF = Fair Fermented; FAO norms: GI = Grade I; GII = Grade II; HS = Hors Standard.

Table.7: Other crops cultivated in that district

Years/ crops	2009	2010	2011
Bananas	104 282	228 803	268 330
Cocoa beans	101 875	101 827	99 901
Coffee	9 548	12 564	8 563
Rubber	52 497	50 215	50 983
oil Palm	89 560	99 562	100 758
Peanut	32 413	5 981	37 601
pineapple	1 968	2 069	2 098
Plantain	130 636	153 977	293 375
Maize	156 747	172 740	154 948
Rice	1 407	1 947	1 874

Source: INS, 2015

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Appendix

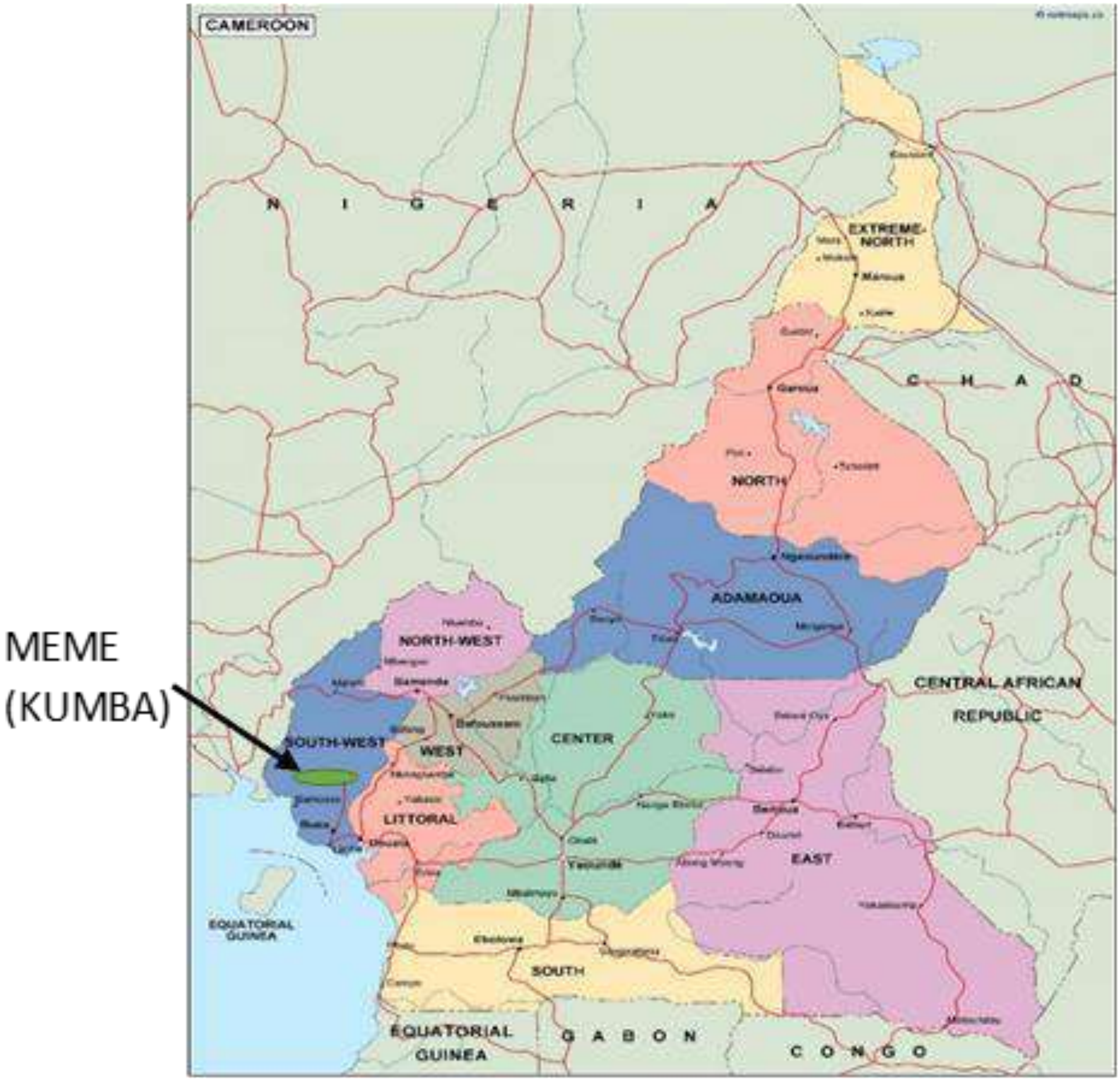

Fig.1: Survey location. Source: INS 2008

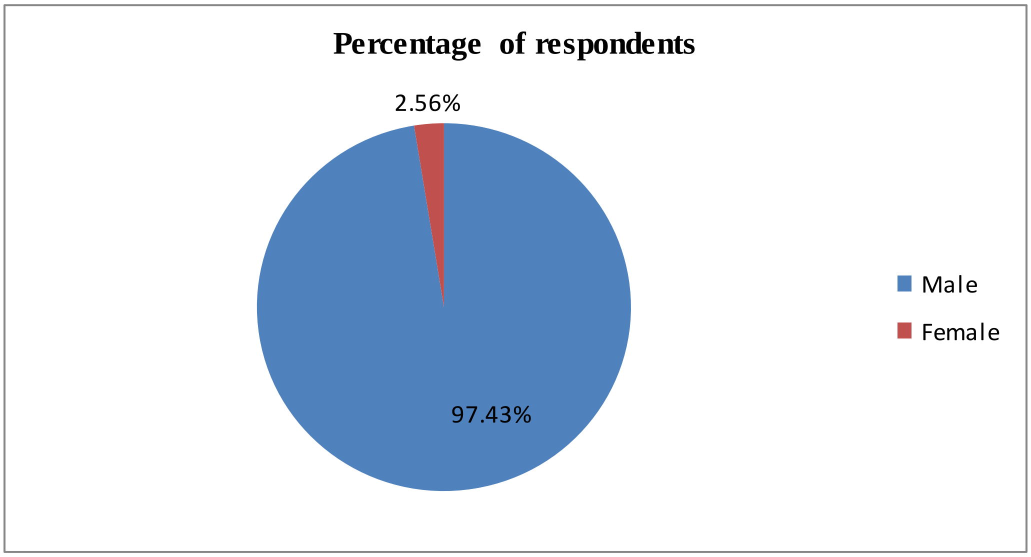

Fig.2; Percentage of respondents by gender

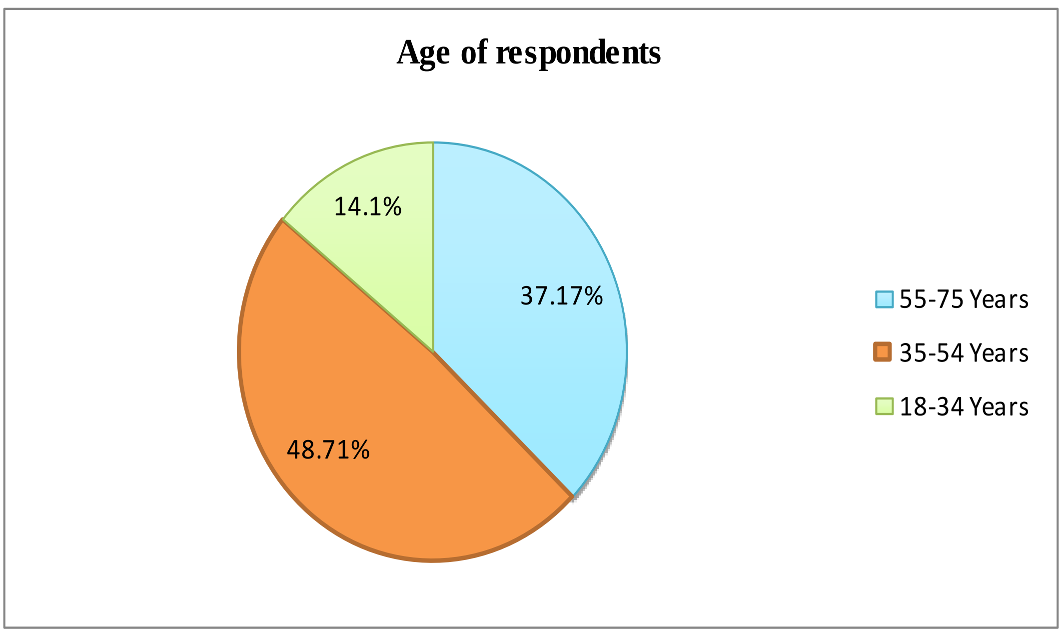

Fig.3; Percentage age of respondents

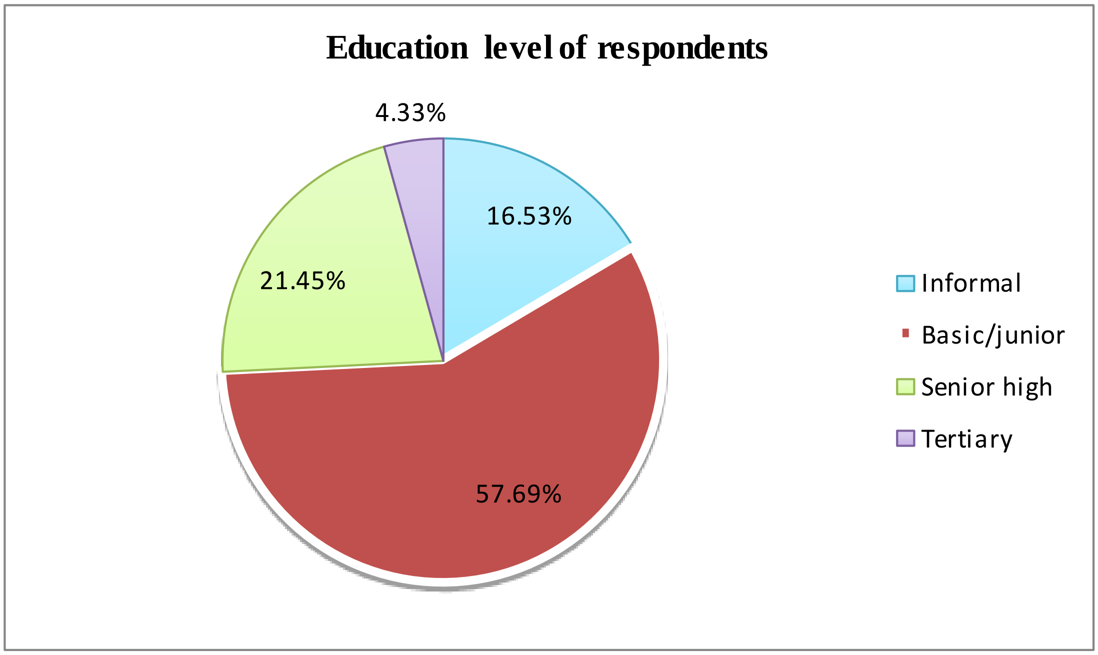

Fig.4; Percentage of respondents by education level

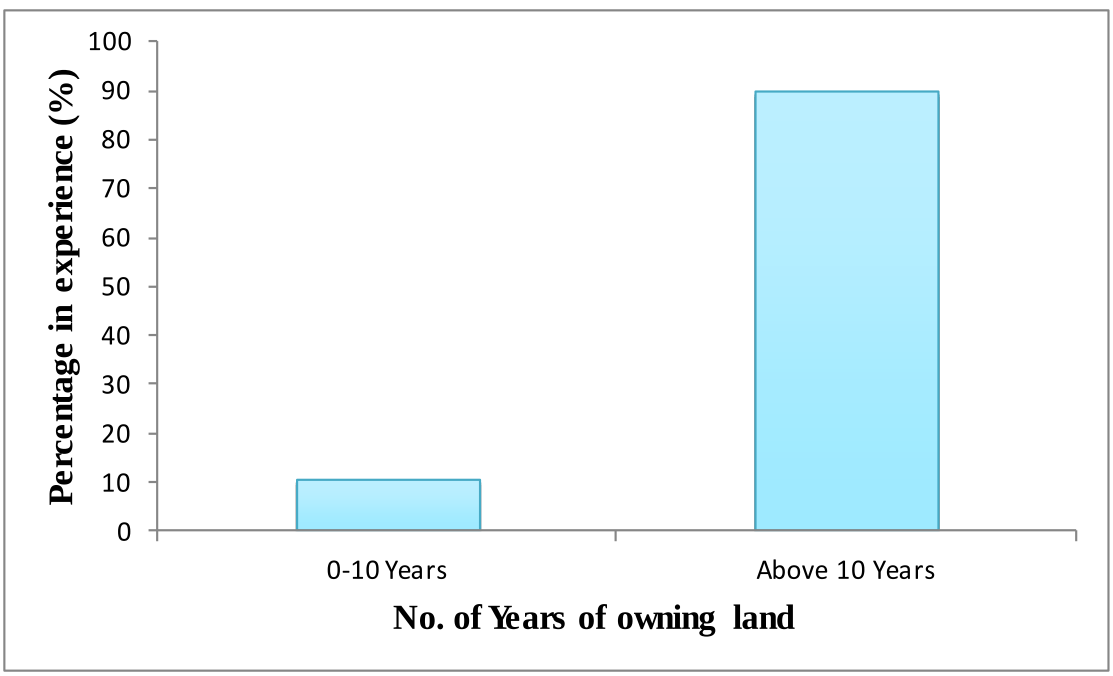

Fig.5; Percentage of land ownership by average age

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Natural Farming System Sustainability of Paddy Fields in Morotai Island Regency

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Abstract— *Various parties intensively conduct the increasing awareness trend and mindset change on environmental and healthy food consumption patterns. The environmental resources damage problem, watertight agricultural land, human health, unhealthy food, contributes to global warming and in aggregate has an impact on diversity, sovereignty, independence and sustainability of food sources.Back to nature is a developed solution to overcome the negative externalities various effects. These research conducted to assessing natural paddy fields farming systems sustainability as a natural local food source in Morotai Island Regency.The analysis method using indicator basis was adapted from the Sustainability Assessment of Farming and the Environment (SAFE) model and the Farmers Sustainability Index (FSI) model on 200 respondents samples. The results explain that the natural farming system of paddy fields sustainable on economic, socio-cultural and environmental dimensions. There are assessment indicators attributes which are full local wisdom of economic, socio-cultural and environmental dimensions based on farmers and regions specific indicators in determining sustainability of paddy field natural farming system level holistically.Therefore, the novelty of these study is that the natural farming system is a world agricultural tradition that has been crushed by agricultural globalization but is still sustainable in Morotai Island Regency and has sustainability indicators which are full specific farmers and regions local wisdom.*
Keywords— Sustainability index, local wisdom, natural farming system, paddy fields, Morotai Island.

I. INTRODUCTION

Starting from population theory of Malthus that population growth is faster than food availability, so famine disasterfears encourage experts to find a solution. Various agricultural systems are developed, one of which is green revolution technology known as inorganic (conventional) farming systems. Studies that are not holistic and mature give rise to a variety of negative externalities. Various problems occur due to green revolution technology, that isenvironmental resources damage problem, watertight agricultural land, human health, unhealthy food, and contribute to global warming and in aggregate have an impact on inequality in diversity, sovereignty, independence and sustainability of food sources.

Mindset changes and increasing awareness trends of an environment and healthy food consumption patterns are intensively carried out by various parties. Back to nature is a developed solution to overcome the negative externalities variety due to environmental damage and unhealthy food. Saragih (2016) explained that global warmingconcerns threat, hungercatastrophe, and food security continue to be discussed. The Global Hunger Index report (2016), there are 795 million people (10.6% of the earth's population) sleeping hungry. On the other hand, WHO states that 1.6 billion people who are overweight and 600 million obese people. These means that hunger occurs not because of insufficient food production throughout the world, but due to inequality in food distribution, inability to produce due to the lack of land to plant and the failure to buy healthy food.

Supporting world orientation changes to increase food availability is no longer affected by quantity trends productivity but instead on food production quality sources. Wigenasantana and Waluyo (1991) had voiced the natural farming system as the best alternative agricultural system in Indonesia. The natural paddy farming system is one of the oldest farming systems in the world. Originally a primitive agricultural system developed into a system of shifting, subsistence farming, as known as the dry land traditional farming system and was crushed by agriculture globalization. Fukuoka (1978) has introduced the concept of natural agriculture, both vegetables, beans, paddy field, rye, and others. Further explained by Fukuoka that natural farming systems are holistic agricultural systems.

The natural agricultural system principle implies that natural power can regulate plant growth. There are four basic principles in developing the natural farming system according to Fukuoka (1978), that is; 1)without-tillage; 2) do not use fertilizers, herbicides and synthetic chemical pesticides; 3) don't use hybrid seeds. Land naturally developed does not respond to hybrid seeds, so it must use local seeds; 4)do not do intensive irrigation water. Explained by Rope and Umasugi(2014) that the paddy fields natural farming system developed in Morotai Island Regency is used without any fertilizer. The average production reaches 4.5 tons, economically efficient because it uses seed inputs and labor.

Based on developing the natural agricultural system principles by farmers on Morotai Island, it has low productivity but is thought more sustainable in meeting farmer householdfood needs. The sustainability allegations of natural paddy fields farming systems are related to several important things, among others; 1)the farming has characteristics of tropical forest dryland ecosystems, meaning that paddy fields developed resistant to drought; 2)economically efficient, because it minimizes external inputs, and maximizes internal inputs; 3) preservation of local seedsqualityvariety, where the best solution to maintain genetic authenticity of seeds variety to be preserved in their original habitat; 4)having initial knowledge and social traditions with mutual cooperation culture and joint harvesting in the future time dimension so it not only measures short-term but long-term interests; and 5) allegedly as the natural and nutritious food source can meet farmer households food needs and easy access to reach specific communities in the border area, because as an area that requires high transportation costs to supply rice products from regionoutside. Thus the sustainability research of natural paddy fields farming systems in Morotai Island District is essentialto be preserved.

II. LITERATURE REVIEW

Economic and Social Culture Values of Farmers

The farmer's phenomenon and the non-market-oriented agricultural system still applies today natural farming systemsregional communities, when information and technology access has developed rapidly, but changes in agricultural globalization do not crush paddy fields natural farming system farmers' perception. Paddy field is not commercialized because there is a strong socio-economic value of farmers' culture as life culture. These phenomenon affected by farmers' perceptions of their farming lives. In Galela farmers, North Halmahera Regency, farmers' perceptions were classified based on the concept of farming and agricultural crop type known as *Doro de Raki*. *Doro* means the subsistence tradition and *Raki* are estate-based assets or investments.

The fulfillment of income needs derived from annual plants, perennials, gardening plants such as coconut, cloves, nutmeg, chocolate, coffee, and others are farming concepts known as *Raki* or plantations which also means investment or farmer's family future life assets. Seasonal plants such as food secondary crops, tubers, and vegetables are fulfillingsource non-market-oriented daily consumption needs that are subsistently lived. These were done from generation to generation as explained by Bareta (1917) that the cultivation system developed in Halmahera and Morotai was not adhering to the plantation method. So that, it is not known the profit or loss in paddy field farming. The farming communities are mixed social organization entities in social structures because the communities have many relationships with various social organizations (Rouf et al., 2015). Families, groups, kinsman, households and homes, and others are farming communityelements. The traditional farming communities characteristics using manual labor, individual production facilities, the unplanned division of labor, readily available land, from families and using natural raw materials and autonomyachieving.

Empirically, the natural farming systemscharacteristics are farmers have *Bari*wisdom or cooperation when planting paddy fields and profit sharing during harvesting activities (Rope, 2013). There is socio-cultural wisdom of economic value. The *Bari*Wisdom can overcome labor shortages experienced by the agricultural sector, especially for planting workers who require a considerable amount of labor and without wages. Likewise at harvestingstage activities taken during harvests for months due to traditional harvesting tools used, but with shared harvesting wisdom and profit sharing being a solution without expensive technology capital can meet harvest labor needs and is socio-cultural wisdom economical regarding meeting fellow food needs. Every human being is responsible for other human food needs (Creswel and Martin, 1998).

Agricultural SystemsSustainability

Various sustainability studies have been conducted. Sustainability measurementis very complicated. The measuring is not measurable: sustainability index survey (Bohringer and Jochem, 2007). Reviewing 11 sustainability index methods to measure sustainability indexes, but failing to fulfill the study based on scientific criteria. The development trend that requires sustainability aspects is vital to develop various studiescontinuously. Likewise in the dialysis agricultural system sustainability study by referring the consistent and objective approach indicators identification and selection (PC & I) using the Sustainability Assessment of Farming and the Environment (SAFE) framework assessment (Van Cauwenbergh et al.,. 2007). The framework is designed in three spatial levels and includes three pillars of sustainability, that is the environmental, economic and social.

Farming sustainability research was also conducted previously by Gowda and Jayaramaiah (1998) using indicators grouped in three dimensions of sustainabilityby developing a Farmer Sustainability Index (FSI). A framework similar to SAFE, but FSI is equipped with an analysis formula. Gowda and Jayaramaiah explained that composite indicators greatly determine the accuracy of sustainability. There were ten traditional agricultural indicators on ecological, economic and social aspects which tended environmental compared than conventional farming systems (Rasul and Thapa,2003). The behavior of adding more organic content, higher local inputs, and providing balanced food.

Sustainability research refers to the Farmer Sustainability Index (FSI) in India with 40 items, including insect control, disease control, weed control, soil fertility management, soil erosion control, and related practices relevant to local farmerconditions. The FSI was developed to measure sustainable practices application by traditional Indian rice farmers. The results illustrate the various values of the Farmer Value Index between two groups of rice farmers; conventional farmers and more sustainable farmers. The first farmer type of index score is 23.95, and the last is 70.06, indicating that farmers who implement sustainable farming systems are more durable than conventional farmers. Likewise, the new agricultural sustainability indicators system is proposed in dynamic weight calculations in China. The three main indicators are used to measure the index valuethat is ecosystems that are indisputable, economically viable, and socially acceptable. The three indicators are then integrated into the overall systemindexvalue using geometric averages (Mohamed et al., 2016).

In principle, the number of sustainability indicators must be carefully studied and determined so that they can measure and describe thesustainability condition. The useful indicatorsselection is the success key of each sustainability evaluation. Hayati et al., (2010) explained that as much as possible efforts to evaluate sustainable agriculture on the aggregate level with some indicators that are almost comprehensive have not been able to measure sustainability capability at the farm level. Therefore, Hayati et al., (2010) further recommended the indicators used for agricultural sustainability must be site-specific. The criteria for selecting 14 agricultural sustainabilityindicators in developing countries are clearly from economic, social and ecological dimensions (Zhen and Rotray, 2003). Thus, the farm system sustainability is assessed as representative if it is based on specific indicators of farmers and regions.

III. ANALYSIS METHOD

The study was conducted in Morotai Island Regency, North Maluku Province. The research areaselection is made by purposive sampling, in 5 districts; East Morotai, Morotai Jaya, North Morotai, South Morotai, and South West Morotai. In the whole region, some farmers preserve the natural farming system of paddy fields. The farmer selection using random sampling in 20% of the 1000 farmer populations that maintain the natural farming system of paddy fields. So that a sample of 200 respondents was obtained.

Answering the aim of the study to assess natural farming systems sustainability in the economic, socio-cultural and environmental dimensions using farmers-specific analysis of the region adapted to the combination concept of Van Cauwenbergh's, N., et al (2007) through SC & I models using The Sustainability Assessment of Farming and the Environment (SAFE) framework and the farmers sustainability index (FSI) model refer to Gowda and Jayaramaiah (1998); Rasul & Thapa (2004); Terano et al. (2015); Mohamed et al., (2016). Indicators Formulated consists of 13 indicators that are developed, modified and adapted based on agricultural sustainability consensus and findings of various research results adapted to potential regional specific conditions in preserving natural paddy fields. For this reason, the formulation of sustainability indicators as shown in Figure 1.

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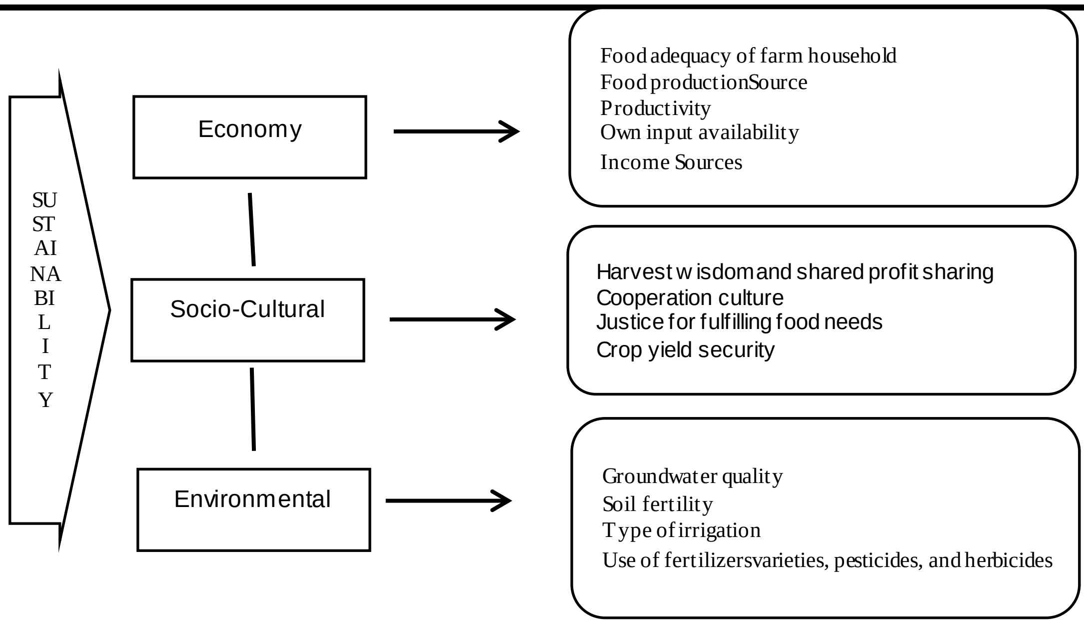

Fig.1: Sustainability indicators formulation of natural paddy fields farming systems

The sustainability indicator formulation of paddy field natural farming system is analyzed by the sustainability index using Gowda and Jayaramaiah (1998); Rasul & Thapa (2004); Terano et al. (2015); Mohamed et al., (2016) formula developed as follows:

$$Zij = \left(\frac{Yij - MinYj}{MaksYj - MinYj} \right)$$

Where is:
Zij = Unit of standard value on respondent *i* on the component to *j*.
Y*ij*: Respondent's value to *i* to component *j*

The further formula used is:

$$Sli = \frac{\Sigma(Zij.Sj)}{TotalNilai} X100\%$$

Where is:
S*Ii*=Sustainability index of farmers to *i*
Zij =Standard value unit on the respondent to *i* on the component to *j*
S*j*=Value scale of the element to *j*
The sustainability measurement is based on the index value criteria as shown in Table 1.

Table 1. The index value criteria of sustainability indicators

Index Value	Criteria
80.01 – 100	Very Sustainable
60.01 – 80	Sustainable
40.01 – 60	Less Sustainable
20.00 - 40	Unsustainable

Furthermore, the testing was an analysis based on the *t*-test where H_o: $\mu<60.01$; H_1: $\mu\geq 60.01$. If $F_{count}>F_{table}$: reject H_0 means sustainable; If $F_{count}\leq F_{table}$: accept H_0 means unsustainable.

IV. RESULTS AND DISCUSSION

Natural, human and capital resources which unique characteristics on Morotai Island are God's gift to be grateful. Many things can be explored to become future agricultural models. The future that is described and heralded at this time, by world geographers that in 2045 there will be a drought due to the dryness of the ten largest springs in Asia followed by increasing population, requiring adequate food availability. Indirectly affecting Indonesia as one of the Asian countries to think about the sustainability of the region-specific agricultural system.

Future phenomena require drought-resistant agricultural systems, maximizing internal and minimizing external inputs are a necessity in agriculture current globalization. But in Morotai Island, which underdeveloped, backward, and poorestregions, evidentlystored natural resources which are truly extraordinary and require human resources in developing representative research. Its physical nature, its farmers, are full of wisdom, its genetic diversity is maintained from destructive modern technology. So that, it can be claimed that the natural paddy farming system is the only agricultural system that can fulfill sustainability criteria holistically. Is the natural paddy farming system characterized by environmentally friendly and conditions with farmers' wisdom, can be economically sustainable, socio-cultural and environmental?

The results will discuss in detail onpaddy fields natural farming system sustainability in three dimensions that is economical, socio-cultural and environmental. There are 13 indicators described in the method section and are assessed based on the number of attributes. The detailed analysis results are shown in Tables 2 and 3.

Table 2. The results analysis of sustainability index

	SustainabilityDimensions		
	Economy	Socio-Culture	Environmental
N	200	200	200
Mean	63,64	72,41	67,19
Median	68,49	72,38	63,33
Modus	82,64	100,00	100,00
Standard Deviation	17,32	24,26	18,97
Minimum	23,78	22,74	30,00
Maximum	97,63	100,00	100,00
Total	12727,47	14481,55	13438,89

Source: Primary data processed, 2018

Table 3. Results of one sample t-test for sustainability in economic, socio-cultural and environmental aspects

Sustainability index	Mean	*t*count	P value
Economy	63,64	11,128	0,000
Socio-Culture	72,41	13,058	0,000
Environmental	67,19	12,808	0,000

Source: Primary data processed, 2018

The results of the economic, socio-cultural and environmental index analysis as the aggregate obtained mean values seen in Table 1 in intervals of 60.01-80.00. The results of one sample *t*-test in Table 2, obtained $t_{count} > t_{table} = 2.576$ at the confidence level of 1%, so it was concluded that H_0 was rejected and H_1 has accepted, ie, $\mu \geq 60.1$ means that the natural farming system of paddy fields is economical, socio-cultural and environmentally sustainable.

The results explained that the indicator criteria characteristics in economic dimension are met with five indicators, that is food sufficiency, food productionsources, productivity, own inputsavailability, and incomesources —the farmerwisdom in fulfilling household foodadequacy with thefoods variety available besidespaddy fields. Various food sources include bananas, cassava, sweet potatoes, taro, and sago. These are supported by multiple food consumption patterns wisdom from generation to generation and still maintained. The farmerorientation to anticipate food production sources is not focused on monoculture productionquantity but is oriented towards polyculture. Low productivity but maximizes internal inputs wisdom with own inputs availability and minimizes external inputs. Thus low productivity is not claimed to be low quality because household food needs fulfill it. If more deeplystudied, why does it reach high production but farm household food needs are not sufficient? These means, it is economically fulfilled if food needs are adequate and not economical if shortage. Thus the mindsetchange about productivity and income is not the permanent indicator in sustainability measurementof the economic dimension but is adjusted to farmers and region-based indicators.

The socio-cultural dimensionsustainability has four indicators. Farmers have socio-cultural wisdom in preserving the natural farming system of paddy field. The wisdom Indicators of shared harvesting are one of many wisdom empires that strongly support the natural farming system of paddy fields sustainability. When the harvest season arrives the landowner'sfarmer, who are ready to harvest invite kinsman and neighbors who do not plant paddy fields to participate in collecting together and sharing their crops, this means that every farmer has a humanitarian responsibility towards the food needs of kinsman and neighbors. Every human being is responsible for other human food needs (Creswell and Martin, 1998). Besides, harvest labor need will be fulfilled without cash wages but with the intended wisdom. So that, the socio-cultural tradition can be economic value because it maximizes internal potential.

The *Bari*Wisdom or cooperation culture is the world peasant tradition carried out when planting paddy fields. For generations, it is still sustainable in Morotai Island

Regency. This can streamline labor without being paid for planting stageneeds. The socio-cultural values achieved from this stage are influential personalities that remain sustainable. So that, shortages of laborneed or farm labor is not experienced by paddy field farmers. Other socio-cultural values that support the natural paddy rice farming systemssustainability are justice fulfillment of food needs and crop yield security. The natural paddy farming system with the wisdom variety protects farmers from not qualified food availability, protected from various imported products because farmers can meet food needs in quantity and quality.

Environmental dimension sustainability indicators consist of groundwater quality, soil fertility, irrigation systems, and fertilizers type used pesticides and herbicides. Farmers have near gardens, and water sourcessurrounded. These affect the groundwater quality is still maintained, thus forming the non-intensive perception-behavior in irrigation systemsuse. Likewise with soil fertility, because the groundwater quality supported indirectly affects the soil fertilitylevel. Wisdom does not use fertilizers, pesticides, and herbicides to keep agricultural lands protected from the negative externalities of using fertilizers, pesticides, and synthetic chemical herbicides. These support the natural paddy field farming systemsustainabilityin Morotai Island Regency; it is sustainable on the economic, socio-cultural and environmental dimensions. Therefore,the only agricultural system claim of inputs, processes, and outputs used is truly sustainable holistically.

V. CONCLUSIONS

The natural paddy farming system is theworld agricultural systemtradition and crushed by agriculture globalization but is still sustainable in Morotai Island Regency. The results and discussion concluded that the natural farming system of paddy fields is economical, socio-cultural, and environmental dimensions sustainable. There are assessment indicators attributes which are full local wisdom of economic, socio-cultural and environmental dimensions based on farmers and regions specific indicators in determining sustainability of paddy field natural farming systemlevel holistically.

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Identification and Controlling of Stem Bulging of Passion Fruit (*Passiflora Edulis*) in Sri Lanka

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Abstract— *Stem bulging is one of the major biological constrains of passion fruit cultivation especially in Low country wet zone areas in Sri Lanka. Green bark split as a result of bulging, discoloration of bark and also stained internal tissues are the symptoms of this stem bulging. A survey was conducted in those infected areas to find out the causal factors of passion fruit stem bulging. Soil samples were collected from disease infected and disease free locations and analyzed for soil pH, major nutrients and some micro nutrients. Examination of the internal tissues of stem showed at first inspection, looked like fungal hyphae. Microscopic observations were consistently indicated the Fusarium species association with infected tissues. Also, Passion fruit stem bulging samples were taken and dispatched to the CABI (Centre for Agriculture and Bioscience International) for further diagnosis of causal organism. The field trail - 1 was conducted at Agro ecological zone WL1a to observe the disease transmission of stem bulging from infected plants to healthy plants. The field trail – 2 was conducted at Agro ecological zone WL1a based on fungicides, insecticides and foliar fertilizer to control the stem bulging of passion fruit. The experiment was laid out in a Randomize Complete Block Design and five treatments were used with 3 replicates. The treatments were, T1- Foliar application of insecticide - Thiamethoxam 25WG 3g/10 l, T2- Foliar application of fungicide - Thiophanate methyl 70 WP 6g/ 10 l, T3- Alternative foliar application of Thiamethoxam 35 WG + Thiophanate methyl 70 WG, T4- Alternative foliar application of Thiamethoxam 25 WG + Thiophanate methyl 70WP + Albert solution (commercial formulation of nutrient mixture) 5g/10 l and T5- Control. Results numerated that, stem bulging can be transmitted from infected plant to healthy plants as a disease. Fusarium species were consistently found in bulged area of the samples tested. The lowest disease incidence was observed in Thiophanate methyl 70 WP treated plots of field trials indicating Fusarium species may be the causal factor of the problem. Insecticide applications were failed in controlling stem bulging in the field conditions. According to the CABI report No: IMI 504215 & IMI 504216, the isolation made from internal tissues and two fungal cultures were subjected for molecular identification and Gibberella fujikuroi and Fusarium sp. were identified as associate fungi of stem bulging of passion fruit.*

Keywords— Stem bulging, Passion fruit, Gibberella fujikuroi, Fusarium sp.

I. INTRODUCTION

Stem bulging is one of the major biological constrains of passion fruit cultivation especially in Kalutara and Ratnapura districts (Agro ecological zone WL1a) in Sri Lanka since 1980s. Since then, this problem has gradually spread. Symptoms were observed in most of the farmer fields with different severity levels. The main symptoms observed in the affected vines were presented in Passion fruit stem branches. Green bark split as a result of bulging, discoloration of bark and also stained internal tissues. However initial examination showed no sign of fungal fruit bodies or insects.

Fig.1: Passion fruit stem bulging

Attempts have been made to develop some solutions for this problem. It was suspected that the symptoms resemble damaged caused by Boron deficiencies. However, application of Boron as a soil treatment (2g of borax per vine) at planting failed to manage stem bulging problem of passion fruit. Preliminary field observations numerated that, stem bulging can be transmitted from diseased plant to healthy plants. So, we were identified stem bulging as a disease. Therefore, it was decided to carryout studies to correctly identify the causal factors and develop control measures for stem bulging of passion fruit in low country wet zone area.

Fig.2: Green bark split as a result of bulging

Fig.3: Longitudinal Section of an affected stem

II. MATERIALS AND METHODS

A survey was conducted in stem bulging reported areas of Ratnapura and Kaluthara districts to find out the causal factors of passion stem bulging. Soil samples were collected from disease infected and disease free locations and analyzed for soil pH, major nutrients and some micro nutrients.

The field trail - 1 was conducted to observe the disease transmission of stem bulging from infected plants to healthy plants. In here, 100% infected field was selected for the experiment. 60 healthy plants were grown adjacent to the diseased plants and 60 healthy plants were grown in disease free field. Disease incidence level was recorded at two months after planting.

Microscopic observations were made in bulged tissues to detect fungal infection. Affected tissues were microscopically inspected and it was observed fungal mycelium and two types of morphologically different conidia. Pieces of bulged tissues were kept on Potato Dextrose Agar (PDA) medium and incubated 7 days under room temperature. The mycelia development and conidia development on PDA was observed. Then single spore inoculants of fungi isolates were made. Ten isolates were collected and each isolate also wound inoculated into healthy passion fruit vines but and observed for development of bulging symptoms on inoculated stems to confirm Koch’s postulates. Also, Passion stem bulging samples were taken and dispatched to the CABI for further diagnosis of causal organism.

The field trail - 2 was conducted at Ratnapura District (Agro ecological zone WL1a) to develop control measures for stem bulging. The experiment was laid out in a Randomize Complete Block Design and five treatments were used with 3 replicates. The treatments were as follows.

Treatment	Description
T1	Foliar spraying of insecticide - Thiamethoxam25 WG at the rate of 3g/10 l, two months after planting and spraying was continued 3 times in 2 weeks intervals
T2	Foliar spraying of fungicide - Thiophanate methyl 70 WP at the rate of 6g/ 10 l, two months after planting and spraying was continued 3 times in 2 weeks intervals
T3	Alternative foliar spraying of Thiamethoxam 25 WG and Thiophanate methyl 70 WP, two months after planting and spraying was continued 3 times in 2 weeks intervals
T4	Alternative foliar spraying of Thiamethoxam, Thiophanate methyl and Albert solution (liquid fertilizer) 5g/10 l, two months after planting and spraying was continued 3 times in 2 weeks intervals
T5	Control

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III. RESULTS AND DISCUSSION

3.1 Analysis of soil samples collected from passion stem bulging infected fields

Table 1: Analysis of soil samples collected from passion stem bulging infected fields

Factor	Results
pH	4 – 5 Acidic
N,P	Normal
K	Higher than normal(800-1000 ppm)
Electrical conductivity	Very high
Micronutrients (Fe, Cu, Mn, Zn)	Normal

Note: Soil samples collected from 10 locations of healthy and disease infected passion fruit cultivation were used for analysis.

Results revealed that soils of those areas were acidic and P^H of soil samples was varied 4 - 5. K level is higher than normal level. N and P levels are within normal range and no Fe, Cu, Mn, Zn deficiencies observed in the tested soil samples.

3.2 Transmission of stem bulging from infected plants to healthy plants

Table 2: Passion Stem bulging incidence of newly planted passion fruit plants which were planted in adjacent to the diseased field and new field

Treatment	Percent disease incidence after 2 months of field planting
T1- Healthy plants growing adjacent to the diseased plants	33.3
T-2 Healthy plants growing in new land	1.7

Stem bulging symptoms were first observed in T1 treatments within 5 weeks after field planting and later symptoms progressively developed and 33 % plants showed stem bulging symptoms. However, only one plant showed mild symptoms on stem of T2 treatment during the period. Results numerated that, stem bulging can be transmitted from infected plant to healthy plants.

3.3 Microscopic observations and characters of isolation of *Fusarium* collected from bulged stem samples of passion fruit.

Colony colour on PDA was white, and then turns into brown when older. Reverse colony colour on PDA was light brown and then turns into brown when older. There were two types of conidia. Macro conidia were abundant, strait and pointed at ends 3-6 cells. Larger micro conidia were few, cylindrical, 1-2 cells and comparatively smaller. Isolates of fungi were identified as *Fusarium spp* by comparison of their colony characters and morphological features of conidia with published data [1]. Further, it was noticed that re-inoculation of purified *Fusarium* isolates failed to development of bulging symptoms of passion fruit vines. However, it was identified by culturing test that *Fusarium* fungi remained viable in tissues of inoculated sites of vines, indicating that the *Fusarium* fungi survives in stem tissues of the passion fruit vines without showing bulging symptoms.

3.4 The field trial based on fungicide and insecticide

Table 3: Passion Stem bulging incidence five months after field planting

Treatments	Mean Passion Stem Bulging incidence per vine at 5 months after planting
T1	11.1
T2	4.2
T3	13.2
T4	9.5
T5	13.7

Lowest disease incidence was observed in Thiophanate methyl 6g/ 10 l i.e. T-2 treated plots of field trials indicating *Fusarium* species may be the causal factor of the problem. Thiophanate methyl is an approved EU fungicide used widely to control fungal diseases on crops. A systemic fungicide is effective with protective and curative activity against a broad spectrum of diseases in fruits, vegetables and other crops [2]. However, Thiophanate methyl is highly effective on *Fusarium sp.* [3]. Foliar spraying of insecticide application, alternative foliar spraying of insecticide and fungicide and alternative foliar spraying of insecticide, fungicide and Albert solution were failed in controlling stem bulging in the field conditions. However, according to the CABI report No: IMI 504215 & IMI 504216, the isolation made from internal tissues and two fungal cultures were subjected for molecular identification and *Gibberella fujikuroi* and *Fusarium* sp. were identified as associate fungi of stem bulging of passion fruit[4].

The fungus, *Gibberella fujikuroi* produces plant growth hormones called gibberellins. Some strains of the fungus *Gibberella fujikuroi* (perfect stage of *Fusarium moniliforme*) are the industrial source of gibberellic acid [5]. These strains infect rice and cause the disease known as bakanae in Japan. Bakanae disease of rice is caused through infection by this fungus and in rice this causes an exaggerated growth response and the plants to become

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extremely tall with a pale spindly appearance [6]. Other hosts are also infected by this fungus although we are unable to find any references concerning passion fruit. The fungus was only isolated from within the bulging areas and therefore we suspect the fungus colonization of the tissues is a localized infection.

IV. CONCLUSION

The isolation made from internal tissues of passion fruit and two fungal cultures were subjected for molecular identification and *Gibberella fujikuroi* and *Fusarium* sp. were identified as associated fungi of stem bulging of passion fruits. Also, these results are compatible with field trails' data. However, Thiophanate methyl 70 WG 6g/ 10 l can be recommended as an effective fungicide to minimize passion fruit stem bulging.

V. ACKNOWLEDGEMENT

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Comparative Studies of Heavy Metals and Mineral Residues in Some Farm Crops around Mining Community of Ribi, Awe Local Government Area of Nasarawa State

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***Abstract*— *This work investigated the level of heavy metals and other elements present in two agricultrural crops (millet and maize) cultivated in mining community of Ribi, Awe LGA of Nasarawa State, Nigeria. Samples were collected from four (4) different farms at the peak of rainy season (between July and August). Samples were analysed at the Chemistry Advance Research Centre, Sheda Science and Technical Complex (SHESTCO) Gwagwalada Abuja. The atomic absorption spectrometer (thermo Scientific, ice3000AA02134104v1.30) was used. All analyses were performed in triplicate. Data were analysed using Minitab Statistical software (16.0). In the rainy season, millet had higher concentration of all the residues quantified except in cadmium which was more concentrated in maize (0.11mg/L). In other heavy metals, lead was 2.83mg/L in millet and 2.54mg/L in maize. Copper was 1.32mg/L in millet and 0.83 in maize. Magnesium was 10.47mg/L in millet and 10.43mg/L in maize. In the dry season, maize had higher concentrations of lead (2.67mg/L), copper (0.925mg/L), nickel (0.134mg/L) and iron (1.688mg/L) whereas cadmium, magnesium, and zinc were more in millet than in maize. Dry season millet was higher in some residues than the control millet. Some millet residues were higher in rainy season than the control level. In millet, lead was highest in raining season (2.831 mg/L) whereas copper and cadmium were very high in dry season (0.586 mg/L and 0.213mg/L respectively), even more than the control. Magnesium residues recorded the highest values among all heavy metals present in millet. Magnesium also had the highest concentration among the residues present in maize in the following order: rainy season (10.43mg/L) > dry season (9.33mg/L) > maize control (9.23mg/L). Iron was more concentrated in the control maize (2.65mg/L) than in both dry and rainy seasons whereas zinc recorded higher seasonal concentrations (0.44mg/L) than the control level (0.46mg/L The values of Pb, Zn, and Ni in millet and maize are above the WHO's standard guideline while other residues are within or below the regulatory limits. Mining activities taking place around the in the study location might have impacted negatively on the safe consumption of agricultural crops cultivated by farmers.*

Keywords*— *Heavy metals, Mineral residues, Millet, Maize, Mining.

I. INTRODUCTION

Heavy metals are metallic elements which have a high atomic weight and a density much greater (at least 5 times) than that of water and is harmful to most organisms even when present at low concentration (Amin *et al*., 2003). Toxic heavy metals comprise a group of minerals that have no known function in the body and are harmful to humans (Amin *et al*., 2003). Heavy metals exist in natural and contaminated environments and cannot be easily detoxified via degradation, resulting in their persistence in the environment.

Many of these metals, such as Cd, Pb and Cr, are carcinogens and are involved in several diseases, including Alzheimer's, Parkinson's, multiple sclerosis, osteoporosis, developmental disorders and failure of several organs (e.g., heart, kidney, lungs, immune system) (Duruibe *et al*., 2007). In mining areas, where exploration activities are carried out, there has been an increased level of toxic metals in water and agricultural soils with a resultant increased uptake and deposition on foods (including processed foods) such as cereals, and vegetables thus posing serious health implications to the consumers (Duruibe *et al*., 2007).Different studies have shown varying amounts of heavy metals in various food sources in Nigeria (Edward *et al*., 2013).

Contamination and subsequent pollution of the environment by toxic heavy metals have become an issue of global concern due to their sources, widespread distribution and multiple effects on the ecosystem as well as their cumulative behavior, toxicity and potential hazardous effects not only on crop plants but also on human health (Oluyemi *et al*., 2008). The situation is even more worrisome in the developing countries where research efforts towards monitoring the environment have not been given the desired attention by the stake holders (Oluyemi *et al*., 2008). Excessive accumulation of heavy metal in agricultural land through vehicular emissions may results in soil contamination and elevated heavy metal uptake by crops, and thus affects food quality and safety and human health (Miclean *et al*., 2007). This work therefore investigated the heavy metals and concentration of toxic elements present in two agricultrural crops cultivated in mining community of Ribi, Awe LGA of Nasarawa State, Nigeria.

II. MATERIALS AND METHODS

The samples were randomly selected within the community where mining and agricultural activities are predominantly practice ‟ collections were done around four notable areas within the community

Experimental samples

The cereal crops (maize and millet) analyzed were collected from four (4) different farm sat the peak of rainy season (between July and August) and dry season around November in Ribi Community in Awe local government area of Nasarawa state. The reason for choosing Ribi community was because its mining nature as well as persistence farming activities. A total of (4) four samples were gathered with each sample randomly hand-picked in a big brown envelope and labelled. The samples were cleaned by sieving and hand separation to remove extraneous materials.

Sample preparation

Analytical reagents (AnalaR) grade chemicals and distilled water were used throughout the study. All glassware plastic containers that were used in this work were washed with detergent solutions followed by 20% (u/v) nitric acid and then rinsed with tap water and finally with distilled water. At the end of the drying, the oven was turned off. The sample was left overnight to enable the sample to cool to room temperature. Each sample was grounded into powder, sieved and stored in a 250cm^3 screw capped plastic jar and appropriately labelled.

Digestion procedure

The 2g of grounded sample was weighed out into100ml standard flask made up to mark with de-ionized water The grounded sample was mixed with 10ml of concentrated nitric acid.

Determination of heavy metal concentration in plants samples

Samples were taken to Chemistry Advance Research Centre, Sheda Science and Technical Complex (SHESTCO) Km 10 Kwali-Abuja Road Gwagwalada for analysis.The mineral elements were analyzed with atomic absorption spectrometer (thermo Scientific, ice3000AA02134104v1.30) equipped with air-acetylene flame. 100ml standard flask was used to filter the digest. The concentration of metals Mg, Pb, Cu, Cd, Fe and Zn samples digests was determined by interpolation while the coding, A= millet control, B= millet dry season, C= maize rainy season, D= maize dry season, E= maize control, F= millet rainy season were used.

Statistical analysis

All analysis was performed in triplicate. Results of heavy metal concentrations in the analysed Crop samples were entered into Microsoft Excel, 2011. Data were transferred into Minitab Statistical software (16.0) for analysis. Data were grouped into segments and described appropriately. Results were presented in Tables, Bar charts, Line plots and Box plots. Inferences were made using: Two ways ANOVA and Two sample T-tests at 95% confidence limit Pearson’s correlation was carried out to determine the relationships among heavy metal residues.

III. RESULTS AND DISCUSSIONS

In millet, lead was highest in raining season (2.831 mg/L) whereas copper and cadmium were very high in dry season (0.586 mg/L and 0.213mg/L respectively), even more than the control. Nickel content in both dry and rainy seasons were lower than the control level of 0.162mg/L (Table 1).

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Table 1: Selected Heavy Metal Residues (Lead, Copper, Cadmium and Nickel) in Millet in Dry and Rainy Seasons

Residues	Millet control (mg/L)	Millet dry season (mg/L)	Millet rainy season (mg/L)
Pb	2.678	2.197	2.831
Cu	0.112	0.586	1.132
Cd	0.108	0.213	0.091
Ni	0.162	0.111	0.15

F (Control, Dry and Rainy seasons) = 1.16, P>0.374 (P>0.05)

As presented in Table 2, magnesium residues recorded the highest values among all heavy metals present in millet. Magnesium in millet was 10.361 mg/L in dry season, lower than the 10.47mg/L in rainy season. Both residues were higher than the control residue (10.329 mg/L). Millet iron contents in dry and rainy season (1.577 mg/L and 1.523 mg/L) were lower than the control level of 2.517mg/L. Similarly, zinc contents in both seasons were also lower than the control level of 0.694mg/L. Seasonal variation and control variables yielded similar results in heavy metal residues (F= 1.20, P>0.05). Figure 1 shows the spectrum of heavy metal residues in millet across two seasons and the control. The top three residues were magnesium, lead and iron. Nickel and cadmium levels were very minute. In rainy season, copper level was far higher than in both dry season and control millet. Among the seven heavy metals investigated, only iron, zinc and nickel residues were higher in control millet than either or both seasonal millets. In other four residues, seasonal results were higher than the control millets.

Table 2: Selected Heavy Metal Residues (Magnesium, Iron and Zinc) in Millet (Dry and Rainy Seasons)

Residues	Millet control (mg/L)	Millet dry season (mg/L)	Millet rainy season (mg/L)
Mg	10.329	10.361	10.47
Fe	2.517	1.577	1.523
Zn	0.694	0.513	0.54

F (Heavy metal residues) = 831.34, P=0.000 (P<0.05)
F (Control, Dry and Rainy seasons) = 1.20, P=0.391, P>0.05

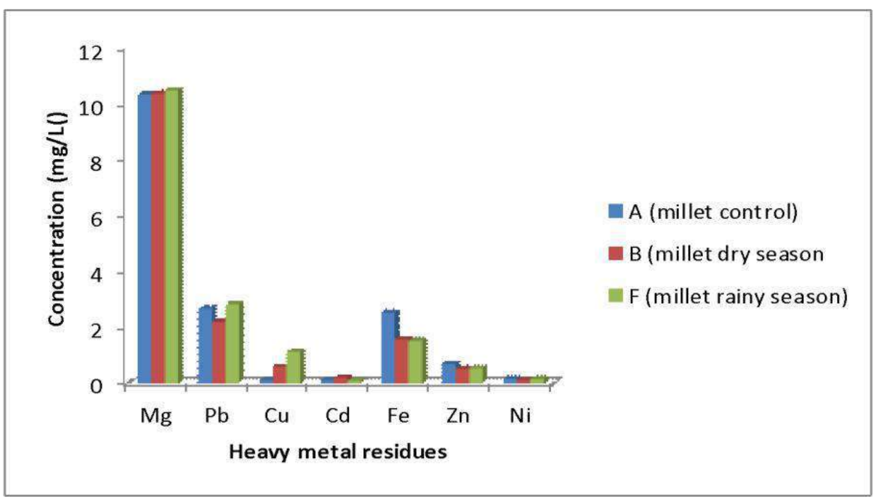

Fig.1: Spectrum of Heavy Metal Residues in Millet across two Seasons

Table 3 gives the concentrations of lead, copper, cadmium and nickel in maize. Lead content was the highest among them recording 2.666mg/L in dry season and 2.538mg/L in rainy season and both values were higher the control maize

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(2.49 mg/L). Copper level was higher in control variable (1.132mg/L) than in seasons. Although cadmium level was relatively low in maize, the amount obtained in rainy season (0.112mg/L) was above the dry season and control. Maize nickel level (slightly raised above cadmium) was almost the same in control, dry and rainy season (0.13mg/L). Seasonal variables and control gave similar results in heavy metal residues in maize (F= 0.42, P=0.677).

Magnesium had the highest concentration among the residues present in maize in the following order: rainy season (10.43mg/L) > dry season (9.33mg/L) > maize control (9.23mg/L) as shown in Table 4. Iron was more concentrated in the control maize (2.65mg/L) than in both dry and rainy seasons whereas zinc recorded higher seasonal concentrations (0.44mg/L) than the control level (0.46mg/L). Statistically, control variable, rainy and dry season produced similar results (F= 0.17, P>0.05). Figure 2 reveals the spectrum of heavy metals in maize. Similar to those in millet, maize had very high concentration of magnesium followed by lead, iron and copper and zinc. Cadmium and nickel levels were much reduced. In copper and iron, values in rainy and dry seasons were below the control level. In other residues, seasonal values were above the control.

Table 3: Selected Heavy Metal Residues (Lead, Copper, Cadmium and Nickel) in Maize (Dry and Rainy Seasons)

Residues	Maize control (mg/L)	Maize dry season (mg/L)	Maize rainy season (mg/L)
Pb	2.49	2.666	2.538
Cu	1.132	0.925	0.831
Cd	0.092	0.091	0.112
Ni	0.134	0.134	0.136

F (Heavy metal residues) = 424.95, P=0.000

F (Control, Dry and Rainy seasons) = 0.42, P=0.677

Table 4: Selected Heavy Metal Residues (Magnesium, Iron and Zinc) in Maize (Dry and Rainy Seasons)

Residues	Maize control (mg/L)	Maize dry season (mg/L)	Maize rainy season (mg/L)
Mg	9.23	9.33	10.43
Fe	2.645	1.688	1.326
Zn	0.441	0.455	0.455

F (Heavy metal residues) = 176.03, P=0.000

F (Control, Dry and Rainy seasons) = 0.17, P=0.851

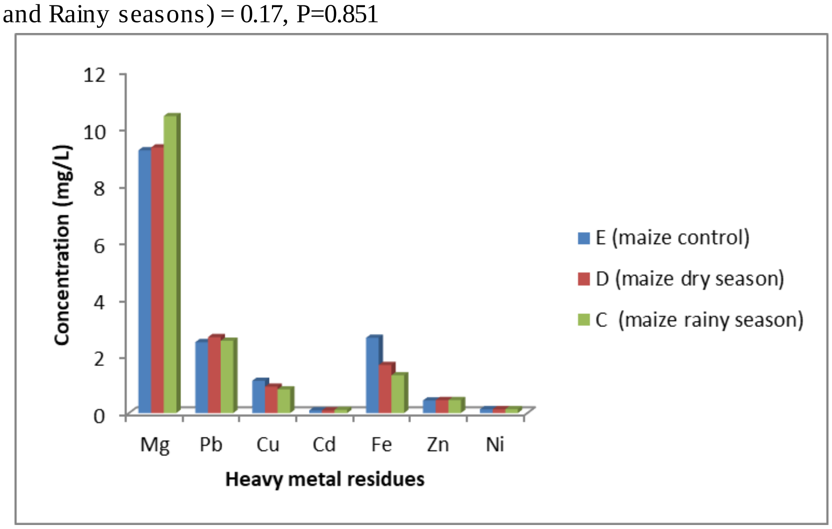

Fig.2: Spectrum of Heavy Metal Residues in Maize across Two Seasons

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Comparing heavy metal residues in maize and millet in rainy season (Table 5) showed that millet had higher concentration of all the residues quantified except in cadmium that was more concentrated in maize (0.11mg/L). In other heavy metals, lead was 2.83mg/L in millet and 2.54mg/L in maize. Copper was 1.32mg/L in millet and 0.83 in maize. Magnesium was 10.47mg/L in millet and 10.43mg/L in maize.

Table 6 compares heavy metal residues in maize and millet in dry season. Maize had higher concentrations of lead (2.67mg/L), copper (0.925mg/L), nickel (0.134mg/L) and iron (1.688mg/L) whereas cadmium, magnesium, and zinc were more in millet than in maize.

Table 5: Comparison of Heavy Metal Residues in Maize and Millet in Rainy Season

Residues	Maize rainy season (mg/L)	Millet rainy season (mg/L)
Pb	2.538	2.831
Cu	0.831	1.132
Cd	0.112	0.091
Ni	0.136	0.15
Mg	10.43	10.47
Fe	1.326	1.523
Zn	0.455	0.54

T= -0.07, P-Value = 0.949, DF = 11

Table 6: Comparison of Heavy Metal Residues in Maize and Millet in Dry Season

Residues	Maize dry season (mg/L)	Millet dry season (mg/L)
Pb	2.666	2.197
Cu	0.925	0.586
Cd	0.091	0.213
Ni	0.134	0.111
Mg	9.33	10.361
Fe	1.688	1.577
Zn	0.455	0.513

T = -0.02, P = 0.984, DF = 11

As given in Table 7, dry season millet was higher in some residues than the control millet. These include: magnesium (10.36mg/L), copper (0.586mg/L) and cadmium (0.213mg/L) in dry season. However. Millet control was higher than dry season millet in lead, iron, zinc and nickel. No statistical significant differences exist in heavy metal concentrations between millet control and the dry season type (t= 0.08, P = 0.941).

Table 8 reveals some millet residues that were higher in rainy season than the control level. Magnesium level was 10.47mg/L and 10.33 in control. Others are: lead (2.83mg/L in rainy season as against 2.68mg/L in control), copper (1.13mg/L in rainy season as against 0.11mg/L in control). Heavy metals below the control level in millet are: cadmium, iron, zinc and nickel . There are no significant differences in the values of heavy metal residues recorded in both millet types (control and rainy season) (t = 0.01, P = 0.992). Statistical uniformity in quantification of heavy metals in the two millet types.

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Table 7: Comparison of Heavy Metal Residues in Millet (Control and Dry Season)

Residues	Millet control (mg/L)	Millet dry season (mg/L)
Mg	10.329	10.361
Pb	2.678	2.197
Cu	0.112	0.586
Cd	0.108	0.213
Fe	2.517	1.577
Zn	0.694	0.513
Ni	0.162	0.111

T-Value = 0.08, P = 0.941, DF = 11

Table 8: Comparison of Heavy Metal Residues in Millet (Control and Rainy Season)

Residues (mg/L)	Millet control (mg/L)	Millet rainy season (mg/L)
Mg	10.329	10.47
Pb	2.678	2.831
Cu	0.112	1.132
Cd	0.108	0.091
Fe	2.517	1.523
Zn	0.694	0.54
Ni	0.162	0.15

T = -0.01, P = 0.992, DF = 11

As shown in Figure 9, the control line (blue line) is below many residues but slightly raised in copper. The control line is widely raised in iron level. Generally, the two lines (control and total maize residues in combined season) are convergent at many points but divergent around magnesium, copper and iron.

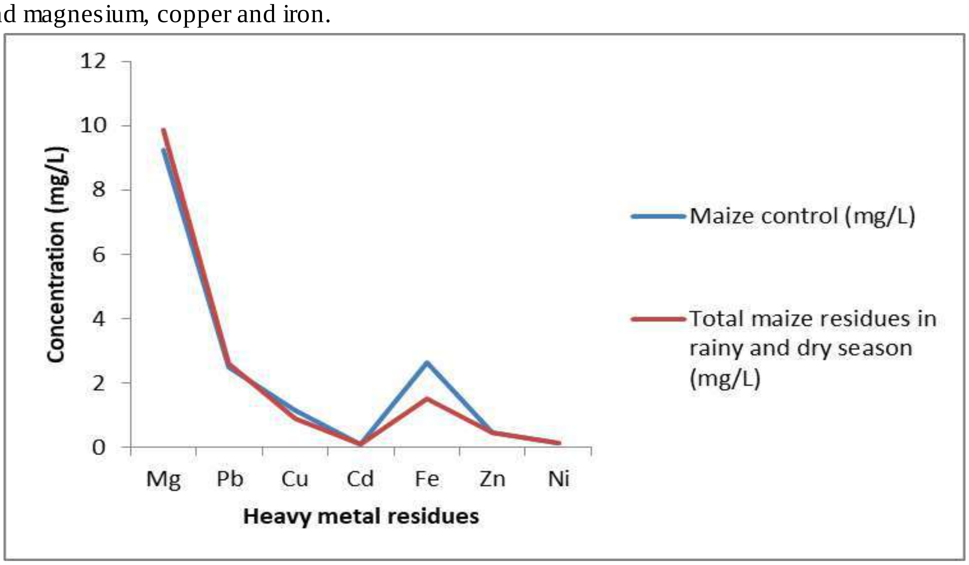

Fig.9: Total heavy metal residues in maize (combined seasons)

Figure 10 shows much similarity in the millet control line (blue) and the total residues in combined season line (red) most especially in magnesium and lead. Copper residues were far above the control line. However, iron level was far belo w the control line. Zinc and nickel levels are slightly below the control line

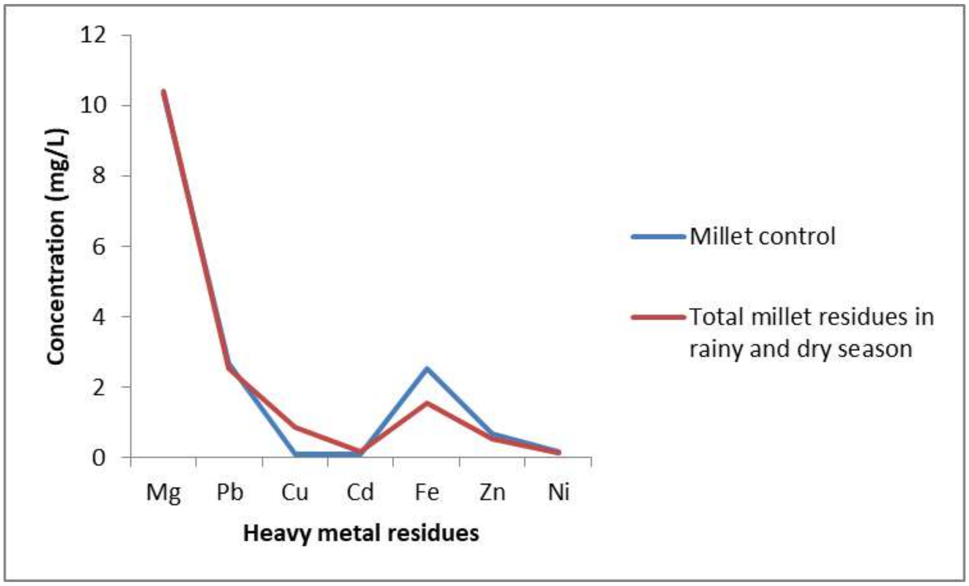

Fig.10: Total heavy metal residues in millet (combined seasons)

According to (Turpeinen (2002), sources of metals contamination can be divided into five major groups: mining, smelting, industry, atmospheric deposition, agriculture and waste disposal The Lead concentration in millet across season was higher than WHO permissible limit (0.01mg/l). This could be as a result of mining activities that introduced Lead to the soil and subsequently to the Millet in the study area. Young children are particularly vulnerable to the effect of Lead and can suffer profound and permanent adverse health effect, particularly affecting the development of the brain and nervours system. Lead also causes long term harm in adults including increased risk of high blood pressure and kidney damage. Similar findings were reported by Ahmed and Mohammed (2005) and Okoye *et al*. (2009).

The concentration of Copper in Millet is highest during dry season when compared with rainy season and the control. Aremu *et al*. (2006) reported similar finding on the assessment of some heavy metal content in some selected agricultural products planted along some roads in Nasarawa State. The result shows that millet has higher Copper content during dry season than in rainy season. The high content of Copper (Cu) in millet during the dry season could be due to deposition of Copper on the surface of these grains during mining or production. Although the deficiency of Copper is rare but it can lead to cardiovascular disease and other problems however most Copper in the body is found in the liver, brain, heart and skeletal muscles and it helps the body to form collagen and absorb iron and play a role in energy production (Okoye *et al*., 2009).

The concentration of Cadmium in the millet is lower when compared to Lead and Copper content across season. This aligned with the work of Wyasu *et al*. (2010). Also, the values of concentrations of Cd in cereals are below the WHO standards (Dahiru *et al*, 2013) (Orisakwe *et al*., 2012). The values are also below NESREA standard for Food, Beverage and Tobacco, (2009).

Higher concentration of Magnesium was found in millet during rainy season similar to the finding of Adamu and Bhagwan (2017). This may offer useful benefits to the body. Deficiency of this element causes growth retardation, nausea, muscle weakness and it may affect cardiac function (Mohammed and Ahmed, 2014). The recommended daily intake of magnesium in male adult is 420mg/day and that of female adult is 320mg/day (Mohammed and Ahmed, 2014).

IV. CONCLUSION

The values of Pb, Zn, and Ni in millet and maize are above the WHO's standard guideline while other residues are within or below the regulatory limits. Mining activities taking place around the in the study location might have

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impacted negatively on the safe consumption of agricultural crops cultivated by farmers.

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Assessment of Pesticide Residues in Some Commonly Cultivated Vegetables in Doma Metropolis, Nasarawa State, Nigeria

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***Abstract*— *Commonly cultivated vegetables (Amaranthus hybridus, and Corchorus olitorius) in Doma and environs were analyzed to determine the residual level of pesticide. The samples extracts were subjected to High Performance Liquid Chromatography (HPLC) using Acetonitrile, Anhydrous MgSO4, NaCl and Ultrapure water used as mobile phase. Data obtained were analyzed using Minitab 16.0.The following tests were applied: Chi square test of association, Mann Whitney U-test of comparing two non parametric systems, Kruskal Wallice H-test and the Independent t-test of significance. All hypotheses were tested at 95% level of significance. Analysis showed highest concentrations residual level of Cypermethrin in the Doma wet Corchorus olitorius sample at 2240.94mg/L with no traces in Igbabo sample. For the dry Corchorus olitorius sample, highest value was recorded in Doma (61.046mg/l) while Igbabo sample showed the lowest (9.285mg/l). The highest residual value (226.39mg/l.) of Cypermethrin in Amaranthus hybridus appeared at Igbabo with the lowest (12.18mg/l) in Alwaza Lamda Cyhalothrin, Alwaza wet Corchorus olitorius sample recorded highest value of 0.00593mg/l with lowest value (0.00058mg/l) in Doma. Dry sample jute recorded highest (0.0159mg/l) in Igbabo; lowest value (0.0002mg/l) in Doma. In spinach samples, the highest value (0.00171mg/l) appeared in Alwaza while igbabo (0.00051mg/l) recorded the lowest. The results showed that Cypermethrin values were well above the Japan Research Foundation MRLs of 0.03mg/l. This has serious implications on the consumers of the vegetables cultivated in the study area. However, for Lamda Cyhalothrin residues results occurred on levels well below MRLs of 0.5mg/l.*

***Keywords*— Health Risk, Jute, Pesticide, Residue, and Vegetables.**

I. INTRODUCTION

Pesticide is an umbrella for all insecticide, herbicides, fungicides, rodenticides, wood preservatives, garden chemicals and household disinfectants that may be used to kill some pest [10]. Pesticides are mostly man-made chemicals agents use to control insects, weeds, fungi and other pests that destroy crops [17] [23]. Different kind of pesticides are used for pest management and vector control in agricultural farms, even though many farming communities are not adequately informed about the hazards associated with the chemicals [26]. Pesticide has considerably help to reduce loses and gives a better yield in agricultural produce [4]. Therefore, knowledge on the use of pesticide is considered to be a fundamental factor in maintaining high agricultural productivity [26]. However, reliance on pesticide is difficult to sustain due to adverse effect on the environment. As a result to their high degree of toxicity, most pesticides are harmful to both human and the environment [13]. Although pesticides are manufactured under very strict regulations processes to function with logical certainty and minimal impact, excessive application of these pesticide results into bioaccumulation of its residues [19]. Pesticide residues are the deposit of the active constituent. Its metabolites or breakdown products are present in some component of the environment after its application, spillage or dumping [25].

More than a few reports show that the usage of pesticide has increased significantly during the last three decades consequent with changes in farming practices and the increasing intensive agriculture. This extensive use of pesticides has resulted in the presence of their residues in various environmental matrices, especially food stuff proving the high risk of these chemicals to human health and the environment. Studies have shown that vegetable intake is positively related in urinary metabolites levels of pyrethroid pesticides [20]. Other hazardous pesticide is Organochlorines pesticides and are the most persistent class of these pesticides; however, due to their adverse effects on human health and the environment, they have been banned in most countries [23].

In Nigeria, Vegetable farmers use a wide range of pesticides at different levels to reduce losses from pest and diseases. However, despite the contribution of pesticide to agricultural production, evidences in other studies showed presence of pesticide residues that could be detrimental to human health and the ecosystems [26]. Among the traditional vegetables in Nigeria are *Amaranthus hybridus (spinach) Corchorus olitorius (Jute), Talinum Triangulare (water leave), Telfairia occidentalis (ugu.)*. Most of these vegetables are consumed in the rural areas or in the communities where they are being planted and sold in the open markets [3]. Nowadays, most vegetables are grown all over the world as climate permits, [15]. Pesticide residues most commonly found in food samples of vegetable farms are pesticides that are intentionally applied to the plants to attack pests and plant diseases [21]. When these pesticides are applied to destroy pests and pathogens, only 15% of the applied amount hits the target, with the remaining 85% being distributed in soils and air [11]. Also, the improper implementations of hazardous chemicals and pesticide regulations and lack of awareness on technical knowhow among the farming communities leaves most of the pesticides active ingredients in vegetables. The present study aimed to investigate and determine pesticide residues in commonly cultivated vegetables grown in three selected communities (Alwaza, Doma, and Igbabo) of Doma Local government Area of Nasarawa state, Nigeria.

II. MATERIALS AND METHODS

Description of the Study Area

The study area Doma metropolis, is located in the North central Nigeria, it is marked by dry season from the month of November to May. Rainfall is mostly conventional type caused by persisting heating of land air mass. The months of December to February are characterized by slight cold harmattan, while March- April hottest period with temperature above 30^{0}C. It covers an area of 2726sq kms with an estimated population of over 98,803 people (2006 census).

Samples collection

To determine the residual pesticides in the vegetables of the study area, fresh samples of vegetables (*Amarathus hybridus* and *Corchorus olitorius)* were collected from the three communities' farms randomly; Alwaza (8^{0}22'40.88''N 008^{0}23'06.79"E), Igbabo (08^{0}12'36.69"N 008^{0}17'55.60"E) and Doma (08^{0}23'42.09"N 008^{0}2119.81"E). The vegetables samples were taken in plastic bags at room temperature to Chemistry Advance Research Center, Sheda Science and Technical Complex (SHESTCO) km 10 Kwali -Abuja Road Gwagwalada for analysis.

Reagents and materials

Residues of pesticides were determined in two types of vegetables samples by using High Performance Thin Layer Chromatography (HPLC). Analytical-grade (BEST and ATTAKE) pesticides standard were ordered from certified seller for the standard solution in liquid form. 1% solution of the standard was prepared. Acetonitrile of HPLC grade was purchased from FINLAB. Deionized water, dimethyl formamide; anhydrous magnesium sulfate, 500 ml Erlenmeyer flask was selected and Primary Secondary Amine (PSA)-bonded silica were used for the sample preparation procedure. Anhydrous MgSO4, and NaCl were obtained from Sheda Science and Technical Complex (SHESTCO) Ultrapure water was used as mobile phase, which were filtered through a 0.45 mm polyvinylidene difluoride (PVDF) filter before injection. Standard stock solution was prepared by dissolving the liquid standard in acetonitrile to reach the final concentration of 1000 to 4000 mg/ml. For method optimization, standard solutions were used, which were prepared by diluting the stock solution to a concentration of 1 to 4 mg/ml. A standard mix solution in acetonitrile for preparation of calibration standards was prepared to yield 10 mg/ml.

Sample preparation

For the detection of Cypermethrin concentrations in the samples, the acetate-buffered sample preparation method for pesticides (AOAC Official Method 2007.01) was applied to all the samples. After homogenization with a house-hold (equipped with stainless steel knives). 10 g of the well-chopped, homogenized sample was weighed into a 40 mL polypropylene (PP) centrifuge tube followed by addition of 10 mL of acetonitile and shaking the sample vigorously for approximately 1 minute. Next, an addition of 4 g anhydrous MgSO4 and 1g NaCl is followed by intense agitation. Afterwards, a 1 mL aliquot of the upper acetonitrile layer is transferred into a centrifuge vial containing 25 mg of PSA sorbent and 150 mg of anhydrous MgSO4. Then, the sample is shaken by hand or with the vortex mixer for 30 seconds and centrifuged. The obtained supernatant is taken from the centrifuge vial and as a final extract can be analyzed directly using LC-techniques coupled with mass spectrometry detectors. To determine the concentration of Lamda Cyhalotrhrin in the vegetable samples, approximately 10g of each sample was also weighed and macerated and 10 ml of water-methanol (50/50) was added. It was soaked overnight. The filtrate was partitioned with 10 ml acetone- hexane and the hexane layer was collected into a sample bottle. HPLC analysis Mobile phase: Acetonitrile/water (20/80 v/v) Wavelength 250nm Flow rate: 1ml per mins; sample injected 20µl

High Performance Liquid Chromatographic (HPLC) Analysis

Extracted samples of vegetables were analyzed by high performance liquid chromatography (HPLC) following the method of [16] HPLC analyses were performed in isocratic system using a PerkinElmer Chromatograph including Series 200 pump, Series 200 UV/VIS detector, and a Supelco C18 analytical column (25 cm x 4.6 mm (i.d)). Acetonitrile/water was used as mobile phase. 20 µl sample was injected through auto sampler. The column temperature was kept 30 ºC with a flow rate of 1ml min-1**.**

Statistical analysis

Data obtained from structured questionnaires were collated and entered into Microsoft Excel Workbook (2010 version). Descriptive statistical operations were applied using frequency counts, percentages, tabulations pie chart and bar chart. Data obtained from quantification of pesticides in plant samples were analyzed using Minitab 16.0. Appropriate unit conversions were done to ensure uniformity. The following tests were applied: Chi square test of association, Mann Whitney U-test of comparing two non parametric systems, KruskalWallice H-test as a non parametric One-way ANOVA analog and the Independent t-test of significance. All hypotheses were tested at 95% level of significance

III. RESULTS AND DISCUSSION

Table 1 presents the quantity of Cypermethrin found in wet jute plant. The Control wet Jute plant had 0.00mg/L of Cypermethrin. At Igbabo, the concentration of this pesticide was also 0.00mg/L. Wet Jute at Doma had 2240.94mg/L of Cypermethrin. About 29.22mg/L of the pesticide was found in the Alwaza jute sample. Significant association exists between Jute sample location and quantity of Cypermethrin found (χ^2 =6579.73, P=0.000). Table 2 gives the quantity of Cypermethrin in dry jute plant. The control dry sample had 1.753mg/L of Cypermethrin. Quantities of this pesticide found in all other samples were higher than the control samples as stated in order of magnitude: Doma jute (61.05mg/L), Alwaza jute (58.96mg/L) and Igbabo jute (9.29mg/L). Significant association exists between quantity of cypermethrin and the dry jute sample collected from different locations (χ^2 = 91.5468, P=0.000).

Table 3 compares Cypermethrin level in wet and dry jute samples. No particular pattern of relationship was established in the pesticide level in the wet and dry samples across the locations. At Igbabo, cypermethrin level was nil in wet jute whereas the dry type recorded 9.285mg/L of the pesticide. At Doma, the wet jute had skyrocketed level of 2240.94mg/L far higher than the 61.05mg/L of the dry sample. At Alwaza, the dry state had higher level of the pesticide than the wet state. Hence, Mann Whiteney U test recorded significant differences in the level of Cypermethrin in wet and dry jute sample depending on the location (U= 16.0, P<0.05).

Table 4 gives the quantity of cypermethrin found in spinach in different locations. The control spinach had zero level of cypermethrin. Igbabo spinach had the highest level of the pesticide with 226.39mg/L followed by Doma spinach with 135mg/L and Alwaza spinach with 12.18mg/L of cypermethrin. Significant association exists between cypermethrin level and the locations of spinach samples (χ^2 = 371.880 P= 0.000). Table 5 compares cypermethrin level in wet jute and spinach across different locations. Cypermethrin contents were higher in wet jute collected from Doma and Alwaza than in spinach samples collected from the same locations. However, the Igbabo spinach had 226.39mg/L of cypermethrin whereas the Igbabo wet jute did not contain cypermethrin. Mann-Whitney U test showed significant differences in pesticide levels in wet jute and spinach sample (U=17.0, P<0.05).

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Table:1 Quantity of Cypermethrin in Wet Jute (Corchorusolitorius)

Sample	Quantity of Cypermethrin Pesticide (mg/L)
Wet Jute (Igbabo)	0.00
Wet Jute (Doma)	2240.94
Wet Jute (Alwaza)	29.22
Wet Jute (Control)	0.00

χ^2 =6579.73, P=0.000 (P<0.05)

Table 2: Quantity of Cypermethrin in Dry Jute (Corchorusolitorius)

Sample	Quantity of Cypermethrin Pesticide (mg/L)
Dry Jute (Igbabo)	9.285
Dry Jute (Doma)	61.046
Dry Jute (Alwaza)	58.964
Dry Jute (Control)	1.753

χ^2 = 91.5468, P=0.000 (P<0.05)

Table 3: Comparison of Cypermethrin Level in Wet and Dry Jute Samples (Corchorusolitorius)

Location	Cypermethrin in Wet Jute (mg/L)	Cypermethrin in Dry Jute (mg/L)
Igbabo	0.00	9.285
Doma	2240.94	61.046
Alwaza	29.22	58.964
Control	0.00	1.753

U= 16.0, P<0.05

Table: 4 Quantity of Cypermethrin in Spinach (Amaranthus)

Sample	Quantity of Cypermethrin Pesticide (mg/L)
Spinach (Igbabo)	226.39
Spinach (Doma)	135.69
Spinach (Alwaza)	12.18
Spinach (Control)	0.00

χ^2 = 371.880 P= 0.000 (P<0.05)

Table 5: Comparison of Cypermethrin Level in Wet Jute and Wet Spinach

Location	Cypermethrin in Wet Jute (mg/L)	Cypermethrin in Wet Spinach (mg/L)
Igbabo	0.00	226.39
Doma	2240.94	135.69
Alwaza	29.22	12.18
Control	0.00	0.00

U=17.0, P<0.05

Table 6 presents the quantity of lamdacyhalothrin in wet jute samples obtained from different sites. Lambacyhalothrin levels were generally diminutive in wet juice across plant samples. It ranged from 0.00003mg/L in Alwaza wet Jute to 0.00593mg/L in control wet jute being higher than other samples. Based on KruskalWallice H-test,

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no significant differences exist in the pesticide level of the samples analysed (H = 3.00, P = 0.392). As presented in Table 7, the quantities of lamdacyhalothrin in dry jute samples are diminutive and insignificant across samples (H = 3.00, P = 0.392). It ranged from 0.00001mg/L in Alwaza dry jute to 0.00159 in Igbabo dry jute. The control level (0.00108mg/L) was higher than two samples but lower than the Igbabo dry jute.

Lamdacyhalothrin contents in spinach samples were also diminutive and low (Table 8). The control sample had higher pesticide content (0.00171mg/L) than other samples in Doma (0.00121mg/L) and in Igbabo (0.00051mg/L). The Alwaza spinach had no trace of lamdacyhalothrin. No significant differences exist in the pesticide levels of the various samples analysed (H = 3.0, P = 0.392). Table 9 compared lamdacyhalothrin contents of wet jute and spinach despite the diminutive level. In both plant types, the control levels were higher than other samples. In Igbabo, pesticide was higher in wet jute (0.00104mg/L) than in spinach (0.00051mg/L). In Doma, the level was higher in spinach (0.00121mg/L) than in wet jute (0.00058mg/L). In Alwaza, no trace of the pesticide in spinach whereas wet jute had very slight amount (0.00003mg/L). Statistically, no significant differences exist in the lambacyhalothrin content in the two plant types (wet jute and spinach) as obtained from various sites (T = 0.73, P = 0.516).

Table: 6 Quantity of LamdaCyhalothrin in Wet Jute (Corchorusolitorius)

Sample	Quantity of LamdaCyhalothrin Pesticide (mg/L)
Wet Jute (Igbabo)	0.00104
Wet Jute (Doma)	0.00058
Wet Jute (Alwaza)	0.00003
Wet Jute (Control)	0.00593

KruskalWallice H = 3.00, P = 0.392 (P>0.05)

Table 7: Quantity of LamdaCyhalothrin in Dry Jute (Corchorusolitorius)

Sample	Quantity of LamdaCyhalothrin Pesticide (mg/L)
Dry Jute (Igbabo)	0.00159
Dry Jute (Doma)	0.00020
Dry Jute (Alwaza)	0.00001
Dry Jute (Control)	0.00108

KruskalWallice H = 3.00, P = 0.392(P>0.05)

Table 8: Quantity of LamdaCyhalothrin in Spinach (Amaranthus)

Sample	Quantity of LamdaCyhalothrin Pesticide (mg/L)
Spinach (Igbabo)	0.00051
Spinach (Doma)	0.00121
Spinach (Alwaza)	0.00000
Spinach (Control)	0.00171

H = 3.0, P = 0.392 (P>0.05)

Table 9: Comparison of LamdaCyhalothrin in Wet Jute and Spinach

Location	LamdaCyhalothrin in Wet Jute (mg/L)	LamdaCyhalothrin in Wet Spinach (mg/L)
Igbabo	0.00104	0.00051
Doma	0.00058	0.00121
Alwaza	0.00003	0.00000
Control	0.00593	0.00171

T = 0.73,P = 0.516 (P>0.05)

IV. DISCUSSION

The study revealed excessive applications of the pesticides within the life cycle of the vegetables attributing to the high percentage of residual content in the vegetables. Our study was in agreement to research done by [16]. Also in the research by [26], it was revealed that the effects of pesticide exposure even for a short duration has the possibility of cumulative effects which farmers are themselves unaware of the health hazards associated with it. The detected cypermethrine residues in wet jute plant from the two communities (Doma and Alwaza) were above MRLs as prescribed by FAO/WHO. In all the three communities of the study area, the dry jute samples showed concentrations of cypermethrin residues ranges from 9.285mg/L-61.046mg/L. The study also showed that the concentrations of cypermethrin in Doma wet jute was higher than the dry sample whereas the Alwaza dry jute sample was higher than the wet sample. The findings had similarity with researched by [16] which established that the levels of cypermethrin and lamda cyhalothrin residue in vegetables were above their respective MRLs values. Consequently, this work has confirmed the work of [16] regarding the observation that some pesticide residue in vegetables are above the MRLs. Similarly, [13] experimented on Cabbage, collected samples from different markets cape coast Ghana to assess the pesticide residues in the vegetable. The detected Cypermethrin and Lamda Cyhalothrin were below the residues detected in this research work. Unlike the study by [9] which indicated that Lamda Cyhalothrin was the most used pesticide in the control of pest in vegetable farms, in this study majority of the farmers applied Cypermethrin.

Also in the study by [17] Cypermethrin residue was determined in high concentrations when compared with other pesticides. This collaborated with this study where Cypermethrin residues in the selected vegetable samples were higher than the concentrations of lamda Cyhalothrin. As a result of these, the high level of the residues in the study suggested that pesticides were applied in excess; it is therefore plausible to state that farmers were not following proper precautions with regards to the use of pesticide in appropriate dosages and standards attributing to lack of training. The results also showed that cypermethrin residues in almost all the spinach samples were high in concentration which is in agreement with [16], who found out that cypermetrhin residues were found higher in almost all the vegetables analyzed.

Previous work done by [18] showed that Lamda Cyhalothrin was widely used on vegetables farms because of their effectiveness. The level of residues detected in this work was lower compared to [18]. Also the result from the study showed that Lamda Cyhalothrin residues were diminutive in wet juice across plants samples of the three communities of the study area. The low levels of the residue could be due to minimal usage of the pesticide by farmers and the unavailability of the products in the market. The result collaborated with [16] [13] research on cabbage, where lamda cyhalothrin residues detected was lowest in concentrations when compared to Cypermethrin residues. Also the findings in this study indicated diminutive and insignificant values across both wet and dry jute plants samples ranging from 0.00020 to 0.00159mg/l. In the spinach samples, the study showed little value of lamda cyhalothrin across the plant samples. The residues range from 0.00051 to 0.00171mg/L. The study showed slight variations of Lamda cyhalothrin in wet jute and spinach samples. The concentrations of lamda cyhalothrin residues in both wet jute and spinach of Alwaza sample were higher than Doma and Igbabo. Although pesticide helps to protect our vegetables during growth, however they may remain in or on foods as small amount after applications. In general the excessive applications of cypermethrin and lamda cyhalothrin in vegetables farms indicated high concentrations of residues when compared with other studies; these imply that there is increase trend in Nigeria about pesticide usage which can lead to significant health problems.

V. CONCLUSION

The two vegetables studied contained residues of Cypermethrin pesticide in high concentrations when compare to Lamda Cyhalothrin pesticides mostly detected above MRLs. This calls for strict implementation of regulations and proper monitoring by relevant stakeholders. Public enlightenment and training of farmers also by relevant agencies and nongovernmental organizations is necessary to reduce the hazards associated with excessive applications of pesticide in vegetables as these may affect the food chain. The need for possible alternative to agro chemicals rather than applications of pesticides in vegetables should be encouraged so that the extent of pesticide damage on public health and the environment could be regulated.

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Load Capacity of Water Pollution of Jaing River in Tabalong

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***Abstract*— *Jaing River is part of the 39 km long Barito watershed in South Kalimantan which disembogues into Tabalong River. Jaing River is classified as class I (one) river that is designated as a source of drinking water. The large number of activities in the bank of the river has a potential to reduce the quality of the river. Field observations revealed that some activities created several pollutions and also run off of production waste into the river. Thus, the purpose of this study was to analyze the load capacity of pollution of Jaing River. The study was carried out in Jaing River as far as 39 Km in TabalongRegency and the river water quality was analyzed at 3 sampling points using physical, chemical and biological parameters. The analysis of quality and determination ofload capacity of water pollution used Mass Balance Method. Finally, the results obtained from this study are (1) the BOD pollutant load value of 418.87 kg/day, the value of COD pollution load of 2018.90 kg / day and the value of TSS pollution load of1698.14 kg/day. (2) The value of pollution load capacity (DTBP) for BOD parameters is 246.07 kg/day, COD parameters are 1154.90 kg/day and for the TSS parameters are 2621.86 kg / day.*

Keywords*— *Jaing River, load capacity of pollution, mass balance method.

I. INTRODUCTION

The rapid development in Tabalong Regency has a significant impact, both positive and negative. The river becomes the media that received this impact as a result of the intense exploration of natural resources.

Sahabuddin et al (2014) stated that the input of waste to the environment from human activities without regarding to the ability of supporting capacity and load capacity of the environment causes a negative influence on the quality of ecosystems either physical, chemical or biological as well as the sustainability of the aquatic environment.

The life of the people of Tabalong Regency cannot be separated from the existence of river; most of them use river water as a source of clean water. JaingRiver is one of the rivers that pass in TabalongRegency with a length of 39 km which *disembogues* into Tabalong River. The Jaing watershed has an area of ± 298 km^{2}as parts of the Barito watershed in South Kalimantan. Jaing River is classified as class I (one) river that is designated as a source of drinking water and other purposes which require the same quality (Tabalong Regency Environmental Agency, 2017).

Transfer of land functions is quite large around Jaing River watersheds, for land clearing of coal mining sector, oil and gas sector, industrial sector, plantation and agriculture sector. The large number of these activities causes theriver potentially experiences a decline in quality. Data from the TabalongRegency Environmental Service stated that the water quality of Jaing River has decreased every year. Field observations show that some activities have the potential to incorporate pollutants into the Jaing River which is likely to dispose of production waste directly or run off of production waste into the river. Sources of run off pollutants tend to be difficult to control because they are scattered.

Pollution is the entry or inclusion of living things, substances, energy, and/or other components into the environment, or it also means changes in the environment by human activities or natural processes so that the quality of the environment drops to a certain level which causes the environment to become less or unable to function anymore in accordance with its designation.Pollutant sources or pollutants are substances or materials that can cause pollution to the environment either pollution of water, air, soil or others. If it is true that there is a decrease in the quality of Jaing River water, of course this will bring many problems that arise for the community around the banks of the Jaing River, both in terms of the level of health and quality of life of the community. Based on this problem, the purpose of this study is to analyze the load capacity of water pollution of Jaing River.

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II. LITERATURE REVIEW

Water Pollution and Pollution Sources

Effendi (2003) said that pollution loads (pollutants) are materials that are foreign to nature or materials that originate from nature itself that enter an ecosystem order that disrupts the designation of the ecosystem. In contrast to Effendi, Suratmo defined water pollution as starting from a certain concentration of pollutants in water for a long time which is able to cause certain influences (SahabuddinHartina, Harisuseno, &Yuliani 2014).

Water pollution is a result of human activities and actions, which are motivated by various things. Because of pollution, the environmental management of the water is disrupted. Water ecosystems become polluted and damaged after receiving the presence of contaminants originating from humans by their actions (Susila, 2011). Water pollution can occur intentionally or unintentionally from human activities in a water that has been clearly designated(Herlambang 2006).

Manan (1977) stated that river water quality problems are mainly caused by sediment content in river water due to erosion in the watershed, especially in the upstream. Water quality is the level of suitability of water for certain uses in meeting the needs of human life, starting from water to meet immediate needs, namely drinking water, bathing and washing, irrigation water or agriculture, livestock, recreation and transportation.

Capacity of Pollution Load

The terminology of environmental capacity in regulation in Indonesia is usually carried out with environmental supporting capacity. Environmental supporting capacity is the ability of the environment to support human life, other living things, and the balance between the two things. Meanwhile, the capacity of the environment is the ability of the environment to absorb substances, energy, and/or other components that enter or are inserted into it (Abdi et al., 2010).

In terms of load capacity of the pollution refers to the Decree of the Minister of Environment Number 110 of 2003, it is the ability of water in a water source to receive input from pollutant loads without causing the water to become polluted. The pollution load itself is the amount of a pollutant contained in water or waste water (Sahabuddin et al., 2014).

There are 2 (two) calculation methods used in determining the load capacity of water pollution on water sources, namely (1) Mass Balance Method and (2) Streeter-Phelps Method.

III. STUDY METHODS

A. Place and Time of Study

The study area was carried out in JaingRiver as far as 39 Km in Tabalong Regency, South Kalimantan Province by dividing it into 3 (three) segments, namely upstream, middle and downstream. The sampling location can be seen in Table 1 below.

Table 1. Locations of Water Quality Sampling in Jaing River

Segment	Location	Latitude (S)	Longitude (E)
upstream	PangelakVillage, Upau District	02'04"21.1	115'37"25.4
middle	Kasiau Village, MurungPudak District	02'07"41.6	115'27"20.1
downstream	Masukau Village, MurungPudak District	02'07"05.9	115'25"21.7

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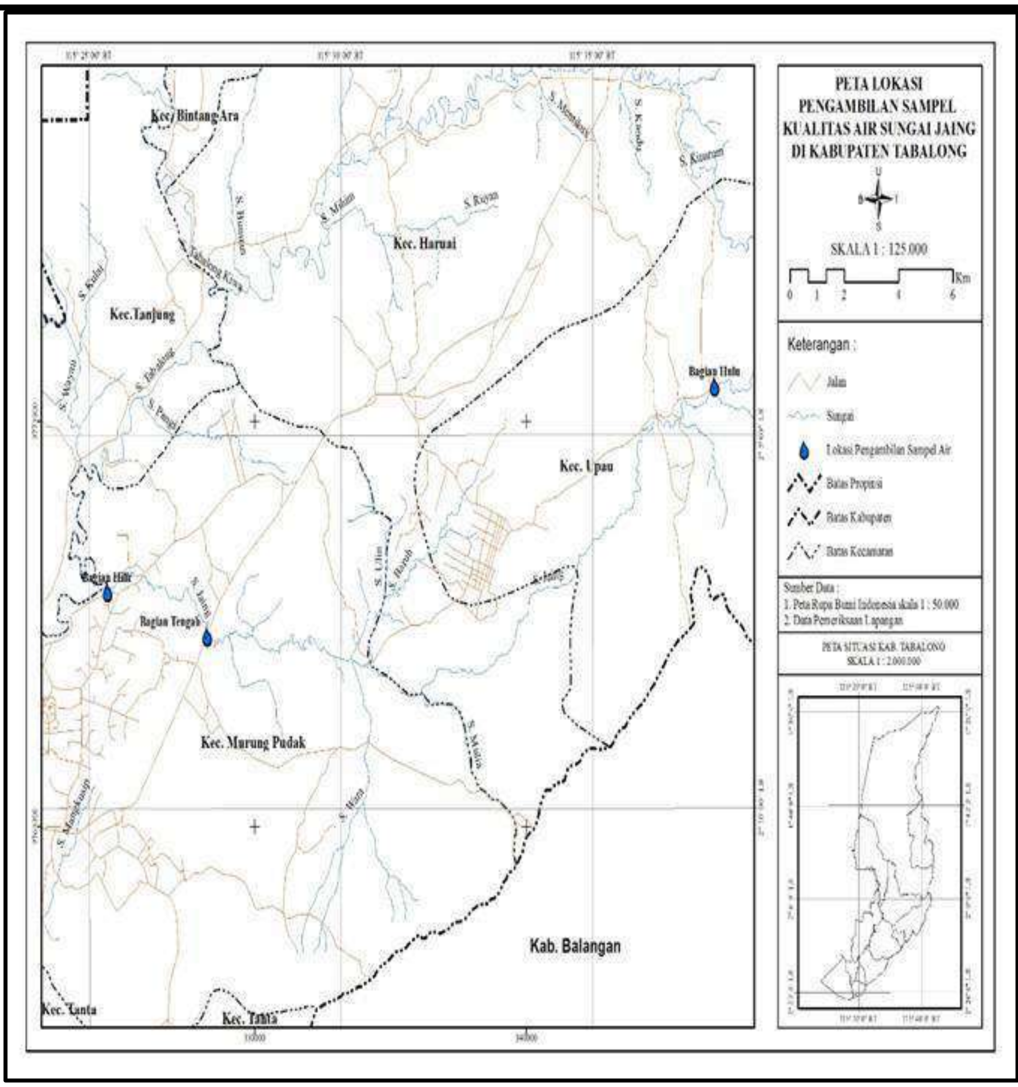

Fig.1: Location of Water Sampling

The time for conducting this studywas 4 (four) months, namely from January to April 2019 and the river water sampling period took place 3 (three) times starting from February to March.

The parameters analyzed include physics, chemistry and microbiology parameters with the analytical method adjusted for the parameters studied as shown in the following table.

Table 2. Physical, chemical, water biology parameters and their analytical methods

No.	Parameter	Unit	Analysis Method
	Site		
1	Temperature	0C	SNI 06-6989.23-2005
2	pH	-	SNI 06-6989.11-2004
3	DHL	µ mhos/cm	SNI 06-6989.1-2004
4	Dissoved Oxygen (DO)	mg/L	SNI 06-6989.14-2004
	Laboratory		
5	Suspended Solid	mg/L	SNI 06-6989.3.2004
6	BOD	mg/L	SNI 6989.72-2009
7	COD	mg/L	SNI 6989.2-2009
8	Phospate	mg/L	SNI 06-6989.31-2005
9	Fecal Coliorm	mg/L	MP N
10	Total Coliform	mg/L	MP N

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B. Determination Analysis of Load Capacity of JaingRiver Pollution

Analysis of capacity determination is carried out by the *Mass Balance* method.

$$C_R = \frac{\Sigma CiQi}{\Sigma Qi} = \frac{\Sigma Mi}{\Sigma Qi} \text{..........................(1)}$$

Where :

C_R = The average concentration of constituents for the combined flow

C_i = Concentration of constituents in the i- flow

Q_i = i-flow rate

M_i = Constituent mass in the i- flow

To determine the load capacity using the Mass Balance Method, the steps that must be taken are as follows:

1. Measuring the concentration of each constituent and the flow rate in the stream before mixing with pollutant sources,
2. Measuring the concentration of each constituent and the flow rate in each pollutant source stream,
3. Determine the average concentration in the final flow after the flow mixes with the pollutant source by calculating:

$$C_R = \frac{\Sigma CiQi}{\Sigma Qi} = \frac{\Sigma Mi}{\Sigma Qi} \text{............................ (2)}$$

In another source, the method for calculating pollution load was based on measurements of river water flow and river waste concentration based on the equations of Mitsch and Goesselink (1993) in Appendix II of the Regulation of the Minister of Environment No. 1 of 2010.

BPs = Qs x Cs (j) x f .... (3)

Information :

BPs = River Pollution Load (kg/day)

Qs = River Water Debit (m^3/sec)

Cs (j) = Pollution Element Concentration (j) (mg/l)

F = FaktorKonversi =

$$\frac{1\ kg}{1.000.000\ mg} x \frac{1000\ liter}{1\ m3} x \frac{84.600\ detik}{1\ hari} = 86{,}4 \frac{kg.liter.detik}{mg.m3.hari}$$

Pollution load capacity (DTBP) can be determined using the following equation:

DTBP = Pollution Load According to Quality Standards - Measured Load Pollution

IV. RESULTS AND DISCUSSION

The analysis of the calculation of pollution load capacity (DTBP) of river water is carried out on 3 (three) parameters, namely BOD (Biological Oxygen Requirement), COD (Chemical Oxygen Requirement) and TSS (Total Suspended Solids). The selection of pollution load capacity parameters (DTBP) is based on the key parameters for the representativeness of JaingRiver water quality conditions while also representing the dominant source in Jaing watershed.

Calculation of pollution load capacity (DTBP) of river water shows the amount of existing or actual pollutant load currently entering the river flow. In addition, it is also showed the amount of pollution load allowed to enter as well as the amount of pollution load that needs to be lowered or the allocation of pollution loads so that the improvement of the quality of the water for these parameters can be achieved.

A. Inventory and Identification of Pollution Loads in Jaing River

Output from the process of inventory and identification of pollutant sources is the amount of pollutant load estimated to be produced by pollutant sources entering into the river flow that potentially enters and pollutes. The results of the inventory and identification of pollution loads entering JaingRiver are shown in the following table 3.

Table 3. Pollution Sources in Jaing River

No.	Sector	Existing BOD (kg/day)	Existing COD (kg/day)	Existing TSS (kg/day)
1	Mining	-	-	4868.51
2	Oil and Gas	1.665	2.978	-
3	Rubber Industry	6.915	15.543	15.543
4	Agriculture	263.12	-	0.35
5	Farm	56.84	137.76	-
6	Fishery	0.136	0.203	-
7	Waste	28.731	43.096	-
8	Household	930.656	1279.652	884.123

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Table 3 shows the value of pollution load from pollutant sources that enter Jaing River flow is a cumulative pollution load from 2 (two) Districts crossed by Jaing River namely MurungPudak District and Upau District. The results of the calculation of the pollution load above shows for the BOD parameter that household activities are the highest contributors to pollutants than other types of activities, then agricultural and livestock activities generally contribute to the second and third pollutant loads. Meanwhile, the waste sector makes a fourth contribution. The smallest contributor is fishery activities, for the exception of mining activities because the data obtained by mining activities do not produce types of waste with BOD parameters.

The results of the calculation of pollution load for COD parameters indicate that household activities are the highest contributors to pollutants than other types of activities, and then livestock activities and general waste contribute to the second and third pollutant loads. Meanwhile, the rubber and oil and gas industry sector contribute the fourth and fifth. The smallest contributor is fishery activities, for the exception of mining activities because the data obtained by mining activities do not produce types of waste with COD parameters.

TSS parameters from the calculation of pollution load shows that there are only 4 (four) sectors that contributed to pollution costs, the highest pollutant contributor is mining activities, then household activities and the rubber industry generally contribute the second and third pollutant loads. Meanwhile, the smallest contributor is agricultural activities.

B. Determination of Load Capacity of Water Pollution of JaingRiver

Determination of load capacity of water pollution (DTBP) of Jaing Riveruses the Mass Balance method. Jaing River is divided into 3 (three) parts, namely upstream, middle and downstream parts. The section describes the existing of points and conditions of Jaing River. The data used for this method uses water quality results for BOD, COD and TSS parameters. In addition to water sampling for water quality, river flow measurements are also carried out in 3 (three) sampling points.

Table 4. Calculation of Load Capacity of Pollution (DTBP) in Jaing River with Mass Balance Method

Jaing River	BOD	COD	TSS
Pollutant Load (kg/day)	418.87	2018.90	1698.14
Quality Standard (Class 1) mg/L	2	10	50
Quality Standard (Class 1) (kg/day)	172.8	864	4320
DTBP (kg/day)	-246.07	-1154.90	2621.86

Table 4 shows that the BOD and COD concentrations in all sampling points in Jaing River have passed the class I (one) water quality standard, so it can be said that the Load Capacity of Pollution (DTBP) ofJaing River has passed BOD and COD parameters. The results of calculations using the mass balance method show that the total BOD pollutant load that has entered the Jaing River from upstream to downstream is estimated at 418.87 kg/day which is distributed almost evenly in 3 (three) segments. Furthermore, the total COD pollution load that has entered the Jaing River from upstream to downstream is estimated at 2018.90 kg/day and the total TSS pollution load that has also entered Jaing River from upstream to downstream is estimated at 1698.14 kg/day.

Based on the South Kalimantan Governor's Regulation No. 5 of 2007 concerning Allotment and Quality Standards of River Water shows the load capacity of pollution (DTBP) of Jaing River water at three monitoring points for BOD and COD parameters (Table 29) has exceeded Class I Water Quality Standards (one) even it has no more capacity. The exceeded value of load capacity of pollution (DTBP) of the river for BOD parameters is 246.07 kg/day and the COD parameter is 1154.90 kg/day. For TSS parameters in JaingRiver it still has capacity or is still within the limits of Class I (one) Water Quality Standards which are permitted according to established regulations which are equal to 2621.86 kg/day.

V. CONCLUSION

Based on the results of study and analysis, it can be concluded that the Load Capacity of Pollution (DTBP) of JaingRiver Water for BOD and COD has exceeded Class I parameters (one) Water Quality Standard and even it has no more capacity. The exceeded value of the load capacity of pollution (DTBP) of Jaing River for BOD parameters is 246.07 kg/day and the COD parameter is 1154.90 kg/day. The TSS parameters that are permitted according to established regulations are equal to 2621.86 kg/day.

VI. SUGGESTION

For the Government of TabalongRegency, the results of this study can be used for programs and activities for Jaing River pollution control asthe development of integrated sanitation system, animal manure processing system, and waste infrastructure.

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Research of Ground Waters and their Impacts in Drinking Water, in Some Villages of the Shala Region

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***Abstract*— *A large number of natural processes and various anthropogenic activities affect the biological, chemical and physical characteristics of the waters, thus altering the normal values of physico-chemical and bacteriological parameters which followed with the change of its quality.Among the activities that influence the change in water quality are: agricultural activities, industrial activities, mining, waste disposal, urbanization and climate change. Through the development of various industrial activities comes the discharge of various waste during water processes such as: heavy metals, various solvents, toxic sludges and many waste of various kinds.*

Kosovo has limited water resources either of surface waters or of groundwater, so their rational protection and use is vital for a sustainable economic development of the country. Most of Kosovo's rivers are seasonal rivers that mostly depend on atmospheric precipitation. Village waters in the Shala region in Mitrovica's hydrographic network represent a country's wealth, but the quality of these waters is not satisfactory.

First of all, the environmental impact of industrial wastes, industrial plants, mining landfills, agricultural landfills, etc. should be highlighted. The study consists of physico-chemical, bacteriological analysis and determination of heavy metals in underground waters in some villages in the region of Shala (Zhazhë, Maxherë, Boletin, Stantërg, Zjaqë and Vllahi).Physico-chemical and bacteriological analyzes were conducted at the National Institute of Public Health in Mitrovica, whereas the determination of metals was done at the Mining Laboratory with Flotation "Trepça" in Mitrovica.

Qualitative assessment consisted of analyzing the most important indicators and comparing them with drinking water standards according to the Standards of Direc. 98/83 EC, WHOs. From the laboratory data it turns out that the water that emanates from these villages is polluted water as a result of high levels of physico-chemical and microbiological parameters, where in some villages the main problem is the presence of bacteria and metals. The source in the well near the elementary school in Stantërg village according to analyzes and the results obtained results as drinking water.

Keywords*— *mines, groundwater, physico-chemical, bacteriological and metals indicators.

I. INTRUDUCTION

Water, soil and air pollution in many parts of the world but also in Kosovo is a serious environmental problem and a permanent threat to public health, so environmental pollution is a large-scale problem that does not have a national border. [1- 4]

In this paper is presented the bacteriological and physico-chemical evaluation as well the determination of the heavy metals of public and domestic wells of some villages in the region of Shala. With the growth and development of the population, the demand for water of a better quality also increases. Poor quality of water is a threat to both the ecosystem itself and the health of people. This is a particularly serious problem and shows a great deal of interest for solutions especially for countries like Mitrovica known as industrial sites, where environmental management practices do not provide for adaptation to economic and

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health development. The quality of water for public use is specified through physical, chemical and microbiological parameters. [5-7]

The main parameters required for drinking water can be divided into these groups:

- organo-leptic parameters,
- physical-chemical parameters,
- undesirable substances,
- bacteriological parameters and
- toxic substances.

This paper presents the mining and metallurgical activity in Trepç, hotspots from mining activities, the spread of natural water and the whole water monitoring process in some villages in the region of Shala. Water monitoring has been conducted at the National Institute of Public Health in Mitrovica, while the determination of the metals has been done at the Mining Laboratory with the Flotation "Trepça" in Mitrovica.

Industrial development that does not respect environmental standards in most cases is the main source of pollution. Through the development of various industrial activities comes the discharge of various waste during water processes such as: heavy metals, various solvents, toxic sludges and many waste of various natures .[8-9]

Kosovo has limited water resources either of surface waters or of groundwater, so their rational protection and use is vital for a sustainable economic development of the country. Most of Kosovo's rivers are seasonal rivers that mostly depend on atmospheric precipitation. Village waters in the Shala region in Mitrovica's hydrographic network represent a country's wealth, but the quality of these waters is not satisfactory.

Groundwaters are sources that lie beneath the surface of the earth, and most of them come from the rains and melting of the ice that fills the spaces between the stones and the soil forming the aquifer and which can be considered as the hidden sources of water. underground are called all the waters that occurred beneath the Earth's surface, in the pits, crevices and other voids of the rocks. They are very widespread and stretch not only in areas with damp climate but also in the dry steppes desert. In the underground is approximately 37 times more water than in all water basins (lakes, swamps, rivers).

The Groundwater Monitoring Program is based on long-term sustainable management of water quality and quantity, which in the future will serve to determine the good ecological status. Systematic water quality monitoring, compliance with local laws in accordance with DKU 2000/60 EC and EU Directives [10-11] To keep the water environment in an industrial region relatively healthy, it should be kept under monitoring by monitoring.

Based on DKU 2000/60 EC and Guidelines no. 16, the protection of water areas used for drinking has affected these steps:

- identification of groundwater bodies (TUN)
- assessment of impacts from human activities
- monitoring
- data analysis
- characterization and division.

The great increase in the use of water for the needs of the population (beverage and personal hygiene), irrigation, industry and other needs has caused two major problems for mankind: lack of water and high pollution. In order to determine the level of pollution, it is necessary to carry out pollution inventory. The designation of a "emission inventory" is to collect and analyze methodologically detailed information on pollutant emissions in certain areas according to local laws and EU standards. [12-14] This inventory contains information on the types of resources and their contribution to pollution.

II. METHODS AND MATERIALS

The paper focuses on physico-chemical, bacteriological analysis and determination of metals in groundwater in some villages in the region of Shala. For research, a number of water samples were collected in 6 villages, where 12 samples were collected for research, 6 samples at the beginning of August and 6 samples at the end of August for 6 villages in the region of Shala (in: Zhazhë, Maxherë, Boletin , Stantërg, Zjaqë and Vllahi) from 1L of each sample. The samples were subjected to physico-chemical, bacteriological and metal-based analysis.

Physico-chemical and bacteriological analyzes were carried out at the National Institute of Public Health in Mitrovica, while the determination of the metals was done in the Minier Laboratory with "Flotation" Trepça in Mitrovica.

The general methods for water analysis used are:

- Water sampling and conservation;
- Analytical methods of parameter setting and working methodology;
- Titrimetric and colorimetric methods of analysis;
- photometric and spectrophotometric methods;
- Atomic absorption spectrophotometry (SAA) method;

One of the most important activities during monitoring of well water is sampling and analyzing them. The water monitoring results are dependent on the manner of sampling.

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The sampling method is important just as much as the analysis, so it is important to ensure that samples are not contaminated during taking and transport to the lab so:

The first step - taking 6 samples in the water wells in the villages around the Trepça mine (Zhazhë, Maxherë, Boletin, Stantërg, Zjaqë and Vllahi) from 1L where the water is collected in clean bottles which, before filling, are cleansed two or three times of the same water to be analyzed.

Step Two - After sampling, the vial closes with a tap, where the sampling point, date, time, water temperature and air are recorded.

Third step - Sampling of samples in the laboratory for the determination of physico-chemical, bacteriological and metal determination.

Samples for research were taken twice, 6 samples in early August and 6 samples at the end of August.

The following table presents sampling procedures for sampling how water monitoring is carried out:

Table 1: Sample analysis procedure.

Analyte	Type of container	Method of conservation	Storage time
Soluble Metals	Plastic , Glass	Field filtration + HNO_3 unitil pH=2	6 month
Metals (total)	Plastic , Glass	Acidification in pH=2 with HNO_3	6 month
Cr (VI)	Plastic , Glass	Cooling in 4 ^{0}C	24 clock
Hg	Q	Acidification in pH=2withHNO_3	28 day
Inorganic anion			
Br, Cl, F	Plastic , Glass		28 day
Cl_2	Plastic , Glass		Immediately
Aroma			In place
J	Plastic , Glass	Cooling in 4 ^{0}C	24 clock
NO_3, NO_2	Plastic , Glass	Cooling in 4 ^{0}C	48 clock
S	Plastic , Glass	Cooling in 4 ^{0}C + acetate zinc + NaOH until pH=9	7 day
Organic Substances			
C	Plastic , Dark Glass	Cooling in 4 ^{0}C + H_2SO_4 until pH=2	28 day
Halogenated compositions	Glass	Cooling in 4 ^{0}C + $Na_2S_2O_3$(0,008%)	14 day
volatile			
Aromatic compounds	Glass	Cooling in 4^{0}C + $Na_2S_2O_3$(0,008%) + HCl until pH=2	14 day
PCB	Glass ,Teflon	Cooling in 4 ^{0}C	7 day
Temperature			In place
pH- ja			In place
BOD	Plastic , Glass	Cooling in 4 ^{0}C	48 clock
COD	Plastic , Glass	Cooling in 4 ^{0}C	48 clock

During the experimental work we used these devices:

- Conductometer
- Turbidimeter
- photometry
- pH meter & thermometer
- Technical scales
- Horizontal stirrer
- Incense burner, 1100 0C

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- Analytical scales
- Absorber Atomic, Model – Thermo

Sampling sites for the determination of metals in public wells in some villages around the mine "Trepça" are: (Zhazhë, Maxherë, Boletin, Stantërg, Zjaqë and Vllahi).

Sampling (in 6 villages) of 250 ml. Filter the samples and place them in 6 wells with 100 ml of distilled water from each sample for the continuation of the process whereby each sample is supplemented with 1mL HNO3 (65%).

Table 2: Types of reagents and their description

Naming :	Formula:	Description :
Hydrochlorid acid	HCl	Tincture , 1:4
Hydroxide of sodium	$NaOH$	(molariteti)
Hidroxide of calium	KOH	(molariteti)
Nitric acids	HNO_3	65%
Amidon	$C_6H_{10}O_5$	1 %
Fenoftalein	$C_{20}H_{14}O_4$	2 %
Destilled water	H_2O	20-100 Ml
Sulfuric acids	H_2SO_4	Tincture , 1:3
Potassium Permanganate	$KMnO_4$	0.01 (molariteti)
Potassium chromium	K_2CrO_4	
Ammonium hydroxide	NH_4OH	25%
Thiosulfate sodium	$Na_2S_2SO_3$	0.01 (molariteti)
Puffer digestion	$NH_4Cl + NH_4OH$	Tincture
Silver nitrate	$AgNO_3$	
Oxalic acids	$C_2H_2O_4$	0.05 (molariteti)
Kompleksion III		0.05 (molariteti)
Barium chromates	$BaCrO_4$	

The laboratory work tools needed to develop the experimental part: Protective equipment (mantillas, handkerchiefs, glasses), Erlenmajer (300 ml), Menzur (100 ml), Automatic Bureta (50 ml), Pipette, Cup Porcelain, Hinka Separate, Quantitative Filter Paper, Chemical Spoons, Metallic Caps, plastic. Samplers and sampling tools should be cleaned each time with distilled water after use.

The samples are placed in the digesters at a temperature of (100 – 160) ° C and last for (18 – 20) min, then cooled to room temperature, (30 – 40) ° C, placed in normal container through the husk and leveled with distilled water (up to the line).

After leveling, the samples are ready for inputting at the Atomic Absorber, initially adjust the processes in the program (PC) for 5 elements: Pb, Zn, Cu, Cd and Fe.

III. DISCUSSION OF RESULTS

Water quality in villages in the Shala region is influenced by natural factors and by anthropogenic activities in the area around water wells. The water quality is estimated for the villages: Zhazhë, Maxherë, Boletin, Stantërg, Zjaqë and Vllahi in the beginning of August and end of August, 2018.

Comparisons of physical-chemical and bacteriological parameters were made with the standards allowed under Direc. 98/83 EC, while monitoring of villages in the region of Shala were made comparisons of metal results with MSHK.

Based on the working methods, the results of the analyzed samples are presented in tabular form and figure.

Tables are divided according to the number of physical-chemical analyzes at the beginning of August and at the end of August.

Table 3: Results of physical-chemical analysis at the beginning of August.

04.08.2018 Settings:	Units:	Stndardet Direc.98/83EC	**Zhazhë:** (Haxhi Voca)	**Maxherë :** (Pusi public)	**Boletin :** (Pusi S. Jonuzi)	**Stantërg :** (Pusi publik)	**Zjaqë :** (Pusi publik)	**Vllahi :**(Shkolla fillore)
Temperature	$^0C/K$	8-12	15	17.1	12	15.2	13	16.9
Wind		Pa	Pa	Pa	Pa	Pa	Pa	Pa
Taste		Pa	Pa	Pa	Pa	Pa	Pa	Pa
Turbidity	NTU	1.2-2.4	0.00	0.00	0.64	0.00	0.00	0.82
Color	Co-Pt	10.-20.0	Pa	Pa	Pa	Pa	Pa	Pa
The value of pH-së	pH	6.8-8.5	7.0	7.87	7.2	7.93	7.6	7.98
Spending $KMnO_4$	mg/L O_2	8-12	7.48	5.72	4.36	2.70	3.36	6.08
Free Chlorine	mg/L Cl_2	0.2-0.5	0.00	0.00	0.00	0.00	0.00	0.00
Clorides	mg/L Cl	200	27	17	92.5	25	43	17
Ammonia	mg/L N	0.1	0.02	0.01	0.01	0.04	0.00	0.02
Nitritets	mg/L N	0.005	0.007	0.005	0.008	0.006	0.014	0.007
Nitratets	mg/L N	10	0.3	0.6	1.5	4.8	0.9	0.7
Elektricity quarrel	ms/cm	1500	450	706	692	506	779	306
Sulfates	mg/L SO_4^{-2}	200	13.44	25.02	30.14	36.86	26.62	36.86
Total hardness	^0dH	30	13.44	23.632	18.32	11.2	11.42	7.56

Table 4: Results of physical-chemical analysis at the end of August.

24.08.2018 Settings:	Units:	Stndardet Direc.98/83EC	**Zhazhë:** (Haxhi Voca)	**Maxherë :** (Pusi public)	**Boletin :** (Pusi S. Jonuzi)	**Stantërg :** (Pusi publik)	**Zjaqë :** (Pusi publik)	**Vllahi :**(Shkolla fillore)
Temperature	$^0C/K$	8-12	15	21.6	9	22.8	13	21.9
Wind		Pa	Pa	Pa	Pa	Pa	Pa	Pa
Taste		Pa	Pa	Pa	Pa	Pa	Pa	Pa
Turbidity	NTU	1.2-2.4	0.00	0.00	0.40	0.00	38.13	0.00
Color	Co-Pt	10.-20.0	Pa	Pa	Pa	Pa	Pa	Pa
The value of pH-së	pH	6.8-8.5	7.7	7.81	7.11	6.83	7.9	7.61
Spending $KMnO_4$	mg/L O_2	8-12	8.47	6.08	9.0	6.44	12.23	5.72
Free Chlorine	mg/L Cl_2	0.2-0.5	0.00	0.00	0.00	0.00	0.00	0.00
Clorides	mg/L Cl	200	20	15	26	46	16	18
Ammonia	mg/L N	0.1	0.01	0.03	0.01	0.03	0.18	0.02
Nitritets	mg/L N	0.005	0.005	0.006	0.010	0.004	0.089	0.006
Nitratets	mg/L N	10	2.4	1.2	2.6	8.6	1.3	1.9
Elektricity quarrel	ms/cm	1500	272	440	617	773	414	427
Sulfates	mg/L SO_4^{-2}	200	5.18	31.1	22.14	102.78	10.62	53.18
Total hardness	^0dH	30	5.6	6.872	14.56	14.672	5.71	8.176

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Table 5: Results of determination of metals during August.

24. 08. 2018 Type of Metal	Unit of:	Zhazhë :	Maxherë:	Boletin:	Stantërg:	Zjaq:	Vllahi:
Pb	mg/L	0.100	0.099	0.223	0.037	0.016	0.093
Zn	mg/L	0.1509	0.1214	0.039	0.026	0.045	0.413
Cu	mg/L	0.0025	<0.001	0.0989	<0.001	0.0021	0.006
Cd	mg/L	0.0022	<0.001	<0.001	<0.001	<0.001	<0.001
Fe	mg/L	2.991	4.308	0.142	0.446	1.996	0.178

Table 6: Maximum allowable values of metals for drinking water according to different international organizations.

		MSHK, 16/2012	OBSH dhe WHO's	EPA (BE)	EU, 2014
Pb	mg/l	0.01	0.01	0.05	0.01
Zn	mg/l	0.00	3.0	3.0	0.00
Cu	mg/l	2.0	2.0	2.0	2.0
Cd	mg/l	0.005	0.001	0.005	0.005
Fe	mg/l	0.2	0.5-50	0.2	0.2

Table 7: Results of bacteriological analysis at the beginning of August.

Samples : 04.08.2018	Results :
Zhazhë (Home well Haxhi Voca)	Spotted with proteus vulgaris and live bacteria.
Maxherë (The village public Well)	Spotted with live bacteria.
Boletin (Home well S. Jonuzi)	Spotted withclostridium, and live bacteria.
Stantërg (The village public Well)	Spotted withacinetobactere.
Zjaqë (The village public Well)	Spotted withcitrobactere.
Vllahi (Well near school)	Spotted withescherichia coli.

Table 8: Results of bacteriological analysis at the end of August.

Samples : 25. 08.2018	Results :
Zhazhë (Home well Haxhi Voca)	Spotted withescherichia coli and clostridium.
Maxherë (Pusi publik i fshatit)	Spotted withescherichia coli and clostridium.
Boletin (Home well S. Jonuzi)	Spotted withescherichia coli and live bacteria.
Stantërg (The village public Well)	Unwanted.
Zjaqë (The village public Well)	Spotted withcitrobactere and clostridium.
Vllahi (Well near school)	Spotted withescherichia coli.

Temperature: in the villages of Maxherë (17.1 - 21.6) 0C, Stantërg (15.2 - 22.8) 0C and Vllahi (16.9 - 21.9) 0C compared to the standard (8 -12) 0C. These levels vary in changing climatic conditions.

Turbidity: the standard is (1.2-2.4) NTU, there was overpass in the village of Zjaqë (38.13) NTU. These high levels of turbidity are observed after rainfall but also as a result of the influence of the anthropogenic factor

Expenditures of $KMnO_4$: 9.0 mg/L in the village of Boletin up to 12.23 mg/L in Zjaqë village compared to standard (8-12) mg/L. The data show that the quality of treated water is moderately good.

Bacteriological: According to the results of the water analysis in the village of Zhazhë, the water is contaminated with live bacteria. In the village of Maxherë there are live bacteria, in the village of Boletin the water is contaminated

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with living bacteria, Stantërgu the public well of the village is contaminated with acinetobactere while the well near the school is unpolluted, in Zjaqë the water is polluted and in the wells of the Vllahi village the water is dirty. The result of anthropogenic factor influence.

Nitrite: standard is 0.005 mg / L, exceeded in the village of Zhazhë 0.007 mg/L, in Boletin village (0.008-0.010) mg/L, in Stantërg village 0.006 mg/L, in village Zjaq (0.014-0.089) mg/L and in the village Vllahi 0.007 mg/L. The result of anthropogenic factor influence.

Metals: Pb standard is 0.01 mg/L, Zhazhë has exceeded 0.100 mg/L, in Boletin exceeds Pb 0.223 mg/L, and in Vllahi village it exceeds the Pb standard where Pb values are 0.093 mg/L . The iron has exceeded in Stantërg 0.446 mg/L limit, in Zijaqë 1.996 mg/L, in Zhazhë 2.991 mg/L and Maxherë 4.308 mg/L compared to the standard of 0.2 mg/L. These exceedance of standards is a result of the Earth's natural composition.

IV. CONCLUSIONS AND RECOMMENDATIONS

From the above, we conclude that the human factor with its activity is endangering life on the ground and every day and more is degrading the nature of man that is causing man economic loss and difficulty in life. Water care means taking care of life on earth so water care means life-time care, so environmental engineers and specialists of this field are confirming the big changes in drinking water from anthropogenic emissions. These changes are in the growth of the polluting elements in the water and the elements of the pathogenic microorganisms which are coming to various infections, where it is noted that the care of the citizens and the maintenance of the environment are almost completely missing in those locations in the region of Shala where these main conclusions were reached:

- The greatest exploitation of these resources occurred during the 70's and 80's and as a consequence, many problems have also been inherited in the field of environment.
- The biggest environmental problem from this mining is polluted mine water that is polluting the area around the mine as a result of land flow and penetration into groundwater.
- The qualitative assessment of groundwater in the villages around the mine "Trepça" consisted in analyzing the most important indicators and their comparison with drinking water standards according to Standards, IA 2/99 (UNMIK).
- From the laboratory data it turns out that the water that emanates from these villages is polluted water since the levels of the physico-chemical and microbiological parameters are high, besides the well near the elementary school in the village of Stantërg according to the analysis resulted as drinking water.

Based on the field survey and the results of the physicochemical, bacteriological analysis and determination of heavy metals from the analyzed samples, we conclude that the following recommendations would be for the preservation of water quality and environmental protection of particular importance:

- Maintain the quality of drinking water from pesticides, waste and agricultural crops.
- Handle sewage and control their discharge into surface waters.
- Setting up information tables for water resource conservation.
- Monitoring the spaces declared as protected areas by the law in force by MESP.
- Approval of laws and regulations for water users in harmony with the EU.
- Protection and preservation of water resources and their use in sustainable development principles.

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Nutrient Assessment of Some Tropical Leaf Meals

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***Abstract*— *Nutrient availability of five tropical leaves were carried out, the tropical leaves were harvested fresh and the leaves were dried under room temperature (air-dry) until a moisture content of less than 11.00% was obtained in all the leaves. The air-dried leaves were milled and analyzed for their; proximate composition, mineral composition, anti-nutritional composition, fibre fractions, amino-acid concentration and biophysical properties. The results revealed that the crude protein content ranged between 30.77% in amaranthus and 44.09% in fluted pumpkin, fat content ranged between 6.11% in fluted pumpkin and 9.13% in moringa, ash content ranged between 9.28% in cotton seed leaf and 19.29% in amaranthus, crude fibre ranged between 8.10% in squash-gourd and 15.32% in fluted pumpkin and CHO ranged between 14.85% in squarch-gourd and 30.57% in cotto seed leaf. The macro and micro minerals analysis and essential and non-essential amino acid analysis revealed that all the parameters analysed for were reasonably high, the anti-nutritional factors of the leaves were very low, the fibre fractions were within range and the biophysical characteristic were normal.This study shows that the leaves were rich in nutrients that are good for poultry production.*

Keywords*— *Nutrient, assessment, Tropical and Leaf Meal.

I. INTRODUCTION

Despite the advances made in poultry nutrition in the last few decades, a lot of nutritional problems still remain unresolved (Igbasan and Olugosi, 2013). One of the most critical areas is amino acid nutrition. Vegetable-based feeds are a rich source of nutrients i.e. essential plant amino acids, vitamins and minerals. Furthermore, it has been established that green vegetable leaves are the cheapest and most abundant source of proteins because of their ability to synthesize amino acids from a wide range of available primary materials such as water, carbon dioxide and atmospheric nitrogen (Agbede and Aletor, 2004 and Fasuyi, 2006). Natural resources are available for the synthesis and polymerization of amino acids into less mobile forms and stored as such in plant leaves. However, the build-up of the amino acids in plant leaves is also accomplished with other anti-nutritional factors that render them less nutritious for consumptive purpose in man and animal. Such factors limiting the nutritive value of leaf protein are the high fibre content and other anti-nutrients (Aletor and Adeogun, 1995).

One of the major problems encountered in the tropics by poultry farmers who wish to formulate their own rations is the high prices of the feed ingredients, and lack of data on the average nutrient contents of the many local foodstuffs in the ration. Therefore, there is great renewed interest in developing natural alternatives to supplement and maintain animal performance and wellbeing, leaves from some tropical plants such as Moringa (*Moringaoleifera*), Fluted pumpkin (*Telfairiaoccidentalis*), Squash-Gourd Melon Pumpkin (*Cucurbita maxima*), cotton seed (*Gossypiumherbaceum*) and African spinach (*Amaranthuscruentus L*), contains appreciable methionine, that could be harnessed for methionine supplementation in chicken diets. The objective of the study is to evaluate the nutritive values of the aforementioned leaves in arbor acre breed of broiler chicken.

II. MATERIALS AND METHODS

2.1 Sources and processing of tropical leaf meals

The tropical vegetables were purchased fresh in the Sasha market in Akure, Ondo State Nigeria. The stalks of the vegetables were removed and the leaves were dried under room temperature (air-dry) for seven days to acquire 11.00% moisture content and to prevent volatilization of the nutrients and milled using the laboratory hammer mill. The milled samples were stored in a cool environment before they were used for chemical and biochemical analyses and digestibility test.

2.2 Determination of the chemical composition of the leaf meals

Proximate analysis of the leaf samples; the percentage of moisture, ash, ether extract, crude fibre, and crude protein content of the leaves were carried out by the methods of AOAC (2006).The gross energy of the samples were determined against thermocouple grade benzoic acid a

Gallenkamp Adiabatic bomb calorimeter (Model CBB-330-01041). The caloric values of the samples were further estimated in Kcal/kg through the use of Atwater conversion factors (4.00, 9.00 and 4.00 for protein, fat and carbohydrate respectively) to multiply the percentage of crude protein, fat and carbohydrate in the sample. The proportion of the energy as contributed by protein, fat and carbohydrate and the Utilizable Energy due to Protein (UEDP) were calculated as described by Oloruntola (2015). The fat content was determined by the method of (AOAC, 2006). Determination of amino acid profiles of the samples was done according to the procedure of Beniter (1989).The Na and K contents were determined by flame photometry (Jewnway Ltd, Dunwow, Essex, UK) and P by Vanadomolybdate method (AOAC, 2006). The Ca, Mg, Fe, Zn, Fe, Mn and Cu were determined after wet digestion with a nitric, sulphuric and hydrochloric acid, using atomic absorption spectro-photometer (Buck Scientific, 2000 A. USA).The fibre fractions (Neutral-Detergent Fibre (NDF), Acid-Detergent Fibre (ADF) andAcid-Detergent Lignin) were determined according to Van Soest and Robertson (1985) procedures. The biophysical characteristics: bulk density and water holding capacity were determined by the methods of Makinde and Sonaiya (2007) and modified by Omede (2010), the specific gravity was determined as a ratio of the bulk density of known mass of each sample to density of water. Oxalate was estimated quantitatively according to the procedure of Day and Underwood (1986). Saponin was determined using the method similar to that of Hudson and El-Difrawl (1981), phytate was determined in accordance with the procedure of Ruales and Nair (1993). Tannic acid was determined in accordance with the procedure of AOAC (1995). Alkaloid determination was done using (Griffiths, 2000), flavonoidwas determined according to the method of Harborne (1973). Phenol was determined using the method of Shubhangi*et al* 2017, trypsin was determined using the method described bySmith *et al*. (1980) and terpenoids was determined using the method described by (Akinmoladun*et al* 2007). All parameter determined were performed in triplicates.

III. RESULTS

3.1 Proximate composition of the tropical leaf meals

The proximate composition of the tropical leaf meals is presented in Table 2. The statistical analysis revealed that there were significant ($P \leq 0.05$) differences among the tropical leaf meals for the parameters analysed. The moisture content values obtained ranged from 7.37 to 10.61%. The highest value was recorded for squash-gourd leaf meal and the lowest value for moringa leaf meal. The crude protein content values that were recorded varied from 30.77% to 44.09%. Amaranthus leaf meal had the highest value while moringa leaf meal had the least value. The fat content values recorded were between 6.11 and 9.13%. Moringa and fluted pumpkin leaf meals had the highest and lowest values respectively. Fluted pumpkin leaf meal had the least ash content value (9.94%), while amaranthus leaf meal had the highest ash content value (19.29%). Squash-gourd leaf meal had the lowest crude fibre and carbohydrate contents; 8.10% and 14.85% respectively. Fluted pumpkin leaf meal had the highest value of crude fibre (15.32%), while cotton seed leaf meal had the highest value of carbohydrate (30.57%). The range of values obtained for calculated energy content was between 2,973.63 and 3,983.94kcal/kg with amaranthus leaf and moringa leaf meals having the highest and lowest values respectively.

3.2 Mineral composition of the tropical leaf meals

The results of the mineral composition of the tropical leaf meals are shown in Table 3. Moringa leaf meal had the highest value recorded for calcium. The values obtained for phosphorus varied between 0.59 and 0.78ppm. The highest and lowest values were recorded in fluted pumpkin and squash-gourd leaf meals respectively. The values obtained for magnesium ranged from 9.44 to 12.04ppm. Amaranthus leaf meal had the highest value, while fluted pumpkin leaf meal had the lowest value. Moringa leaf meal had the highest value recorded for sodium (6.90ppm) while squash-gourd had the lowest value (2.0ppm).

3.3 Amino acid profile of the tropical leaf meals

The results of the amino acid profile of the tropical leaf meals are presented in Table 4, the values of histidine were significantly ($P \leq 0.05$) different with moringa leaf meal having the highest value (2.52g/100g) and amaranthus leaf meal with least value (1.80g/100g). Isoleucine values recorded were also significantly ($P \leq 0.05$) differentwith squach-gold leaf meal having the highest value (4.63g/100g) and moringa leaf meal having least value (1.80g/100g).The values of the leucine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 7.26g/100g in amaranthus leaf meal to 8.74g/100gin cotton seed leaf meal. The values of the lysine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 3.24g/100g in amaranthus leaf meal to 5.12g/100g in cotton seed leaf meal.

The values of the methionine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 1.29g/100g in cotton seed leaf meal to 1.88g/100g in fluted pumpkin leaf meal. The values of methionine recorded in amaranthus, squach-gold, moringa and fluted pumpkin leaf meals were similar ($P \geq 0,05$), however the highest value 1.88g/100g was recorded in fluted pumpkin leaf meal and the least value 1.29g/100g was recorded incotton seed leaf meal.The values of the phenylalaninie contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 3.80g/100g in amaranthus leaf meal to 5.57g/100g in squach-gold leaf meal but there were no significantly ($P \geq 0,05$) different in the values recorded for fluted pumpkin leaf meal and cotton seed leaf meal.The values of the threonine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 3.04g/100g in fluted pumpkin leaf meal to 4.19g/100g in squach-gold leaf meal.

The values of the valine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 3.65g/100g in fluted pumpkin leaf meal to 5.85g/100g in squach-gold leaf meal but there were no significantly ($P \geq 0,05$) different in the values recorded for fluted amaranthus and pumpkin leaf meal. The values of the arginine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 4.41g/100g in amaranthus leaf meal to 5.18g/100g in fluted pumpkin leaf meal but there were no significantly ($P \geq 0,05$) different in the values recorded for squach-gold leaf meal and cotton seed leaf meal. The total essential amino acid content of the leaves were also significantly($P \leq 0.05$) different and the highest value (41.21g/100g) recorded was in squach-gold leaf meals and the least value (32.25g/100g) was recorded in amaranthus leaf meal.

The values of the alanine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 3.57g/100g in amaranthus leaf meal to 4.55g/100g in squach-gold leaf meal but there were no significantly ($P \geq 0,05$) different in the values recorded for moringa and cotton seed leaf meals.The aspertic acid content of the leaves were also significantly($P \leq 0.05$) different and the highest value (9.06g/100g) recorded was in squach-gold leaf meals and the least value (5.18g/100g) was recorded in fluted pumpkin leaf meal.The values of the cysteine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 0.79g/100g in amaranthus leaf meal to 1.08g/100g in cotton seed leaf meal but there were no significantly ($P \geq 0,05$) different in the values recorded for moringa and squach-gold leaf meal and also for values recorded for amaranthus and fluted pumpkin.Also the values of glutamic acid contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 7.00g/100g in amaranthus leaf meal to 11.04g/100g in cotton seed leaf meal but there were no significantly ($P \geq 0,05$) different in the values recorded for amaranthus and fluted pumpkin leaf meals.

The glycine content of the leaves were significantly ($P \leq 0.05$) different and the highest value (5.48g/100g) recorded was in amaranthus leaf meals and the least value (2.98g/100g) was recorded in moringa leaf meal. The proline content of the leaves were significantly ($P \leq 0.05$) different and the highest value (3.36g/100g) recorded was in squach-gold leaf meals and the least value (2.24g/100g) was recorded in amaranthus leaf meal. Also the values of serine acid contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 2.00g/100g in fluted pumpkin leaf meal to 3.63g/100g in squach-gold leaf meal but there were no significantly ($P \geq 0,05$) different in the values recorded for amaranthus and fluted pumpkin leaf meals. The values of the tyrosine contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 2.86g/100g in moringa leaf meal to 3.98g/100g in squach-gold leaf meal but there were no significantly ($P \geq 0,05$) different in the values recorded for moringa and fluted pumpkin leaf meal and also for values recorded for amaranthus and cotton seed leaf meals.

The total non-essential amino acid were significantly ($P \geq 0,05$) different, squash-gourd leaf had the highest value (37.57 g/100g), while fluted pumpkin leaf had the least value (30.08g/100g). The values for grand total amino acid were significantly ($P \geq 0,05$) different. Squash-gourd leaf was observed to have the highest amino acid profile with grand total amino acid content of (78.78 g/100g), while fluted pumpkin leaf had the least value (64.31g/100g).

3.4 Anti-nutrients composition of the tropical leaf meals

The results of the anti-nutrient composition of the tropical leaf meals are presented in Table 5. The statistical analysis revealed that there were significant ($P \leq 0.05$) differences among the tropical leaf meals for the parameters analysed. The values obtained for tannin content ranged from 0.12 to 0.37mg/100g. Amaranthus and moringa leaf meals were recorded to have the least and highest values respectively. The values obtained for phenol content ranged from 0.10 to 0.23mg/100g. Amaranthus leaf meal was recorded to have the lowest value, while moringa leaf meal had the highest value. The values obtained for flavonoids content ranged from 0.40 to 1.38mg/100g, fluted pumpkin leaf meal was recorded to have the lowest value, while Squash-gourd leaf meal had the highest value. Squash-gourd leaf meal was observed to have the highest value of trypsin terpenoid contents; 4.08mg/100g and 4.32mg/100g respectively, while amaranthus leaf meal was observed to have the lowest value; 2.55mg/100g and 1.75mg/100g respectively. The values obtained for oxalate content ranged from 1.88mg/100g in moringa leaf meal to 3.24 mg/100g in cotton seed leaf meal. The result of the Phytate content shows Amaranthus leaf meal (53.06mg/100g) and cotton seed leaf meal (6.92mg/100g) were recorded to have the highest and least values respectively. The values obtained for alkanoids content ranged from 3.00mg/100g in amaranthus leaf meal to 6.01mg/100g in cotton seed leaf meal.

The result of the saponin content shows moringa leaf meal (1.09mg/100g) and cotton seed leaf meal (0.23mg/100g) were recorded to have the highest and least values respectively.

3.5 Dietary fibre content of the tropical leaf meals.

The results of the dietary fibre fraction of the tropical leaf meals are presented in Table 6. The values of neutral detergent fibre NDF were significantly ($P \leq 0.05$) different with moringa leaf meal having the least value (40.40g/100g) and cotton seed leaf meal with highest value (78.25g/100g). Acid detergent fibre ADF values recorded were also significantly ($P \leq 0.05$) differentwith moringa leaf meal having least value (29.54g/100g) and the highest value (47.79g/100g) in fluted pumpkin leaf meal.The values of the acid detergent lignin ADL recorded were significantly ($P \leq 0.05$) different, the values ranged from 14.55g/100g in moringa leaf meal to 29.33g/100gin fluted pumpkin leaf meal however the values recorded for amaranthus and cotton seed leaf meals are not significantly ($P \geq 0.05$) different . The values of the hemicellulose contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 5.77g/100g in amaranthus leaf meal to 21.072g/100g in cotton seed leaf meal.

The values of the cellulose contents recorded were significantly ($P \leq 0.05$) different, the values ranged from 14.99g/100g in moringa leaf meal to 25.66g/100g in squach-gourd leaf meal.

3.6 Bio-Physical Characteristics and pH values of the Leaf Meals

The results of the bio-physical characteristic of the tropical leaf meals were presented in Table 7. The bulk density values recorded in amaranthus, moringa, fluted pumpkin and cotton seed leaf meals were not significantly ($P \geq 0{,}05$) different, however squach-gold leaf meal had the highest value 0.44 and the least value 0.31 was recorded in both amaranthus and moringa leaf meals.The values of water holding capacity recorded were significantly ($P \leq 0.05$) different, the values ranged from 1.59 in moringa leaf meal to 4.68 in fluted pumpkin leaf meal. The values of the specific gravity values recorded in amaranthus, moringa, fluted pumpkin and cotton seed leaf meals were not significantly ($P \geq 0{,}05$) different, however squach-gold leaf meal had the highest value 0.41 and the least value 0.29 was recorded in amaranthus leaf meals.

The pH values of the tropical leaf meal were significantly ($P \leq 0.05$) different, the values ranged from 4.60 in squach-gourd leaf meal to 5.41 in cotton seed leaf meal but there were no significantly ($P \geq 0{,}05$) different in the values recorded for fluted pumpkin cotton seed leaf meals.

IV. DISCUSSION

The values of the crude protein obtained for the air dried leaf meals: amaranthus (30.77%), cotton seed (31.68%), fluted pumpkin (44.09%), moringa (37.20%) and squash-gourd (42.42%) meal was found to be very high compare to what was recorded by Fasuyi and Akindahunsi (2009), Apena*et al* 2004, Ifon and Basir (1980), Makkarand Becker (1997) and Adebayo *et al* 2013 respectively. The higher result may be due to the drying method which cannot denature the amino acid content of the leaf,the richness of the crude protein content can be also attributed to the soil parameters on which leafs were planted. Also, the crude protein of the leaf meals was observed to be lower than that of soyabean meal (44 %) or fish meal (60 %) used conventionally as sources of protein in chicken diets. It was also observed that the crude fibre content were higher in all the tested leaf meals than what was recorded by the following authors; Fasuyi and Akindahunsi (2009), Apena*et al* 2004, Ifon and Basir (1980), Makkarand Becker (1997) and Adebayo *et al* 2013. This may be due to the age of the leaves before harvesting.

It was also observed that the crude fibre content is higher in fluted pumpkin leaf meal than in other leaf meals. The value was recorded to be 15.32%. Meanwhile, Akoroda (1990) recorded the crude fibre content to be 13%. The high crude fibre content of leaves can limit the nutritive value and utilization of protein of the leaf. The crude fibre of amaranthus leaf meal was observed to be 9.71% and this is similar to the 8.8% that was reported by Fasuyi*et al*. (2007). Fasuyi*et al*. (2007) commented that the high fibre and bulkiness of vegetable materials which call for large quantities to be consumed to provide adequate levels of nutrients has been major drawbacks to their use as major sources of nutrients for monogastrics nutrition.

The ash content of amaranthus leaf meal which was recorded to be 19.29 % is higher than the values reported for other leaves. This might have been influenced greatly by soil parameters. This observation is similar to the report of Fasuyiand Akindahunsi (2009) which recorded the ash content of amaranthus leaf meal to be 19.30%. The fluted pumpkin ash content result of Fasuyi and Nonyerem (2007) is similar to the result of this study, the ash content of moringa, cotton seed and squash-gourd leaves as reported by Makkarand Becker (1997), Apena*et al* 2004 and Adebayo *et al* 2013 respectively were lower which may be due to the system of processing and the age of the plant.

The mineral composition determination carried out on the leaf meals revealed that all the micro and macro mineral values were in line with the results of Fasuyi and Akindahunsi (2009), Apena*et al* 2004, Ifon and Basir (1980), Makkarand Becker (1997) and Adebayo *et al* (2013). Amaranthus and fluted pumpkin leaf meals are high in iron 2.87ppm and 2.1ppm respectively. Asiegbu (1998) commented that fluted pumpkin is a good source of iron and fatty acids and it makes the leaves potentially useful as food supplements. The high value of fibre fraction may be due to fibrous nature of the leaf meals. The biophysical characteristics of the leaves were comparable with Eleasu*et al*, (2012) and Fasuyi (2006). The lysine and methionine contents of the tropical leaf meals are considered to be high. This makes them good substitutes for conventional feed sources in animal nutrition. Okereke and Akaninwor (2013) also reported the amino acid profile to be rich in lysine (3.60g/100g) and methionine (0.95g/100g). The significant differences observed indicated that amaranthus had least values of Tannin, Phenol and trypsin. Dietary tannins are said to reduce feed efficiency and weight gain in chicks (Dei *et al.*, 2007) while trypsin causes pancreatic enlargement and growth depression (Aletor and Fetuga, 1987). Therefore, low levels of these antioxidants would amount to better feed efficiency and growth.The observed values of Terpenoids, Oxalate and Phytate for Moringa appeared to be least when compared with other leaf meal. This result agrees with the findings of Makkar and Becker (1996) who reported that the anti-nutrient concentration in *Moringaoleifera*is low. On this basis, consumption of moringa appears safer than that of other common vegetables like spinach and green and purple amaranths. The levels of phytate and oxalate found in the leaf were generally higher than levels reported by Abiodun*et al*. (2012).

The presence of some anitnutritional factors (ANFs) in VLMs (Fasuyi, 2005) is of negative nutritional relevance. The presence of ANFs (phenol, oxalates and tannins) in cotton was a probable factor that militated against the digestibility of crude protein (CP) and amino acids (AAs) in VLM based diets. Higher contents of tannins in the cotton could have contributed to the poor digestibility of their CP and AAs in the cotton based diets compared to the reference Amaranthus that had little of these ANFs

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Table.2: Proximate Composition of the Tropical Leaf Meals (%)

Parameters	Leaf Meals				
	Amaranthuscruentus L	*Cucurbitapepo*	*Moringaoleifera*	*Telfairiaoccidentalis*	*Gossypiumhirsutum*
Moisture Content	9.7±0.69^{ab}	10.61±0.01^{a}	7.37±0.03^{c}	9.35±0.02^{b}	10.77±0.04^{a}
Crude Protein	30.77±0.02e	42.42±0.01^{b}	37.2±0.01^{c}	44.09±0.01^{a}	31.68±0.12^{d}
Ether Extract	6.51±0.01^{c}	6.97±0.02b	9.13±0.03^{a}	6.11±0.00^{e}	6.48±0.08^{d}
Ash	19.29±0.01^{a}	17.01±0.06^{b}	12.75±0.03^{c}	9.94±0.01^{d}	9.28±0.02^{e}
Crude Fibre	9.71±0.01^{c}	8.10±0.01e	8.92±0.01d	15.32±0.01^{a}	11.24±0.01^{b}
Carbohydrate	24.02±0.04^{bc}	14.85±0.05^{d}	24.60±0.04b	15.19±0.08^{c}	30.57±0.06^{a}
Enery (kcal/kg)	2973.63±47.77^{d}	3331.90±100.32^{c}	3983.94±42.99^{a}	3852.58±112.26^{b}	2998±90.02^{e}

a-e = means within the same column having different superscripts are significantly different

Table 3:Mineral composition of the tropical leaf meals

Parameters	Leaf Meals				
	Amaranthuscruentus L	*Cucurbitapepo*	*Moringaoleifera*	*Telfairiaoccidentalis*	*Gossypiumhirsutum*
Macro Minerals (ppm)					
Calcium (Ca)	110.00±0.17^{c}	140.00±0.17^{b}	150.00±0.35^{a}	21.00±0.56^{e}	98±0.23^{d}
Phosphorus (P)	0.73±0.01^{b}	0.59±0.06^{d}	0.69±0.06^{c}	0.78±0.03^{a}	0.49±0.36^{e}
Magnesium (Mg)	12.04±0.01^{a}	9.72±0.13^{b}	9.46±0.02^{c}	9.44±0.01^{c}	7.85±0.12^{d}
Potassium (K)	46.50±0.23^{a}	9.50±0.01^{d}	30.50±0.06^{b}	12.50±0.01^{c}	8.00±0.27^{e}
Sodium (Na)	2.30±0.11^{b}	2.00±0.23^{b}	6.90±0.17^{a}	2.40±0.01^{b}	1.50±0.01^{c}
Micro Minerals (mg/kg)					
Zinc (Zn)	0.52±0.01^{a}	0.31±0.01^{b}	0.32±0.01^{b}	0.52±0.02 ^{a}	0.22±0.23^{c}

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Iron (Fe)	2.87±0.01^a	1.62±0.06^c	1.84±0.01^c	2.10±0.17 ^{b}	0.81±0.04^d
Copper (Cu)	0.08±0.01^a	0.07±0.01^a	0.08±0.01^a	0.08±0.23^a	0.07±0.21^a
Lead (Pb)	0.04±0.01^a	0.03±0.01^a	0.02±0.01^a	0.03±0.01^a	0.04±0.22^a
Manganese (Mn)	0.75±0.01^a	0.60±0.02^b	0.25±0.02^c	0.55±0.02^b	0.24±0.45^d

a-e = means within the same column having different superscripts are significantly different

Table 4: Amino Acid Profile of the Tropical Leaf Meals

	Leaf Meals					
Parameters	*Amaranthuscruentus L*	*Cucurbitapepo*	*Moringaoleifera*	*Telfairiaoccidentalis*	*Gossypiumhirsutum*	*SEM*
Histidine	1.80^e	2.22^c	2.52^a	1.95^d	2.48^b	0.56
Isoleucine	3.61^d	4.63^a	3.50^e	3.99^c	4.33^b	0.23
Leucine	7.26^e	7.92^c	8.08^b	7.59^d	8.74^a	0.17
Lysine	3.24^e	4.11^c	4.75^b	3.73^d	5.12^a	0.25
Methionine	1.73^a	1.72^a	1.79^a	1.88^a	1.29^b	0.11
Phenylalanine	3.80^d	5.57^a	5.06^b	4.22^c	4.47^c	0.34
Threonine	3.59^b	4.19^a	3.48^c	3.04^e	3.26^d	0.12
Valine	3.88^d	5.85^a	4.28^c	3.65^d	5.26^b	1.21
Arginine	4.14^d	5.00^b	4.83^c	5.18^a	4.93^b	0.43
CTEAA	**32.25^e**	**41.21^a**	**37.59^c**	**34.23^d**	**39.88^b**	**3.42**
Alanine	3.57^d	4.55^a	4.03^c	4.25^b	3.95^c	0.65
Aspartic acid	8.25^c	9.06^a	7.88^d	5.18^e	8.60^b	0.32
Cysteine	0.79^e	0.99^b	0.93^b	0.80^c	1.08^a	0.43
Glutamic acid	8.48^c	7.00^d	9.39^b	8.79^c	11.04^a	1.85
Glycine	5.48^a	5.00^b	2.98^e	3.55^d	4.04^c	1.34
Proline	2.24^e	3.36^a	3.05^b	2.64^c	2.49^d	0.32
Serine	2.01^d	3.63^a	2.60^b	2.00^d	2.32^c	0.3
Tyrosine	3.33^b	3.98^a	2.86^c	2.87^c	3.32^b	0.11
CTNEAA	**37.15^a**	**37.57^a**	**33.72^c**	**30.08^d**	**36.84^b**	**5.32**
CGTAA	**69.40^d**	**78.78^a**	**71.31^c**	**64.31^e**	**76.72^e**	**8.48**

a-e = means within the same column having different superscripts are significantly different

CTEAA = Calculated Total essential Amino Acid, CTNEAA = Calculated Total Non-Essential Amino Acid, CGTAA = Calculated Grand Total Amino Acid and SEM = Standard Error Mean

Table 5: Anti-nutrients Composition of Tropical Leaf Meals (mg/100g)

	Leaf Meals				
Parameters	*Amaranthuscruentus L*	*Cucurbitapepo*	*Moringaoleifera*	*Telfairiaoccidentalis*	*Gossypiumhirsutum*
Tannin	0.12±0.00^d	0.15 ±0.01^c	0.37 ±0.00^b	0.17 ±0.01^c	0.42±0.01^a
Phenol	0.10 ±0.00^e	0.14 ±0.00^d	0.37 ±0.00^b	0.23 ±0.01^c	0.46±0.01^a
Flavonoids	0.57 ±0.00^c	1.38 ±0.01^a	0.53 ±0.01^d	0.40 ±0.00^c	1.22±0.01^b
Trypsin	2.55 ±0.01^b	4.08 ±0.12^a	3.95 ±0.01^a	3.95 ±0.02^a	3.92±0.01^a
Terpenoids	1.75 ±0.01^c	4.32 ±0.00^a	4.31 ±0.00^a	3.78 ±0.01^a	4.31±0.01^a
Oxalate	2.7 ±0.10^b	2.37 ±0.02^c	1.88 ±0.03^d	2.03 ±0.03^d	3.24±0.01^a

Phytate	53.06±0.01^{a}	47.97±0.01^{b}	8.65±0.01^{d}	19.69±0.01^{c}	6.92±0.01^{e}
Alkanoids	3.00 ±0.00^{d}	4.99 ±0.33^{b}	5.01 ±0.06^{b}	4.41±0.01^{c}	6.01±0.01^{a}
Saponin	0.81 ±0.01^{b}	0.33 ±0.00^{d}	1.09 ±0.01^{a}	0.66 ±0.01^{c}	0.23±0.01^{e}

a-e = means within the same column having different superscripts are significantly different.

Table 6: Dietary Fibre Fraction Content of the Tropical Leaf Meals

6	Leaf Meals					
Parameters	*Amaranthuscruentus L*	*Cucurbitapepo*	*Moringaoleifera*	*Telfairiaoccidentalis*	*Gossypiumhirsutum*	*SEM*
Neutral Detergent Fibre	46.83^{d}	61.74^{b}	40.40^{e}	57.20^{c}	78.25^{a}	16.45
Acid Detergent Fibre	41.06^{d}	43.48^{c}	29.54^{e}	47.79^{a}	46.53^{b}	11.76
Acid Detergent Lignin	21.64^{b}	17.82^{c}	14.55^{d}	29.33^{a}	21.70^{b}	5.34
Hemi-cellulose	5.77^{e}	18.26^{b}	10.86^{c}	9.43^{d}	21.72^{a}	2.87
Celluose	19.42^{c}	25.66^{a}	14.99^{e}	18.66^{d}	24.83^{b}	5.76

a-e = means within the same column having different superscripts are significantly different
SEM = Standard Error of Means,

Table 7: Bio-Physical Characteristics and pH Values of the Leaf Meals

	Leaf Meal					
Parameters	*Amaranthuscruentus L*	*Cucurbitapepo*	*Moringaoleifera*	*Telfairiaoccidentalis*	*Gossypiumhirsutum*	*SEM*
Bulk density	0.31	0.44	0.31	0.32	0.32	0.03
Water oldingcapasity	4.31	3.22	1.59	4.68	1.77	1.52
Specific gravity	0.29	0.41	0.32	0.31	0.31	0.04
pH	4.94	4.6	5.26	5.4	5.41	0.52

a-e = means within the same column having different superscripts are significantly different SEM = Standard Error of Means,

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Effect of different Soaking media on the Efficiency of Carob Molasses Production

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***Abstract*— S*everal commercial products have been produced from carob pods ranging from food additives to ready-to-eat foods. Examples include carob gums as food thickening agents and carob molasses as traditional sweeteners in the Middle East. During processing, carob is cut into different sizes and soaked in different reagents to yield permeates that undergoes several processing in late production stages. It has been shown that soaking in basic media (Sodium Bicarbonate 1%) yields higher brix values followed by soaking in alcohol (20%), distilled water and acidic media (Citric Acid 1%) respectively. Her we show that powder form yields significantly the highest brix value followed by small, large, mixed and medium, which did not differ significantly from each other. Moreover, no significant difference is noticed between 2 hours, 4 hours or 6 hours of soaking. Sensory analysis shows that base powder was the most favorable reagent-size interaction among all samples.*
Keywords*— *carob; carob pods; carob molasses; brix; permeate; reagent-size interaction.

I. INTRODUCTION

Carob tree, scientifically named "Ceratonia siliqua L.", is cultivated in most Mediterranean countries mainly in mild and dry areas (Papaefstathiou, Agapiou, Giannopoulos, & Kokkinofta, 2018) (Tetik, Karhan, & Oziyci, 2010). It has been cultivated throughout the Mediterranean region for over 4000 years. Carob tree grows to a height of 12-15 meters with a productive life span of more than one hundred years (Karababa & Coşkuner, 2013). World production is estimated at about 160,000 tons per year produced from about 80000 hectares. Yield varies depending on different cultivars and farming practice (Goulas, Stylos, Chatziathanasiadou, Mavromoustakos, & Tzakos, 2016a). Spain is the largest producer of carob followed by Italy, Portugal, Morocco, Turkey, Greece, Cyprus and Lebanon (Papaefstathiou et al., 2018). Lebanon is one of the large consumers of carob products and especially carob molasses because of the Lebanese traditional sweet "carob molasses and tahini". According to FAO statistics (2017), Lebanon witnessed a remarkable decline in both: the production of Carob from around 7000 to 2226 tons/year and the area of harvest from around 700 to 243 hectares, between 1998 and 2017 (HAMADE, 2016).

The fruit is a dark brown pod with a straight, curved or twisted shape (D. Petit & M. Pinilla, 1995). The mass of the pod ranges between 5 and 30 grams with its length and thickness being up to 25 cm and 1.3 cm respectively. Carob bean consists mainly of two parts: the pulp and the seeds (El Batal. H et al., 2016). The fruit consists of: 48%-56% sugar (mainly sucrose, glucose and fructose), 3%-4% protein and 0.2%-0.6% fat content. Carob fruits are rich in dietary fibers and especially in the seeds (Haber, 2002). Pulp is composed of sugars, polyphenols (tannins, flavonoids, phenolic acids) and minerals like potassium, calcium, magnesium, sodium, copper, iron, manganese and zinc. However, seed contains proteins, dietary fibers, polyphenols and minerals (Goulas, Stylos, Chatziathanasiadou, Mavromoustakos, & Tzakos, 2016b). The fruit is rich in vitamins E, D, C, niacin, B6 and folic acid (Papaefstathiou et al., 2018). Carob fruit has many health benefits and is an excellent natural remedy (Azab, 2017). Not only does it act as an anti-diabetic drug, but also it enhances lipid metabolism. Carob lowers total and LDL cholesterol (Wursch, 1979). Moreover, carob relieves gastrointestinal complications and favors lipid oxidation (Rtibi et al., 2015). In addition, it has anti-cancer and anti-diarrheal effects (Goulas et al., 2016a).

Being a natural sweetener, carob can be used as a substitute for cocoa based on the fact that carob does not contain high levels of caffeine and theobromine as cocoa does (Yousif & Alghzawi, 2000). On the market's shelves, carob products are exposed with wide varieties including: powder, creams and molasses. Carob molasses with tahini is the Lebanese sweets signature (Tounsi, Kchaou, Chaker, Bredai, & Kechaou, 2019).

Molasses is produced from sugar rich fruit juices by boiling until achieving 70%-80% soluble dry matter content (Akbulut & Özcan, 2008). Fruit is cut into

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different sizes (small, medium, large and mixed) the mixed equally and soaked for several hours, keeping in mind that no significant difference is seen between brix values of one hour and three hours or between two hours and six hours or between three hours and nine hours (Dimassi, Fawaz, & Rached, 2019). Thus, there is a need to develop new methods to increase the yield of carob molasses via processing modifications without the addition of sucrose or glucose syrup (Dimassi, Rached, Fawaz, & Akiki, 2019).

Based on the fact that carob molasses are done by boiling for few hours after cutting and soaking carob pods in water (Dimassi, Fawaz, et al., 2019). It is interesting to study whether soaking carob pods with different reagents and starting from different sizes of pods can affect the yield of the product or not. Another important point to pinpoint in this study is the sensory evaluation and the product acceptance of the consumers.

Aim is to study the effect of sizes, soaking media and their interaction on the permeate Brix value. Furthermore, sensory evaluation and consumer's acceptance for the acceptance of products resulting from each soaking media and size would be tested using sensory analysis.

II. MATERIAL AND METHOD

2.1. Materials

The materials used in this study are carob pods, which were broken into small, medium and large pieces at the carob production facility at Tier Felsay, South district, Lebanon. Furthermore, the soaking media were distilled water, acidic media using 1% citric acid, basic media using 1% sodium Bicarbonate, 20% alcohol soaking media.

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).

Powder Production: Powder was attained by using Moulinex uno 2-in-1 blender of 350 W power and stainless steel blade model LM2211 (SEB group, Ecully, France)

2.2. Methods

2.2.1 Effect of different ground carob sizes and soaking reagents on brix value of permeate

To study the effect of different ground carob sizes, carob pods were cut into four different sizes (small, medium, large and powder). Small, medium and large sizes were attained using mechanical carob pods cutter of 0.1 cm, 1 cm (rounded), and 1 cm (squared) sieves respectively.

To study the effect of different soaking reagents, citric acid), sodium bicarbonate, 95% ethanol alcohol and distilled water were used as acidic, basic, alcoholic and control mediums. Acid and base were added to prepared mediums as 1% by mass while Alcohol is diluted to 20% by volume.

Cups were used to soak ground carob with assigned reagents. For each reagent, tubes are prepared using different sizes. For acid, 3 tubes were put in a group as triplicate samples to be measured at 2 hrs for each size (small, medium, large and mixed). Another 3 tubes were prepared to be measured at 4 hrs and an additional 3 tubes were prepared to be measured at 6 hrs. Procedure is repeated for each reagent (base, alcohol and control).

For small, medium large, and powder sizes, 10 grams of ground carob is weighed and added to each tube with 30 ml distilled water. Mixture is stirred and covered.

For mixed size, 10 ± 1 grams were weighed (3x3x3) from small, medium and large ground carob and added to the tube with 30 ml distilled water. Mixture is stirred and covered.

2.2.1 Effect of different ground carob sizes and soaking reagents on sensory analysis.

Seven samples were prepared to undergo testing. First ample is prepared by the addition of distilled water to commercial carob molasses bought from local producer. Second and third samples were prepared using powder size and mixed size soaked in distilled water. Fourth and fifth samples were prepared from powder and from mixed sizes soaked in acidic media. The sixth and seventh samples were prepared from powder and from mixed sizes soaked in a basic media. Mixtures were soaked and stirred intermittently. Brix value was measured recurrently until a value of 20 °B is attained. In addition to that the commercial carob molasses attained from the carob production facility was diluted till a 20 °B was reached.

The sensory attributes include: 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, bitterness with 0 having no bitterness and 5 having the highest bitterness sweetness with 0 having no sweetness and 5 having highest sweetness, sourness with 0 having no sourness and 5 having the highest sourness, mouth feel with 0 being very thin and 5 being very thick, non-mouth feel with 0 having no grittiness and 5 having the most grittiness. Due to cultural reasons, integrating alcohol in our sensory evaluation was avoided.

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2.3. 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 treatment effect (size as covariate) and size effect (treatment as covariate).

Furthermore, general linear model was used to study the effect of source of carob molasses on sensory evaluation taking soaking media as covariate. The general linear model was also used to study the soaking media effect on the scores of the sensory attributes taking source of carb molasses as covariate.

III. RESULTS

3.1. Effect of soaking time on permeate brix value

Average brix value of different soaking medium at 2, 4, and 6 hrs is presented in figure 1. No significant effect is seen with increased soaking time (Fig. 1). There is a significant difference between acid and base treatment where the brix of permeate from soaking in a basic medium is significantly higher than Brix of permeate resulting from soaking in water and basic medium and tended to be higher, although not significant, than the Brix resulted from soaking in alcohol.

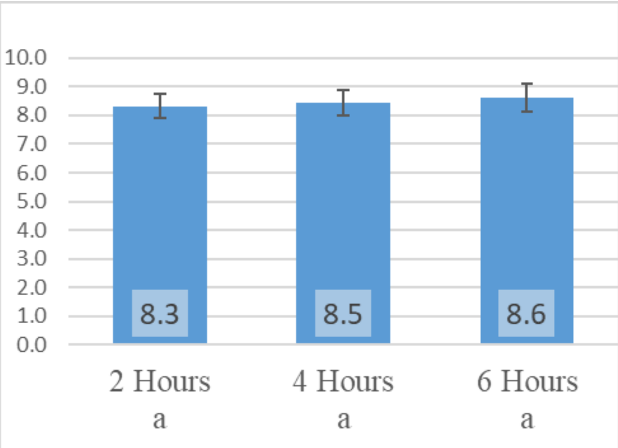

Fig.1 Effect of Soaking Time on Brix of Permeate

The Brix resulting from soaking in alcohol was significantly higher than that resulting from soaking in acidic medium, being the lowest, and tended to be higher than that resulting from soaking in water. The brix resulting from soaking in water did not differ significantly from that of Brix resulting from soaking in acidic medium

3.2. Effect of different forms of ground carob on permeate brix value.

There is no significant difference between brix of permeate resulting from soaking small, medium, large and mixed forms. Powder is the only form that showed the significantly highest brix compared to the Brix resulting from the soaking of the different forms, which did not differ significantly. Brix values from soaking Powder is higher by a factor of 2, 2.96, 2.42 and 2.54 compared to Brix of permeate resulted from soaking small, medium, large and mixed forms respectively. (Fig. 3).

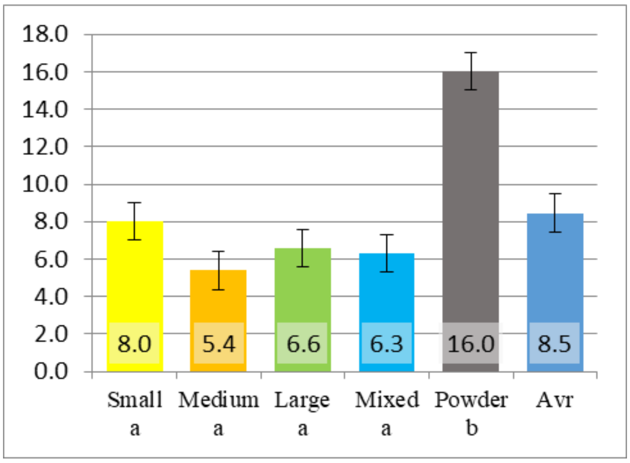

Fig.2 Effect of ground pod form on permeate brix

3.3. Effect of different forms of ground carob on permeate brix value.

The permeate brix value obtained from soaking in base and alcohol medium was significantly the highest followed by the those obtained from soaking in water and acidic media which did not differ significantly (Fig. 2)

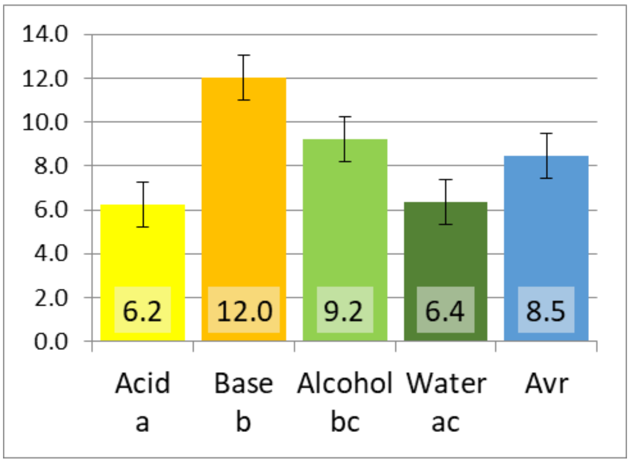

Fig.3 Effect of Soaking Medium on Permeate Brix

3.4. Sensory analysis.

Panelist of n=37 aged between 16 and 55 years old with 37 participants underwent scoring process (0 to 5 scoring). When running sensory evaluation, different parameters were studied.

3.4.1. Effect of source of carob molasses on sensory attributes

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Carob molasses drink produced from soaking powder in acid had the significantly highest score compared to molasses drink produced from acid mixed, base powder, base mixed, water powder and water mixed (Table 1). The first word being the soaking medium te second word being the form of ground carob pods. The carob score of the carob molasses drink resulting from the commercial product scored significantly the lowest and only comparable to the carob molasses drink resulted from water mixed method, which is a simulation of what is done in the carob producing facility (Table 1).

Concerning the color the carob molasses drink resulting from the acid and base powder methods scored significantly the highest. While carob molasses drink from the acid mixed method scored significantly the lowest followed by the carob molasses drink from the commercial and the water mixed source (Table 1).

Aroma followed the a similar pattern where carob molasses drink resulting from the acid powder source possessed the significantly highest value followed by the carob drink resulting from the base powder, base mixed and commercial source and ended by the drinks resulting from the acid mixed, water powder and water mixed sources (Table 1).

Table 1 Score, Color and Aroma of carob molasses from different sources

Carob Drink Source*	Score			Color			Aroma		
	Mean		SE	Mean		SE	Mean		SE
Acid Mixed	1.86	a	0.10	1.16	a	0.19	1.46	a	0.24
Acid Powder	3.19	b	0.10	4.11	b	0.19	4.00	b	0.24
Base Mixed	2.22	c	0.10	3.54	c	0.19	2.49	c	0.24
Base Powder	2.73	d	0.10	4.24	b	0.19	3.24	d	0.24
Commercial	1.57	e	0.10	2.22	d	0.19	2.65	cd	0.24
Water Mixed	1.78	ae	0.10	1.95	d	0.19	0.86	a	0.24
Water Powder	2.81	d	0.10	3.51	c	0.19	1.30	a	0.24

Means with different letters among columns are significantly different

*: First word refers to the soaking medium the second word to the form.

Commercial is diluted carb molasses attained from the carob production facility.

The second sets of tested sensory attributes were bitterness, sourness and sweetness. As for bitterness the carob molasses drink from Acid Mixed and Acid Powder were significantly the highest followed base mixed, water powder, then by the scores of the base powder, water mixed and ended by the score of the drink from the commercial molasses, which is the lowest (Table 2).

The sourness scores of the drinks from the Acid Powder, Acid mixed and water mixed were significantly the highest followed by the water powder which was significantly higher than the carob drinks originating from the base mixed and ended by the drinks resulting from the base powder and commercial sources being the significantly lowest (Table 2).

As for the sweetness the drink obtained from the commercial source scored significantly the highest, followed by the molasses drink from the water powder, base mixed and base powder and ended by the drinks from acid powder and acid mixed being the significantly lowest (Table 2).

Table 2 Bitterness, Sourness and Sweetness of carob molasses from different sources

Carob Drink Source*	Bitterness			Sourness			Sweetness		
	Mean		SE	Mean		SE	Mean		SE
Acid Mixed	2.84	a	0.30	4.70	a	0.19	0.54	a	0.20
Acid Powder	2.03	ab	0.30	4.73	a	0.19	0.81	ab	0.20
Base Mixed	1.68	bc	0.30	2.43	b	0.19	1.65	ce	0.20
Base Powder	1.19	ce	0.30	1.49	c	0.19	1.54	ce	0.20
Commercial	0.68	de	0.30	1.38	c	0.19	3.30	d	0.20
Water Mixed	1.08	ce	0.30	4.46	a	0.19	1.14	be	0.20
Water Powder	1.41	bce	0.30	3.30	d	0.19	1.78	c	0.20

Means with different letters among columns are significantly different

*: First word refers to the soaking medium the second word to the form.

Commercial is diluted carb molasses attained from the carob production facility.

The third sets of sensory attributes were the Mouth-Feel and the non-mouth-feel. The first sensory attribute the Mouth-Feel the molasses drink from water Powder is significantly the highest followed by the drinks originating from base powder, acid powder and base mixed then by the scores of the drink obtained from the water mixed and the acid mixed and ended by the score originating from the commercial molasses being significantly the lowest (Table 3).

As for the non-mouth-feel the molasses drink done from water powder and base powder source were significantly the highest, followed by the drinks obtained from acid powder source and consequently the molasses from the water mixed, base mixed and the acid mixed, ending with the drinks from the commercial source, which is the significantly lowest (Table 3).

Table 3 Mouth Feel and Non-Mouth Feel of carob molasses from different sources

Carob Drink Source*	Mouth-feel		Non-Mouth-Feel	
	Mean	SE	Mean	SE
Acid Mixed	1.11 a	0.18	1.03 a	0.16
Acid Powder	3.05 b	0.18	3.24 b	0.16
Base Mixed	2.57 b	0.18	1.08 a	0.16
Base Powder	3.51 bd	0.18	4.27 c	0.16
Commercial	0.51 c	0.18	0.14 d	0.16
Water Mixed	1.59 a	0.18	1.35 a	0.16
Water Powder	3.73 d	0.18	4.35 c	0.16

Means with different letters among columns are significantly different

*: First word refers to the soaking medium the second word to the form.

Commercial is diluted carb molasses attained from the carob production facility.

3.4.2. Effect of soaking reagents on sensory evaluation

The score, aroma and non-mouth-feel of molasses drinks soaked in acidic and basic media were significantly higher than those drinks done by soaking in water. The color and mouth feel of molasses drinks soaked in acidic medium is significantly higher than those obtained from soaking in water and basic medium. As for the bitterness score of drinks soaked in acidic media were significantly the highest compared to those soaked in water and basic medium which did not differ significantly. As for the sweetness score of drinks soaked in acidic media were significantly the lowest compared to those soaked in water and basic medium which did not differ significantly. Different from each other but they are both significantly different than base. As for the sourness score of a drink coming from soaking in an acidic medium was significantly the highest and that of the drink coming from soaking in basic medium is significantly the lowest.

Table 4 Sensory Attribute of Molasses from Different Soaking Medium

Sensory Attribute	Water		Acidic		Basic	
	Mean	SE	Mean	SE	Mean	SE
Score	1.99 a	0.08	2.58 b	0.09	2.52 b	0.09
Color	2.36 a	0.14	2.79 a	0.16	4.04 b	0.16
bitterness	1.15 a	0.18	2.36 b	0.21	1.36 a	0.21
Sweetness	1.86 a	0.13	0.84 b	0.15	1.76 a	0.15
Sourness	3.38 a	0.12	4.46 b	0.14	1.71 c	0.14
Aroma	1.28 a	0.15	2.98 b	0.18	3.11 b	0.18
Mouth-feel	1.91 a	0.15	2.11 a	0.18	3.07 b	0.18
Non-Mouth Feel	1.80 a	0.18	2.24 ab	0.22	2.78 b	0.22

Attributes with different letters among rows are significantly different

IV. DISCUSSION

As for the soaking time it showed no significant difference in the permeate brix values between 2 hours, 4 hours and 6 hours. This is in accordance with the results found by Dimassi el. 2019 (Dimassi, Fawaz, et al., 2019). Brix values from soaking Powder is higher by a factor of 2, 2.96, 2.42 and 2.54 compared to Brix of permeate resulted from soaking small, medium, large and mixed forms respectively. Thus making powdered carob to be used in food processing may be useful, although a formation of clumps may pose a major problem. This might be solved by doing two stages mixing. As for the small, medium and large showed no significant difference which is not in accordance with Dimassi et al. 2019 where the medium and the mixed ground carob pods form tended to possess the highest permeate brix. This might be explained by the fact that the sample size in this study was much less than that of the later study and thus could not capture the difference. In addition to that, powdered carob pods were proven to high levels of dietary fibers, brown color and water/oil retention capacity compared to molasses done from soaking ground pods which were characterized by reducing sugars content, dark color and functional properties with high antioxidant activity and emulsifying capacity. Thus, it would be interesting to study if soaking using the powder form would combine the benefits of both (Leila Tounsi, Sirine Karra, Héla Kechaou, & Kechaou, 2017).

Furthermore, soaking in basic and alcohol base medium resulted in significantly highest brix values. As for the basic medium it is done by sodium bicarbonate which is available in the market and is relatively used at low concentration. The only concern is the pH value which would make the product a low acid food thus increasing its constrain by considering it a safe food according to the FDA and thus the end product should have a water activity lower than 0.6. Furthermore, one of the uses of carob molasses is as a sweetener in ice cream (Leila Tounsi et al., 2017). If it is to be used as such a basic medium is beneficial since it will make the molasses pH value nearer to the normal milk value and away from the milk proteins isoelectric point.

As for the sensory attributes the acidic medium and the powder form scored the highest. The alcohol as a soaking medium was not done due to cultural reasons. And all the forms and soaking medium sources possessed higher overall score compared to the drinks obtained from the commercial and the water mixed.

V. CONCLUSION

The results of this study suggested the fixation of soaking time to 2 hours and thus lowering the operation times needed in the carob molasses production. Furthermore,

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basic and alcoholic as a soaking media will increase the brix of permeate thus would increase the efficiency of carp molasses production. The main problem is with the alcoholic soaking medium, which would affect the consumption of carob molasses in Lebanon and Arab world due to cultural reasons. Furthermore, soaking using the powder form should be seriously considered, but care should be taken as not to have development of clumps.

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A Review of Pellet Production from Biomass Residues as Domestic Fuel

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***Abstract*— *Burning fossil fuels and deforestation are the major contributors to anthropogenic climate change. As a result of climate change threat, the use of biomass and biomass residues have become extremely important to created a new industry focused on the production of clean energy through the use of renewable sources. However, factors such as low density, high moisture content, ease of handling, storage and transport are some disadvantage from the use of biomass. Pelletizing is a promising technology which converts it into a more useful form through densification in order to minimize these disadvantages. Between 2006 and 2012, pellet production worldwide grew from 7 to 19 million tons. However, the use of pellets is insignificant in developing countries. Many of the developing countries produce huge quantities of wood and agro residues with an interesting potential for biomass energy production, but they are used inefficiently causing extensive pollution to the environment. This paper presents a synthesis on what pellet is, the characteristic of pellet, the raw materials used for pellets production, biomass pelletizing process and description of a typical biomass pelletizing operation. Previous research that has been carried out on pellet production from biomass residues and application as domestic fuel has also been reviewed and cited in this paper.*

Keywords— Biomass residue, Pelletizing, Characteristics, Domestic fuel.

I. INTRODUCTION

It is known generally that burning fossil fuels and deforestation are the major contributors to anthropogenic climate change. The use of biomass as an alternative energy source provides substantial socio-economic and environmental benefits, compensating its localized nature for its high availability and carbon-neutral raw material for the production of energy.

However, bio-fuels have low bulk densities of 80–150 kg/m^3 for herbaceous and 150–200 kg/m^3 for woody biomass (Tumuluru *et al.* 2010). This limit their use to areas around their origin; plus, their heterogeneity is considered when it comes to moisture and loose nature, among others. These drawbacks are restrictive factors for their energy use (Arranz, 2011).

Many of the developing countries produce huge quantities of agro residues but they are used inefficiently causing extensive pollution to the environment. The major residues are rice husk, coffee husk, coir pith, jute sticks, bagasse, groundnut shells, mustard stalks and cotton stalks. Sawdust, a milling residue is also available in huge quantity (Grover and Mishra 1996).

The least-expensive biomass resources are these residues from wood or agro-processing operations since they are basically considered as waste. These residues have been highly promoted to be used in various heating systems, during the past decades. Compared to fossil fuel, most biomass residues have higher moisture content and lower density, thus making them technically unsuitable for direct use due to combustion and handling problems. Nevertheless, densification of biomass minimizes these disadvantages being a process that compress these raw materials in order to obtain denser fuels, with homogeneous properties and size. It improves biomass handling characteristics, increases the volumetric calorific values, and reduces transportation, collection, and storage costs (Markson *et al.* 2013; Grover and Mishra 1996). Among the different techniques that are available, pelletizing is currently the most extended one (Poddar, 2014).

The global pellet production has considerably increased for the last few years. Between 2006 and 2012, pellet production worldwide grew from 7 to 19 million tons (Duca, 2014), with Europe and North America being responsible for, practically, the whole production and consumption of densified products. The use of pellets is insignificant in developing countries: The pellets market for Africa, Asia and South America combined production is only 0.3 million tons/year in 2009 (Pirraglia *et al.*, n.d. cited by Deepak, 2012).

The growth in pellet consumption has resulted in more diversity, when it comes to the use of raw materials for pellet manufacture. Consequently, the industry has started looking for products, such as wastes obtained from forestry, agriculture or a combination of the latter, currently obtaining a wide range of these products (Sepúlveda, 2014).

In spite of the huge raw materials, pelletizing technologies is yet to get a strong foothold in many developing countries because of the technical constraints involved and the lack of knowledge to adapt the use of these technologies to suit local conditions (Grover and Mishra 1996). Overcoming the many operational problems associated with this technologies and ensuring the quality of the raw material used are crucial factors in determining its commercial success for use as domestic fuels. In addition, the importance of these technologies lies in conserving wood - a commodity extensively used as domestic fuel in developing countries, leading to the widespread destruction of forests.

This research report aims to review pellet production from biomass residue and characterization for application as domestic fuel in developing countries. It is a key to the problem of environmental pollution caused by the inefficient use of biomass residues and long term solution to mitigate the problems of deforestation.

II. PREVIOUS RESEARCH

It is difficult to find information about the levels of pellet production and application in the developing countries. This is because the use of pellet is insignificant in developing countries. However, there is some global information on densified biomass fuels (DBFs) available, but most of the information does not separate between different kinds of DBFs. Hence, previous research on the production of pellets and application as domestic fuel will include both pellets and briquettes from wood and agricultural residue.

Gravalos *et al. (*2010), conducted an experimental study on calorific energy values of biomass residue pellets for heating purposes. The fuel samples used, were biomass residues of agricultural (cotton, cardoon, etc.) and forest (pine, fir, beech, etc.) wastes. The experimental results obtained are encouraging and show that these materials can be used as alternative fuels.

Roos and Brackley (2012), examines the three major wood pellet markets in Asia: China, Japan, and South Korea. In contrast to the United States, where most wood pellets are used for residential heating with pellet stoves, a majority of the wood pellets in Asia are used for co-firing at coal-fired power plants. A consistent Factor in these nations is that their governments are promoting renewable energy, leading to policies that are driving demand for wood pellets. As these countries strive to meet their renewable energy targets, their wood pellet consumption is projected to grow.

Raju *et al.* (2014), in a work "Studies on development of fuel briquettes using locally available waste" stated that Briquettes of small size can be used in gasifies for power generation. If the plant sites are chosen properly for easy availability of raw material, the agricultural residues can be briquetted to reduce further transportation costs and associated pollution. This also improves the handling characteristics of biomass. The briquettes so obtained are very good fuels for local small scale industries and domestic purposes.

Trangkaprasith and Chavalparit, (2011) in a study "Heating value enhancement of fuel pellets from frond of oil palm" palm fronds were used as raw materials to produce pelletized fuel and waste glycerol as adhesive to reduce biodiesel production waste. The result from heating value analysis of frond is 17.25 MJ/kg. Therefore it is potential to make them to be useful by pelletizing. These pellets could be used for alternative energy in the industrial segment by mixing with glycerol to get higher heating value. The aim of the research was to find optimum ratio of ingredients (ratio of raw material, waste glycerol, and water) for producing fuel pellet from such materials.

In a study "Characterization and feasibility of biomass fuel pellets made of Colombian timber, coconut and oil palm residues regarding European standards", Carlos *et al.* (2012), assessed the main properties of Colombian timber industry residues, coconut shells and oil palm shells and compare the characteristics of pellets made from these raw materials with European standards. Pellets made from these feedstocks have an average density between 850 and 1025kg·m^{-3}, low ash contents and heating values around 18000kJ·kg^{-1}. Coconut shell pellets have low compression ratios and problems during pretreatment; whereas, sawdust, wood shavings and oil palm shell pellets proved to be an attractive opportunity for pellet industry development in Colombia.

Tokan *et al.* (2016), tested 9 samples of rice husk pellets; P_1 – P_9 using water boiling test, 100g of pellet sample P1, achieved 100^{0}C in 6 minutes to boil 500ml of water while 100g of pellet samples P6 and P7each achieved 100^{0}C each in 8 minutes to boil 500ml. Comparative studies of rice husk pellets and charcoal was also conducted, the results showed that 100g of pellets burns uniformly under free convection with pale yellow flame and very little smoke while 100g of charcoal burns irregularly and would require forced convection. With water boiling test, 100g of charcoal sample achieved 100^0C in 14 and 20 minutes to boil 500ml of water for C1 and C2 respectively. C3 did not achieve 100^0C. With calorific value ranging from 15.129 – 17.589 MJ/kg, and good physical and combustion characteristic of the rice husk pellet, it can conveniently substitute for charcoal as a domestic fuel.

Comparative thermal analysis of the properties of coal and corn cob briquettes was conducted by Ikelle and Chukwuma (2014). The work involved the production of smokeless briquettes of various compositions from coal and corn cob using CaSO4 and starch as binders, while Ca(OH) was used as desulphurizing agent. The briquettes were produced in the following ratio of coal and rice husk such as 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 respectively. The proximate analyses of the raw coal sample yielded the following: ash content 12.56%, moisture content 7.03%, volatile matter 39.21%, fixed carbon 41.2% and calorific value 117.18 KJ/g. The corn cob gave the following values, ash content 12.56%, moisture content 7.03%, volatile matter 39.21%, fixed carbon 41.2% and calorific value 61.46 KJ/g. The prepared briquettes were sun dried for seven days, subjected to various tests to assess their fuel quality. Of the briquettes produced, the 80% coal: 20% corn cob briquettes produced using starch as binder had the following values; ash content 21.70%, fixed carbon 45.01%, moisture content 2.87%, density 0.482 g/cm 32, volatile matter 30.42%, porosity index 40.12%, calorific value 153.23 KJ/g, water boiling test 1.65 minutes, burning time 24.42 minutes, ignition time 41.22 seconds and sulphur content 6.05%. For briquettes produced with CaSO as binder, 80% coal: 20% corn cob had the following values; ash content 27.69 %, fixed carbon 41.63 %, moisture content 2.77 %, density 0.503 g/cm 43, volatile matter 27.91 %, porosity index 41.11 %, calorific value 134.46 KJ/g, water boiling test 1.71 mins, ignition time 41.40 secs, burning time 25.91 mins and sulphur content 7.42 %. The briquettes showed improved properties but with regards to combustible property, the briquettes made using starch as binder do have better qualities than those produced with CaSO as binder.

In a study "Using Agricultural Residues as a Biomass Briquetting: An Alternative Source of Energy", Maninder *et al*. (2012), showed that, raw material including rice husk, coffee husk, saw dust, ground nutshell and cotton stalks etc. were densified into briquettes at high temperature and pressure using different technologies. And also discuss the various advantages, factors affecting the biomass briquetting and comparison between coal and biomass briquetting. Maninder *et al*, (2012) concluded that apart from the transportation, storage and handling problems biomass briquetting have several advantages over coal, oil etc. so we have to use it for our domestic purposes like heating and cooking. Thus, biomass briquetting is an alternative source of energy.

In a work "Production and comparative study of pellets from maize cobs and groundnut shell as fuels for domestic use" Kyauta *et al*. (2015), handles the production and comparative study of solid fuels from agricultural waste (i.e. maize cobs and groundnut shell) that can serve as alternative energy sources for domestic use, using the densification process. The characteristics of the pellets determined were moisture content, ash content, combustion rate and calorific value. The result showed that groundnut shell pellets attained a higher temperature than maize cobs. The temperatures attained by 100g of each type of fuel were 756 0C and 600 0C for ground nut and maize cob pellets respectively. The result of the net calorific value test for maize cob was found to be 13.8MJ/kg while that of groundnut shell pellets was 13.9MJ/kg. These results showed that the pellets are capable of generating heat that is sufficient for domestic use if appropriate appliances are used.

Sánchez *et al*. (2014), presents the results of a project focused on the development of briquettes from the waste wood (sawdust) resulting from the main waste from timber companies located in the Piura Region of Peru. This waste wood currently lacks a useful purpose, and its indiscriminate burning generates CO and CO_2 emissions. Through a drying and compression process, sawdust briquettes were obtained with the following features: 19.8 MJ/kg, 10% of humidity, 894 kg/m3, 1.3% of ashes, 15,29% of fixed carbon, and 83.41% of volatile matter. The results achieved show that sawdust briquettes are a perfect substitute for the fuels coming from illegal logging of the dry forest reserve in Piura that are currently used in domestic stoves (e.g. charcoal, firewood) by 55.81% of families in the region. In order to investigate the acceptance of the substitute product, eleven communication and awareness workshops were conducted reaching over 600 families, in addition to product testing for 127 families in five low-income areas of the Piura region.

Production and characterization of rice husk pellet was investigated as an alternative source of energy by Japhet *et al*. (2015), Pellets were produced from rice husk at three (3) pressures of compaction of 28MPa, 31MPa and 34MPa and three (3) particles sizes of 212µm, 300µm and 425µm. The effects of compaction pressure on the properties of pellets were determined. The results showed that, the higher the compaction pressure the lower the porosity index and consequently the higher the bulk density. The fuel pellet's density affects its bulk thermal properties. This effect is seen, when 100g of each pellet sample were combusted. Increased burning time of pellets was observed as the bulk density increases. The result also showed that the maximum calorific value of 17.589MJ/kg was achieved with a compaction pressure of 34MPa and with particle size of 425µm. also the minimum calorific value of 15.129MJ/kg was achieved with a compaction pressure of 34MPa and with particle size of 212µm.

Golinski and Foltynowicz (2012), in a study "Pellet – a Key to Biomass Energy" state that Pellet production is a rapidly growing business in many European countries. This fact is strongly connected with increasing role of biomass as a resource of clean energy. Future of pellet market is influenced by different political, economical, environmental and social aspects which create complex relations between suppliers of raw material, pellet producers and consumers. That is why standardization and quality control is being introduced in many countries, that allows to deliver better product which can compete with other fuels in terms of efficiency and impact on environment.

III. MATERIALS AND METHODS

3.1 What Is Pellet

Pellets are closely related to briquettes except that they have a smaller diameter and are more adapted to small scale use. Ashden (2011) refers to pellets as very small briquettes. There are a few different definitions of a pellet, but the one used in this study is as follows; *"A Wood briquette (pellet) is a mass of ground fuel stuff moulded or pressed into a convenient unit with or without the aid of a binder"* (written by Natividad, 1982 cited by Vinterbäck, 2000).

Pellets are a form of densified biomass with interesting opportunities for development of renewable energy. This solid fuel is mainly produced from wood residues but other biomass residues could be used. Pellets are an important renewable energy source that can easily be used in small-scale domestic systems. The dimensions of fuel pellets vary between 3 and 25 mm in diameter depending on the die block that is used in production. The length generally varies between 5 and 40 mm. If the product exceeds 25 mm in diameter it is called a briquette (Morten *et al*., 2009). Two major factors have promoted the growth of the pellet fuel market. The first is the instability in price and consistent rise in the cost of fossil fuels, and the second is the increasing attention given to the effect of climate change on the environment caused by the use of fossil fuels. Other factors supporting the use of pellets are that they are a fuel that can be produced locally, from local wood and biomass residues. The local production of pellet can produce an affordable fuel, while creating local jobs and mitigating the problem of deforestation in developing countries.

3.2 Characteristics of Pellet

The main purpose of pelletizing a raw material is to reduce the volume and thereby increase the energy density. When densification has taken place, there are two quality aspects that need to be considered. Firstly, the pellet has to remain solid until it has served its purpose (handling characteristics). Secondly, pellet has to perform well as a fuel (fuel characteristics). The energy characteristics are other important issues when describing and comparing pellets with other fuels (Karlhager, 2008).

3.3 Biomass Raw Materials for Pelletizing

Biomass raw material base for the production pellets (briquettes) has been thoroughly described by Hirsmark (2002). There are a number of biomass materials that can be used for pellet (briquette) production. Wood residues as saw dust, wood chips, planer shavings, recycled wood and pure wood can all be used after milling. Agricultural residues as straw, hemp or reed canary grass can be used. Short rotation coppice, e.g. Salix can also be used in pelletizing (briquetting) processes. Peat is another raw material suitable for pelletizing (briquetting) (Hirsmark 2002). There is no data of which raw material is the most important for briquette production (Karlhager, 2008). Hirsmark showed that saw dust and planer shavings are the two most common raw materials for pellet and briquette production though. In many developing countries which produce huge quantities of agro residues, the potential agro-residues which do not pose collection and drying problems, normally associated with biomass are rice husk, groundnut shells, coffee husk and coir waste (obtained by dry process). At present, loose rice husk, groundnut shells and other agro-residues are being used mostly by small scale boilers in process industries (Grover and Mishra 1996). "Fig." 1, shows some biomass sources for pelletizing.

Fig.1: Biomass sources for pelletizing (Kiss and Alexa, 2014).

3.4 Biomass Pelletizing Process

Pelletizing is the process of densification of biomass to produce homogeneous, uniformly sized solid pieces of high bulk density which can be conveniently used as a fuel. The densification of the biomass can be achieved by any one of the following methods: (i) Pyrolysed densification using a binder, (ii) Direct densification of biomass using binders and (iii) Binder-less briquetting (pelletizing) (Karaosmanoglu, 2000). Depending upon the type of biomass, three processes are generally required involving the following steps:

I. Sieving - Drying - Preheating - Densification - Cooling – Packing
II. Sieving - Crushing - Preheating - Densification - Cooling – Packing
III. Drying - Crushing - Preheating - Densification - Cooling – Packing

3.5 Description of a Typical Biomass Pelletizing Operation

A typical biomass pelletizing operations consisting of three major unit operations - drying, size reduction (grinding), and densification (pelletizing) is shown in "Fig." 2.

The biomass is dried to about 10% (wb) in the rotary drum dryer. Superheated Steam dryers, flash dryers, spouted bed dryers, and belt dryers are also common in European countries (Stahl *et al*., 2004; Thek and Obernberger, 2004) but they are not used in North America (to the knowledge of the authors).

After drying, a hammer mill equipped with a screen size of 3.2 to 6.4 mm reduces the dried biomass to a particle size suitable for pelletizing. The ground biomass is compacted in the press mill to form pellets. The individual pellet density ranges from 1000 to 1200 kg/m^3. The bulk density of pellets ranges from 550 to 700 kg/m^3 depending on size of pellets. Pellet density and durability are influenced by physical and chemical properties of the feedstock, temperature and applied pressure during the pelletizing process (Mani *et al*., 2003). In some operations, the ground material is treated with super-heated steam at temperatures above 100°C before compaction. The superheated steam increases moisture and temperature of the mash causing the release and activation of the natural binders present in the biomass. Moisture also acts as a binder and lubricator (Robinson, 1984).

In some operations, binders or stabilizing agents are used to reduce the pellet springiness and to increase the pellet density and durability. Most widely used binders for pelletizing of animal feeds are calcium lignosulfonate, colloids, bentonite, starches, proteins and calcium hydroxide (Pfost, 1964; Tabil And Sokhansanj, 1996). Pfost and Young (1974) Reported that there was a significant increase in pellet durability when using colloids and calcium lingo-sulphonate as additives in the range of 2.6% by weight. Biomass from woody plants contains higher percentages of resins and lignin compared to agricultural crop residues (straw and stover). When lignin-rich biomass is compacted under high pressure and temperature, lignin

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becomes soft exhibiting thermosetting properties (van Dam *et al*., 2004). The softened lignin acts as glue.

The temperature of pellets coming out of the pellet mill ranges from 70°C to 90°C. The elevated temperature is due to the frictional heat generated during extrusion and material pre-heating. Pellets are cooled to within 5°C of the ambient temperature in a cooler. The hardened cooled pellets are conveyed from the cooler to storage areas using mechanical or pneumatic conveying systems. Pellets may be passed over a screen to have fines removed and were weighed before being stored in enclosed storage areas (Mani *et al*., 2006).

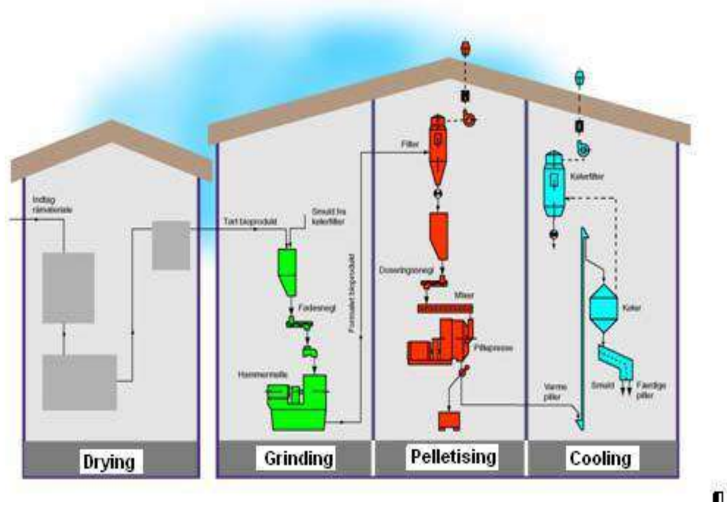

Fig.2: Flow diagram of the pelletizing process (Morten et al, 2009)

IV. DISCUSSION

Biomass residues – wood and agricultural waste, have high potential to contribute to the energy needs of developing countries, but factors such as low density and high moisture content are some drawbacks, making them technically unsuitable for direct use. Densification technologies provide practical options for overcoming some of the inherent drawbacks of biomass (moisture content and low energy density being the most important). Pelletizing can be regarded as one of the well established densification procedure, gaining increasing popularity and acceptance in recent years in the developed countries. Which are mainly due to pellets dimensions (appropriate for automatic feeding and for application in small domestic appliances). In this paper, the characteristics of pellets, biomass raw materials for pelletizing, biomass pelletizing process and description of a typical biomass pelletizing operation were described.

From previous research, it was shown that pellets with good handling and fuel characteristics could be produced from wood and agricultural waste. This will provide other alternatives for reducing problems caused by burning fossil fuels and deforestation which are the major contributors to anthropogenic climate change.

V. CONCLUSIONS

Massive production of fuel pellets from wood and agricultural waste, for application as domestic fuel could give a positive development to developing countries, where there are a lot of these resources and yet lack a sustainable source of biomass fuel supply.

Therefore, more research on different alternatives - combination of raw materials for the production of pellet from wood and agricultural waste, on analysis of their characteristics and their behavior on combustion, and on the appropriate appliance for their application, should be performed, to encourage the use of pellet as domestic fuel in developing countries.

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Analysis of Social Economic Aspect of Farmers Participants of Raskin Program for Food Solid Patterns with Wanatani System in Dry Land in North Central Timor District

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Abstract— *This research aims to: (1) determine the level of acceptance by farmers to Raskin Program with agroforestry systems on dry land, (2) determine the level of application of technology by participating farmers in the Raskin Program with agroforestry systems on dry land, (3) determine the relationship of some social and economic aspects of farmers participating in the Raskin Program with the level of technology implementation, (4) determine the relationship of some of the social and economic aspects of farmers participating in the Raskin Program with the success of the annual crops, (5) know the level of economic welfare of farmers participating in the Raskin program, and (6) understand the constraints faced by farmers in the implementation of Raskin program.The method used in this research is descriptive method with survey techniques. Primary data were collected by interviews based on questionnaires; secondary data obtained from government agencies and institutions associated with the variable of interest and research. Analysis of data usingRevenue Analysis, CorrelationSpearmanandDescriptive Analysis with simple tablature.The study concluded that: (1) Farmers receiveeitherprogram, with an average score of 29 (73%), (2) The farmer is good enough to apply the technology system agroforestry, with an average score of 26 (79%), (3) Aspects duration of formal education, working hours and farmers' income have a relationship (significant) at the application level, (4) Aspects of the number of family members productive, working hours and income of farmers have a relationship (significant) with a success rate of growing perennial crops (5) The level of economic welfare of farmers belonging Almost Poor (HM) with a per capita income (IC) Rp. 486.059 (186 %), (6) Income poor rice farmers participating in the program on the work of food patterns for dryland agroforestry systems in North Central Timor district is Rp. 3,194,347 per farmer or Rp. 3,719,547 per hectare and costs Rp. 1,536,019 per farmer or Rp. 1,788,565 per hectare, (7) The outpouring of working time patterns Raskin program participant farmers intensive dryland agro-food system is HKO 139.92 per farmer or HKO 152.45 per hectare and (8)The technical constraints of cultivation in the form of an attack pest, not the availability of production facilities at the site, and the absence of improved seed and non-technical obstacles such as lack of capital, limited land, distance from markets, natural disasters / wind, low prices and a lack of education.*

Keywords— ***Socio-economic, Raskin program, Agroforestry, Dryland.***

I. INTRODUCTION

The issue of poverty is currently still a concern of the government. One of the poverty alleviation efforts carried out by the central government is by providing rice assistance to the poor (raskin rice). The provision of rice assistance for the poor (RASKIN) aims to help the community, especially in order to fulfill the need for food, especially rice.

According to Data Badan Pusat Statistik (2011), Negara Indonesia 95% of the population consumes rice as the main food, with an average rice consumption of 113.7 kg/person/year. This consumption level is far above the world average consumption of only 60 kg/capita/year. Thus Indonesia is the largest rice consuming country in the world. Rice is a very strategic national commodity. National rice instability can cause turmoil in various aspects of life, both social, political and economic (Coordinating Ministry of People's Welfare of the Republic of Indonesia, 2014).

According to Kementerian Koordinator Bidang Kesejahteraan Rakyat (2014), the challenges facing Indonesia in combating poverty and hunger include: 1) The slowing down of poverty, which is an annual average of only 0.37%; 2) Growth that has not been optimal so that it does not have a significant impact on the poor; 3)

Many isolated areas and underdeveloped areas are limited to meeting their basic needs.

Based on these conditions, the Central Government continued to launch the Raskin Program to all provinces in Indonesia. The Raskin program is an implementation of the President's instructions on national rice policies. The President instructed Ministers and Heads of certain non-Ministry Government Agencies, as well as Governors and Mayors/Regents throughout Indonesia to make efforts to increase farmers' income, food security, rural economic development and national economic stability with the implementation of the Raskin Program.

The East Nusa Tenggara (NTT) Provincial Government is one of the areas targeted by the Raskin program, because it is a region that has poor population and a fairly dry land area for agricultural development. People who live in this dry land area, at a certain time, will lack food, especially rice. The most noticeable situation is during the long dry season, which is in the period from March to November. This situation makes the government need to intervene with the help of Raskin rice to help with community food stocks and poverty alleviation.

According toNTT Province Statistical Data for 2014, NTT Province has a dry land area of 3,527,112 ha or 74.49 percent of the land area of 4,734,990 ha. From the area of dry land, the land use by the people of NTT is allocated for tegal/plantation land with an area of 508,745 ha, field/human land with an area of 312,514 ha, pasture/grassland area of 613,131 ha, and plantation area of 379,913 ha(BPSNTT Province, 2014).

Timor Tengah Utara Regency (TTU), which is one of the districts in NTT Province, also has a wide area of dry land which is an area of 187,650 ha or around 62.79 percent of the total area of the regency.(Badan Pusat Statistik Regency TTU,2014). As a District in NTT Province that received the Raskin Program nationally, the TTU District Government changed the Raskin Program to a Food-Based Solid Raskin Program (PKP) as the flagship program of the TTU District Government and all contained in the Five District Strategic Programs, namely agricultural development programs, development programs education, health development programs, cooperative and SME empowerment programs as well as programs to optimize natural and environmental resources(Pemerintah Kabupaten Timor Tengah Utara, 2011). In the context of community empowerment on dry land in TTU District, the local government implemented the Food Empowerment Raskin Pattern (PKP) program with an agroforestry system with the aim of increasing the welfare of farmers. This program has been implemented since 2011 until now which is the flagship program of the North Central Timor District Government, for more details can be seen in the table below:

Table 1. Data on the Location of the Number of Sub-Districts and Villages of Implementers of the Raskin Program Labor-intensive Patterns in TTU District 2011 - 2014.

No.	The Implementation of the Raskin Program Solid	Number of Subdistricts	Number of villages
1.	Year 2011	24	175
2.	Year2012	24	175
3.	Year2013	24	175
4.	Year2014	24	194

Source: Dinas Pertanian Tanaman Pangan dan Perkebunan TTU Discrit (2014)

From Table 1, the above shows that the implementation of the raskin pattern program on food works in TTU District in 2011 was carried out in 24 sub-districts and 175 villages. While for 2014, the implementation was in 24 sub-districts and 194 villages/kelurahan. The number of villages that became the location of the program implementation increased in 2014 due to the expansion of villages in TTU District.

The Raskin Program with a Solid Work Pattern (PKP) is a maximum effort to streamline and streamline the management of Raskin by giving more weight through organizing farm families, mutual cooperation work to manage land for agricultural enterprises, assistance, coordination meetings, monitoring and evaluation to strengthen impacts cooperation towards food security as well as food sovereignty of the farm family. The work carried out by farmers participating in the program is carried out with agroforestry systems (Dinas Pertanian Tanaman Pangan dan Perkebunan TTU Discrit, 2014).

The Raskin program of labor-intensive patterns is a program where people or households that previously received rice in exchange for money receive free rice from the local government by doing work in their own gardens. The provision of free rice is expected to enable the community to work on their gardens, so that it will suppress shifting cultivation activities carried out by the community. Thus the community will have a permanent garden and not damage the forest.

The work carried out on his garden must be by applying agroforestry systems. The agroforestry system is expected to suppress the production of gardens by implementing slash and burn which can damage the environment. The agroforestry system is carried out with several jobs ranging from cleaning the garden to planting longevity plants. According to the results of Tanu's research (2014), the Raskin program of labor-intensive patterns successfully motivated the community and succeeded in controlling the community in implementing agriculture, thereby increasing agricultural production.

The Raskin program of food-intensive patterns with agroforestry systems has various activities ranging from land clearing to planting carried out by farmers. Thus farmers have their own level of acceptance or perception during the program. In implementing the program, socio-economic aspects affect the lives of farmers participating in the program and their families. Economically, farmers have income that will improve the welfare of farmers. The implementation of the Raskin program of food-intensive labor with agroforestry systems has been running for several years with the application of agroforestry system technology on dry land.

Farmers participating in the program plant annual crops and longevity plants or annual crops in their gardens. This longevity plant or annual plant is the hope of farmers in the future or in the future. When longevity plants have reached the age of production, farmers are expected to earn income from these longevity crops. Thus longevity plants or perennials are the main plants in farming, so the success of annual crops is very important. The success of annual crops or longevity plants related to the socio-economic aspects of farmers participating in the program Raskin labor-intensive patterns of agroforestry systems on dry land.

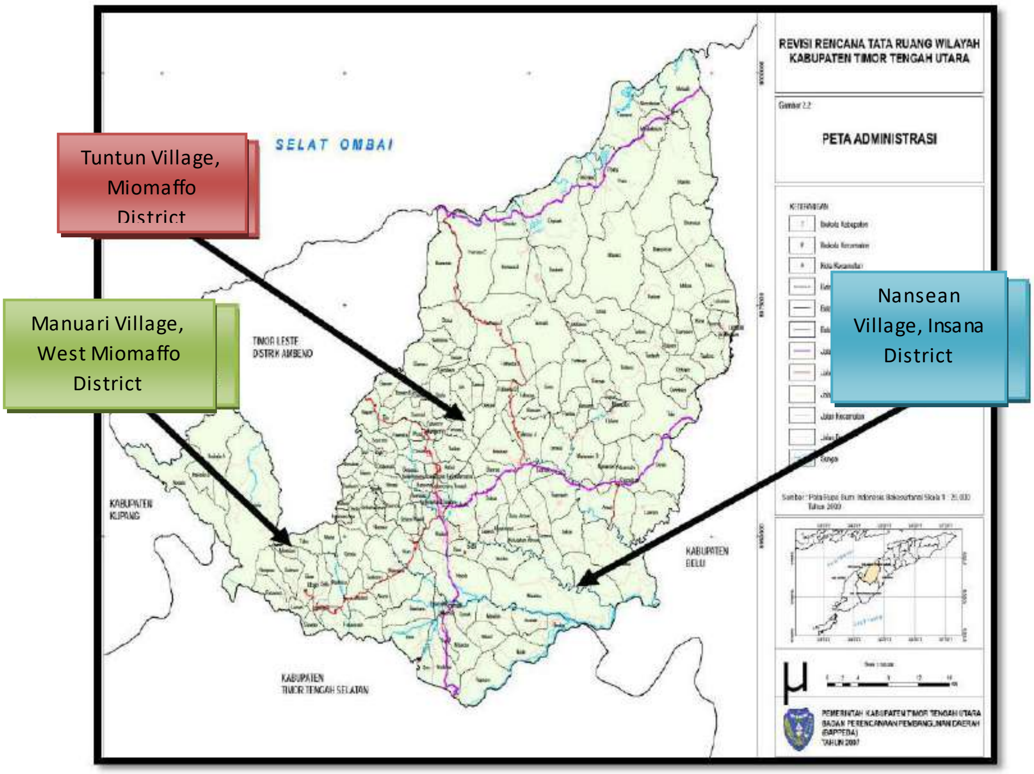
In carrying out program activities, farmers apply agroforestry technology on their farms starting from land clearing activities, making terraces to planting seasonal crops and longevity plants. Besides that, in the implementation of the program with the application of agroforestry technology there were also many obstacles faced both in the form of technical and non-technical constraints.

This study aims to: 1). To find out the level of farmers' acceptance of the Raskin Pattern Solid Food Program with agroforestry systems on dry land. 2). To find out the level of application of technology by farmers participating in the Raskin Program of Solid Labor Pattern agroforestry systems on dry land. 3). To find out the relationship between several socio-economic aspects of farmers participating in the Raskin program, the labor-intensive patterns of agroforestry systems on dry land with the level of application of agroforestry system technology. 4). To find out the relationship between several socio-economic aspects of farmers participating in the Raskin program, the labor-intensive patterns of agroforestry systems on dry land with the success of annual crops. 5). To determine the level of economic welfare of farmers participating in the Raskin program, the labor-intensive patterns of agroforestry systems on dry land. 6). To find out the constraints faced by farmers in the implementation of the Raskin Pattern of Solid Labor Food agroforestry system by program participants.

II. METHOD

This study uses descriptive methods, namely methods that are focused on solving problems that exist at the present time by collecting data and then compiling, explaining, analyzing and drawing conclusions. According to Nasir (1999), descriptive method is a method of examining the status of a group of people, an object, a set of conditions, a system of thought, or a class of events in the present that aims to make descriptions, drawings or paintings systematically, factually and accurately, regarding the facts, characteristics and relationships between the phenomena investigated.

Respondents in this study were farmers who carried out raskin programs on food-intensive patterns with agroforestry systems on dry land. Determination of the location of the study was carried out by "Purposive Sampling" which is spread across 24 sub-districts in TTU Regency which have the widest dry land and have the highest number of program participants so that three sub-districts are chosen, namely West Miomaffo District, East Miomaffo District and Insana District. From each of the three sub-districts each village has the widest dry land and the program participants are quite numerous and good at implementing the program, so Manusasi Village is chosen to represent West Miomaffo District, Tuntun Village representing East Miomaffo District and Susulaku B Village representing Insana District. For more details, see the map of North Central Timor Regency as follows:



Picture ***1.*** *Map of North Central Timor Regency as a Research Location (Arrows).*

The total number of farmer respondents was determined by Quota Sampling so that there were 150 farmers who were respondents, taking into account the cost and ability of the researcher. Data collection techniques are carried out through survey techniques, namely collecting data by direct interviews with respondents from a number of individuals based on a list of questions that have been prepared in advance (Surakhmad, 1990). While analyzing the data using Revenue Analysis, Spearman Correlation and Descriptive Analysis with simple tabulations.

III. RESULTS AND DISCUSSION

General Description of the Implementation of the Raskin Program in the Solid Work Pattern for Dry Land Agroforestry Systems in North Central Timor Regency

The Raskin Pattern Solid Work Program Food agroforestry systems on dry land are the mainstay program of the elected Regent of North Central Timor Regency for the period 2010-2015 in order to improve the welfare of farmers. According to the report on the Office of Food Crops and Plantations in North Central Timor District (2014), the Raskin Program for Food-Intensive Work in the North Central Timor Regency was carried out in 24 Sub-Districts and 194 villages/kelurahan. The Raskin Pattern Solid Food Work Program was held from 2011 to the present, as the elected Regent Program for the period 2010-2015 where agricultural development is a top priority known as the Five Strategic Programs. The raskin program for food-intensive food agroforestry systems is a special program in North Timor Tengah Regency, East Nusa Tenggara Province. In the TTU District 2011-2015 RPJM (Bappeda TTU, 2011) the five Strategic Programs consist of: 1) Agricultural development; 2) Educational development; 3) Health development; 4) Empowerment of cooperatives and SMEs; 5) Optimizing the management of natural and environmental resources.

The Raskin program of food-intensive patterns is included in the Five Strategic Programs, namely the agricultural development program with the slogan "The Movement of Love of Farmers Towards Farmers Pension" and is expected to answer the problem of poverty through the realization of food security and increased cash income of farm families (Pemerintah Kabupaten Timor Tengah Utara, 2011).

The program began with the formation of a district Coordination team, sub-district coordination team, village/kelurahan coordination team and the establishment of district technical implementers, sub-district technical implementers, village/kelurahan technical implementers, and the determination of facilitating partners , assisting and organizing the implementation of raskin programs for food-intensive patterns.

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Based on the results of Tanu's research (2104), the Implementation of the Food-Works Solid Raskin Pattern implemented in two sub-districts, namely Musi District and Kefamenanu City District, turned out this program was implemented with the principle of community empowerment, so that the community became the subject and object of community empowerment through local wisdom owned and actually here lies the real community empowerment. Management of program implementation is based on various binding provisions and well-structured organizational structures involving various elements of the relevant community and NGOs engaged in agriculture. With the synergy of all the related components, all stages of the Food-Intensive Raskin Pattern Program from assistance in the field, distribution of rice to technical evaluation went well. This very systematic implementation management has succeeded in motivating rice recipients of the Raskin Pattern Solid Food Program, so that it always works optimally. This has opened the eyes of the central government in this matter Menkokesra that it turns out that Raskin rice distribution can be done by means of food-intensive labor patterns and making TTU District (including Musi District and Kefamenanu City District) as a national pilot project for a solid pattern of rice distribution works in 2013.

In the management of the Food-Intensive Raskin Pattern Program, funding is provided by the Government of North Central Timor Regency using funds sourced from the North Central Timor Regency Regional Budget. This fund is to finance the purchase of rice and operational activities in the field. The amount of funds provided by the Timor Tengah Utara Regency Government for the program of magnitude varies each year, for more details can be seen in the table below as follows:

Table 2. The Amount of Funds for the Work-intensive Pattern of the Raskin Program in Timor Tengah Utara Regency in 2011-2014

No.	Budget (Year)	Amount of Fund Allocation (Rp)
1.	2011	5.621.000.000
2.	2012	9.037.336.500
3.	2013	7.500.000.000
4.	2014	7.827.512.000
	Total	29.985.848.500

Sumber: Dinas Pertanian Tanaman Pangan dan Perkebunan TTU District (2014)

From Table 2 above, it can be seen that the total funds spent by the North Central Timor Regency Government for four years to finance the program amounted to Rp. 29,985,848,500. In addition to funds from the district government, for the operational costs of implementing sub-district and village/kelurahan program activities using the budget sourced from sub-districts, sub-districts and ADD/APBdes for villages.

From the funds, the use is for funds to purchase rice by the government, NGO assistance funds, villages / sub-districts, sub-districts and Mantri farmers and monitoring funds by the district technical team. Of all these, the largest portion of this fund is for the purchase of raskin rice from Bulog and NGO assistance funds. This is quite good because NGOs as technical assistants in the field will help PPL with farmers to implement the program.

Level of Farmer Acceptance of Raskin Program Food-Intensive Pattern with Agroforestry System on Dry Land

The level of farmer acceptance is the perception of farmers in this case in the form of farmers' responses to the Raskin program of labor-intensive food patterns, which can arise in the form of thoughts, feelings, or emotions, attitudes or actions or behavior. According to Saptorini (1989) perception is a complicated mental process and involves various activities to classify the incoming stimulus to produce a response to understand the stimulus. Perception can be formed after going through various activities, namely physical processes (sensing) and psychological (memory, attention, brain information processing). Next Rahmat (1998) says that perception is also determined by functional and structural factors. Some functional factors or factors that are personal between individual needs, experience, age, problems, personality, gender and others are subjective. Structural factors or factors from outside the individual include the family environment, applicable laws, and values in society.

Furthermore, according to Mantra (2011), in many places agroforestry can mean a relatively small change from traditional production methods that are non-optimal or non-substantial so that they can be more easily accepted by local communities than modern and intensive farming techniques that based on one type pattern. Thus in the perception of farmers the implementation of agroforestry systems will be easily accepted because the model is not much different from what they have done in farming.

In implementing the Raskin Pattern Solid Food Program agroforestry system on dry land in North Central Timor Regency there are 8 (eight) aspects which are perceptions of each farmer, which include: the benefits of the program, the process of rice distribution, the benefits of the coordination team and the technical team, the benefits of the Pendampin NGO, benefits of program socialization, benefits of annual crops, benefits of annual crops, and benefits of land conservation. Of the 8 (eight) aspects, the average score of the farmer acceptance level reached a score of 29 (73%) from a maximum score of 40 and included in the high qualification. The level of acceptance of farmers has not been able to reach the maximum score because all aspects that have not reached the maximum score. Judging from the level of acceptance score, there are three aspects which have the lowest score of 3 (60%) from a maximum score of 5, namely the benefit aspects of the technical team and coordination team, aspects of the benefits of companion NGOs and aspects of annual crops planted by farmers participating in the program. For more details, see Table 3.

Table 3. Level of Acceptance of Raskin Program for Food-Intensive Patterns of Agroforestry Systems in Dry Land in North Central Timor Regency

No.	Level of Farmer Acceptance / Perception	Acceptance Score Value										Score achieved	(%)
		1		2		3		4		5			
		Amount (People)	%	Amount (People)	%	Amount (People)	%	Amount (People)	%	Amount (People)	%		
1.	Benefits of the Food-Intensive Raskin Pattern	-	-	2	1	11	7	137	91	-	-	4	80
2.	Rice distribution process	-	-	-	-	18	12	131	87	1	1	4	80
3.	Benefits of the Coordination Team and Technical Team	-	-	2	1	109	73	39	26	-	-	3	60
4.	enefits of Companion NGOs	-	-	1	1	88	59	58	39	3	2	3	60
5.	Benefits of Program Socialization	-	-	1	1	39	26	110	73	2	1	4	80
6.	Benefits of annual crops	-	-	1	1	104	69	42	28	3	2	3	60
7.	Benefits of Annual Plants	-	-	1	1	36	24	111	74	6	4	4	80
8.	Benefits of Land Conservation	-	-	2	1	32	21	115	77	1	1	4	80

Source: Primary Data processed

Based on Table 3 above the benefits aspects of the technical team and the coordination team only 109 respondents (73%) received benefits from the coordination team and the technical team said they were quite good, while the rest said good and bad. This is because most of the respondents' farmers were not

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familiar with the coordination team and technical team. The coordination team and technical team only went down to the village during monitoring while not at all during the program process.

For NGO companion benefits 88 people (59%) said they were good enough and the rest said good and bad. This is due to the fact that many respondent farmers said that NGO advocates only fostered NGO-assisted groups, while groups outside the NGO-assisted group only occasionally during program data collection and Raskin rice distribution. While NGOs in the technical guidelines have a role as technical assistants for all farmers participating in the raskin agroforestry system food-intensive system on dry land. In addition, companion NGOs have a heavy duty because one NGO facilitator must assist one to two sub-districts, with a large number of farmer groups and a number of villages for each sub-district. For the program it can run well so ideally one village is an NGO companion so the mentoring task will run well. Respondent farmers said that the role of NGOs in their own groups was good enough to always accompany their groups at all times. Many NGO assistants provide technical assistance on agroforestry systems, especially changes in farmer behavior regarding pit processing and lane processing. Assistance is carried out through counseling, terrace making practice, preparation of annual seedlings, making organic fertilizers, improving group administration and making demplot.

While farmer receipts for annual crop benefits, 104 people (69%) said the benefits were quite good while the rest said good and bad. This is because most respondent farmers receive very little from annual crops planted such as maize, rice fields, beans, and cassava. Many farmers say that the results are a little caused by the constraints of unavailability of production facilities such as seeds, medicines and fertilizers on the site and limited capital to buy these production facilities.

For other aspects such as the benefits of the program, the benefits of distributing rice, the benefits of program socialization, the benefits of annual crops and the benefits of land conservation most farmers say good and the rest say quite well. This is because these aspects have been well implemented by the program. For the benefits of socialization the respondent's farmers said it was good, this was because socialization was carried out simultaneously at both the village and sub-district levels. Farmers also said that annual crops and conservation benefits were very good in their farming. Annual crops are plants that in the future will be a source of income for farmers. They also said that conservation can improve soil fertility so that the concept of settled gardens can be done. Meanwhile overall farmer acceptance can be seen in table 4.

Table 4. Criteria for Value of Receipt of Raskin Program for Food-Intensive Patterns of Agroforestry Systems in Dry Land in North Central Timor Regency

No.	Criteria for Farmer Acceptance / Perception Value	Qualification	**Amount (People)**	%
1.	34 – 40	Very high	1	1
2.	27 – 33	high	144	96
3.	21 – 26	Medium	5	3
4.	14 – 20	Low	0	0
5.	8 – 13	Very Low	0	0
	Amount		150	100

Source: Primary Data processed

From table 4, it can be seen that most farmers or 144 farmers (96%) are in high qualifications, while the rest are in moderate qualifications and very high qualifications. This shows that the majority of farmers participating in the Raskin program of labor intensive dry land agroforestry systems in Timor Tengah Utara District received the program, so it was hoped they would implement the program.

Thus the acceptance of the raskin program for labor-intensive patterns of dry land agroforestry systems by the respondents who are of high criteria will automatically run the program, especially the implementation of program aspects. The implementation of program aspects that are running well means that farmers carry out each stage of the program at the level of their farming, from tillage to harvest.

Level of Technology Implementation by Farmers participating in the Raskin Program for Food-Intensive Pattern with Agroforestry System on Dry Land

After the farmer has received a program, the farmers will only implement the program. The application of a program by farmers involves the application of

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aspects of the program. These aspects concern the agroforestry system recommended in the program. The recommended agroforestry system is the agrisilviculture model. Model agrisilviculture is the development of food crops / horticulture and tree / wood plants (annual) in an area of dry land. According to Rianse ed al. (2010), agrisilviculture is a combination of components or forestry activities (trees, shrubs, palms, bamboo, etc.) with agricultural components (annual crops and plantation crops).

What is meant by the level of application of agroforestry system technology is the application of aspects of agroforestry system activities carried out by farmers participating in the Raskin Program for Food-Intensive Patterns on their farming. According to Mubyarto (1985) argued that basically farmers in farming aim to increase production so that they get high income. Farmers need to try to increase production which is closely related to agricultural intensification efforts. In order to be able to carry out agricultural intensification, technology is needed in this case agroforestry system to increase farmers' income. Although technology is available, but if this technology is not implemented by farmers, productivity increases will not occur and eventually will also relate to the income earned.

Furthermore, according to the Office of Food Crops and Plantations in North Central Timor Regency (2014) the agroforestry system developed is by combining annual crops (food and horticulture) and annual crops / timber / forestry with the application of dryland agroforestry system activities. The application of dry land agroforestry systems to the implementation of the Food-Intensive Raskin Pattern Program prioritizes productive activities in dryland farming areas with agroforestry systems. Priorities in productive activities are intended to obtain wider results and impacts on life and survival. Therefore, the Raskin Pola Karya Pangan Program does not prioritize communal activities. The choice of the type of activity of the Raskin Program Food Works is adjusted to the potential of farmers in dryland agriculture.

In the Raskin program labor-intensive patterns of food, with the application of agroforestry system technology in the form of aspects including land cleaning and tillage (PLPT), terrace making, hole making and fertilization (PTPLP), planting annual crops, legume plants and longevity plants (PTSLP), intercropping and weeding of plants (PTSP), and treatment of longevity plants and terrace repair (PTUPT). For more details, see Table 5.

Table 5. Level of Application of Agroforestry System Technology on Dry Land by Farmers in North Central Timor District

No.	Components of Technology Implementation	Maximum score	Score achieved	(%)
I.	Land and Land Treatment Cleaning	6	5	83
	1. Land clearing for gardens	3	3	100
	2. Soil Processing	3	2	67
II.	Porch Making, Hole Making and Fertilization	6	6	100
	1. Terrace Making	3	3	100
	2. Making holes for longevity plants	3	3	100
III.	Planting of annual crops, legume plants and longevity plants	9	7	78
	1. Planting of annual crops	3	2	67
	2. Planting legumes	3	2	67
	3. Planting longevity plants	3	3	100
IV.	Planting of intercropping and weeding plants	6	4	67
	1. Planting intercrops	3	2	67
	2. Weeding plants	3	2	67
V.	Longevity Plant Care and Terrace Repair	6	4	67
	1. Care for longevity plants	3	2	67
	2. Patio treatment	3	2	67
	Average	**33**	**26**	**79**

Source: Primary Data processed

In Table 5, the component of technology implementation of the respondents' respondents in the Raskin program for labor-intensive agroforestry systems on dry land in Timor Tengah Utara District has a score of 26 (79%) from a maximum score of 33 with a fairly good rating category The score for the level of implementation

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does not reach the maximum score because there are several components of technology that have not reached the maximum score. These components include planting and weeding (PTSP), long-life plant maintenance and terrace repair (PTUPT), and planting annual crops, legumes and longevity plants (PTSLP) and land clearing and tillage (PLPT).

In the technological component of intercropping and weeding (PTSP) only achieved a score of 4 (67%) from a maximum score of 6, this is because there are some farmers who plant intercropping and weeding not as recommended. Planting intercrops is important for diversification with longevity plants. Therefore, the selected type of intercrop is a type of plant that is suitable for living in the shade and of high economic value. Some recommended intercrops include: taro / taro, porang, turmeric, ginger and cayenne pepper. Meanwhile weeding must be done twice, but most farmers only do it once.

In the component of long-term plant maintenance technology and terrace repair (PTUPT), most farmers do not do as recommended, namely having to do treatment by pruning and fertilizing longevity plants. Fertilization is done by giving mulch or manure around longevity plants in the form of plant residues in the garden. For repairs to the terrace, you should also repair the terrace and plant terrace reinforcement plants so that one day you will grow terrace reinforcement plants as living terraces and fodder. The recommended terrace reinforcing plants are gamal, kaliandra and lamtoro. In addition, farmers also need to repair contour ditches and pruning terrace reinforcement plants.

For the technological component of planting annual crops, legume plants and longevity plants (PTSLP), it does not reach maximum because farmers do not comply with the recommendations. The activity of planting PKP-recommended annual crops is planting annual crops in a permanent garden. The recommended planting of annual crops is maize, and field rice, peanuts, green beans, yams and red beans specifically for elevation areas, namely West Miomaffo District. While for the land clearing and tillage (PLPT) technology component it has not reached its maximum because there are those who have not yet carried out the recommendations. There is still clearing of land for gardens that clears land of less than 0.25 ha and does cleaning by burning (slash and burn). While the aim of the program is to reduce slash and burn so as not to damage the forest. For land processing, there are also those who do imperfect soil treatment, which is to do soil treatment for only a part of the land.

The level of technology application of farmers in the Raskin program participants in the labor intensive pattern of agroforestry systems is mostly included in the Good Enough assessment category and the remainder is in the Good assessment category. For more details can be seen in table 5.

Table 5. Categories of Assessment of the Level of Application of Agroforestry System Technology in Dry Land by Farmers in North Central Timor District

No.	Application Level Assessment Category	Amount (People)	(%)
1.	Well	74	49
2.	Pretty good	76	51
3.	Not good	0	0
4.	Not so good	0	0
	Amount	**150**	**100**

In Table 5, it can be seen that the distribution of respondents' farmers in the application of dryland agroforestry system technology was the highest, including the category of Good Enough, 76 people (51%) and the least in the Good category as many as 74 people (49%). This is because most of the respondents' farmers have not implemented the technology component according to the recommendations, so the application score does not reach the maximum score.

The application of the raskin agroforestry program to the pattern of food works on dry land with the agrisilviculture model by respondent farmers is a combination of annual crops, plantation crops and forestry plants. Respondent farmers said that in implementing the agroforestry system they planted terrace reinforcing plants with kaliandra, gamal and lamtoro as well as livestock feed and living terraces. For this reason the respondent farmers said that they hoped that there would be a combination with livestock in the raskin program of labor-intensive patterns of dry land agroforestry systems in the future. According to Berek et al (2010), a combination of food crops (annuals), horticultural plants (plantation crops) and livestock, is an agrofilopoporal model of agroforestry systems.

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Relationship between Farmers' Socio-Economic Aspects of Participants in the Raskin Program for Food-Intensive Patterns of Agroforestry Systems on Dry Land with a Level of Technology Implementation

The stage of adoption of technological innovations includes the stages of implementing, trying phase, evaluation phase, interest stage, and conscious stage. With the first stage, farmers have heard, know, and know about a technology, the second stage is that farmers seek further information about a technology, the third stage is that farmers consider that a technology provides more economic benefits, the fourth stage is that farmers have tried the technology is on a small scale, and the fifth stage of farmers has applied the technology continuously.

The relationship between the level of application and the socio-economic aspects of the farmer which includes age, length of formal education, number of family members, outpouring of work time, and income, are detailed in Table 6 below.

Table 6. Relationship of Farmers' Socio-Economic Aspects with the Level of Application of Agroforestry System Technology to Dry Land in North Central Timor Regency

No	Socio-Economic Aspects	Correlation (rs)	*p-value*	Decision
1	Age	0,129	0,058	Non Signifikan
2	Duration of formal education	0,187*	0,011	Signifikan
3	Number of productive family members	0,088	0,141	Non Signifikan
4	Outpouring of work time	0,178*	0,015	Signifikan
5	Income	0,217*	0,004	Signifikan

Source: Primary Data processed

Based on Table 6, it can be seen that the value of the correlation coefficient (rs) between ages with the level of application is 0.129 with p-value 0.058 which is greater than α = 0.05 (Value t-count 1.583 is smaller than t-table 1,660) then Ho is accepted, so that it can be concluded that there is no significant relationship between age factors with the level of application of technology. This means that the increasing age of a person will not affect someone in learning and implementing an innovation technology, and vice versa, young age respondents will not influence them to apply the technology. This insignificant relationship is caused by the age groups both young and old can apply the technology. It is evident from the age of respondents who varied from young to old with a range of 25-69 years. The value of rs 0.102 shows a relatively low trend figure which means the relationship between the age of the respondent and the level of application (adoption) of technological innovation is very weak.

While from the table above shows the relationship between the length of formal education with the level of application of technological innovation has a significant relationship with the value of rs of 0.187 and p-value of 0.011 which is smaller than α = 0.05 (t-count of 2.315 is greater than t- table 1,660) then H1 is accepted, so the higher the formal education of respondents the higher the level of application of technology.

Based on Table 6, it can be seen that the value of the correlation coefficient (rs) between the number of productive family members with the level of application of technological innovation amounted to 0.088 with p-value 0.141 which is greater than α = 0.05 (t-count value 1.074 smaller than t-table 1,660) then Ho is accepted. This means that there is an insignificant relationship between the number of family members and the level of application of technological innovation. This means that the large number of family members of respondents does not necessarily influence the level of application of technology.

From Table 6 it can be seen that the value of the correlation coefficient (rs) between the outpouring of work time and the level of application (adoption) of technological innovation is 0.178 with p-value 0.015 which is smaller than α = 0.05 (t-count 2,200 is greater than t -table 1,660) then H1 is accepted, so it can be concluded that there is a significant relationship between the outpouring of work time and the level of application of technology. This shows that the higher the outflow of work time of respondents, the higher the level of application of technology adoption.

From Table 6 it is known that the value of the correlation coefficient (rs) between the income of farmers and the level of adoption of technological innovation is 0.217 with p-value 0.004 which is smaller than α = 0.05 (t-count 2.690 is greater than t-table 1.660) then H1 is accepted, which means that the relationship of respondents' income is significant with the level of application of technology, so the higher the income of respondents the higher the level of application of technology. This shows that income affects the high and low levels of application of technology. This relationship shows that in cultivating a plant requires a large amount

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of money. This means that with the respondent's high income, the respondent's farmers are more willing to make decisions to implement new innovations.

Relation of Socio-Economic Aspects of Farmers Participants in the Raskin Program Food-Intensive Pattern Agroforestry System on Dry Land with Plant Growth Success

This study also looks at the success of growing annual crops with socio-economic aspects because in cultivating annual crops by farmers related to socio-economic aspects. Farmers in cultivating annual crops need a long time. This long time is related to the age of the plant to be able to produce and produce. In the process of cultivating crops by farmers, from planting to fruiting, it requires certain socio-economic conditions so they can succeed.

The aim of the raskin program for food-intensive patterns with agroforestry systems in Timor Tengah Utara District is to improve the welfare of the community in this case to improve the socio-economic aspects of the community. With the increase in the socio-economic aspects of society, it will certainly affect the success rate of growing plants. The socio-economic aspects of the farmers studied in this study included age, length of formal education, number of family members, outpouring of work time (CWK), and income. The relationship between the success rate of growing plants and the socio-economic aspects of farmers in detail is presented in table 7.

Table 7. Relationship between Farmers' Socio-Economic Aspects and the Success of Annual Plant Growth in North Central Timor District

No	Socio-Economic Aspects	Correlation (rs)	*p-value*	Decision
1	Age	0,028	0,365	Non Signifikan
2	Duration of formal education	-0,108	0,094	Non Signifikan
3	Number of productive family members	0,341*	0,306	Signifikan
4	Outpouring of work time	0,280*	0,166	Signifikan
5	Income	0,149*	0,035	Signifikan

Source: Primary Data processed

Based on Table 7 it can be seen that the value of the correlation coefficient (rs) between age and the success rate of growing plants is 0.028 with p-value 0.365 which is greater than $\alpha = 0.05$ (t-count value 0.341 is smaller than t-table 1,660) then Ho accepted, so it can be concluded that there is no significant relationship between age factors with the success rate of growing plants. This means that the increasing age of a person will not affect the person in determining the success rate of plant growth, and vice versa, the young age of respondents will not affect them to determine the success of growing plants. This is because the nature of annual plant maintenance activities is relatively easy and simple so it does not require a certain age in maintaining plants. Age will affect respondents in ways of thinking and acting, especially in making a decision. The insignificant relationship is caused by the age group both young and old can operate a plant independently. It is evident from the age of respondents who varied from young to old with a range of 25-69 years.

Table 7 above shows the relationship between the length of formal education with the success rate of plant growth has a relationship that is not significant but negative with a value of rs of -0.108 and p-value of 0.094 which is greater than $\alpha = 0.05$ (T-count value is 1.323 smaller from t-table 1,660) then Ho is accepted, so the higher the formal education of the respondent the higher the success rate of growing plants. Respondents can be said to have been aware of formal education. Formal education that has been taken by respondents can influence thinking in responding to new things that have not been known including innovations in crop cultivation.

Based on Table 7 it can also be seen that the value of the correlation coefficient (rs) between the number of productive family members with the success rate of growing plants is 0.341 with p-value 0.306 which is smaller than $\alpha = 0.05$ (t-count value of 4.429 greater than t- table 1,660) then H1 is accepted. This means that there is a significant relationship between the number of family members and the success rate of growing plants. This means that the number of respondent family members that influence the success rate of growing plants. The increase in the number of productive family members of the respondent farmers has led to a tendency to increase the number of farm workers in supervising / maintaining annual crops so that the success of growing plants tends to increase.

From Table 7 it can be seen that the value of the correlation coefficient (rs) between the outpouring of work time (CWK) and the success rate of growing plants

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is 0.280 with p-value 0.166 which is smaller than α = 0.05 (Value tcount 3.547 greater than t -table 1,660) then H1 is accepted, so it can be concluded that there is a significant relationship between the outflow factor of work time and the success rate of growing plants. This shows that the higher the outflow of work time of the respondents, the higher the success rate of growing plants. In addition, there were also many productive family members who devoted their labor and work time to the Raskin program for dry land agroforestry system food systems, especially in the care of longevity plants (13.36 HKO per cultivated land area or 15.56 HKO per hectare) so that time devoted to maintaining more annual crops. Thus increasing the outpouring of working time of farmers has a tendency to increase the success of growing plants.

From Table 7 it is known that the value of the correlation coefficient (rs) between income and the success rate of growing plants is 0.149 with p-value 0.035 which is smaller than α = 0.05 (t-count value 1.8327 is greater than t-table 1,660) then H1 is accepted, which means that the relationship between the income of the respondent's farmer is significant with the success rate of plant growth, so the higher the income of the respondent, the higher the success rate of growing the plant. This shows that farmers' income affects the high and low success rates of growing plants.

The relationship between the number of productive family members, the outpouring of work time (CWK) and significant farmer income with the success rate of growing these crops is in accordance with the results of research by Syaihuddin (1996), which states that productive family members (CWK) and farmers' income significantly associated with the success rate of growing plants.

Economic Welfare Level of Farmers Participants in the Raskin Program Food-Intensive Pattern Agroforestry System in Dry Land in North Central Timor Regency

The level of economic welfare is the level of income of farmers participating in the Raskin food-intensive pattern of agroforestry systems on dry land in fulfilling basic needs based on the rupiah value of minimum needs based on the prices of nine basic needs that apply to the local market. According to Sajogyo (1996) said that there are several levels of poverty, which are divided based on poverty criteria that compare income with the standard price of rice. The poverty variable is seen by measuring household expenditure in rural areas, namely food expenditure and non-food expenditure. In measuring the level of economic well-being of farmers participating in the Raskin program, the labor intensive patterns of dry land agroforestry systems in Timor Tengah Utara Regency were measured by a poverty approach based on the rupiah value of minimum needs based on the prices of nine basic needs in the local market. The minimum standard of living needs (poverty line) per person per year based on the price of nine basic commodities that apply in the local market in Timor Tengah Utara Regency in 2013 is Rp. 261,995.- (BPS Timor Tengah Utara Regency, 2014).

Criteria for the level of economic prosperity of farmers participating in the Raskin program for food-intensive patterns can be identified by comparing income per capita with a minimum standard value of living needs per person per year. While per capita income is obtained by dividing the total income of the farmer by the number of farmer family members. The average level of economic well-being of farmers in the participants of the Raskin program for labor-intensive agroforestry systems on dry land in Timor Tengah Utara Regency is classified as a criterion of Nearly Poor with Capita Income (IC) of Rp. 486,059 (186%) with a range between Rp. 139,926 (53%) up to Rp. 2,044,500 (780%). For more details about the level of economic welfare of respondent farmers, see Table 8.

Table 8. Economic Welfare Level of Farmers Participants in the Raskin Program Food-Intensive Pattern Agroforestry System in Dry Land in North Timor Tengah Regency

No.	Criteria for Economic Welfare	Amount(People)	(%)
1.	Not poor	16	11
2.	almost poor	98	65
3.	poor	29	19
4.	Poor Once	7	5
	Jumlah	150	100

From table 8, it can be seen that the majority of respondents who participated in the Raskin program for labor intensive dry land agroforestry systems in the North Central Timor Regency were almost poor, which were as many as 98 (65%) and 29 people (19). %). While Not

Poor as many as 16 people (11%) and Poor Once as many as 7 people (5%).

From this situation it can be said that the raskin program of labor-intensive dryland agroforestry system food can increase the economic welfare of farmers, where the number of farmers who are above the poverty line (Not Poor and Nearly Poor) is greater than the number of farmers below the poverty line (Poor and Poor once). This is because the longevity of the respondent farmers has started production in 2014 so that there is an increase in income from the annual crop of farmers, especially annual crops planted in 2011. The annual plants that start producing are cashew and orange. The average income obtained from cashew crops is Rp. 138,133.- per arable land area or Rp. 160,845.- per hectare. While the average income obtained from citrus crops is Rp. 296,000.- per arable land area or Rp. 344,667.- per hectare.

While other annual crops that have been planted since the start of the program in 2011 have not produced such products as candlenut and forestry plants such as white teak (gamalina) and mahogany. This is because the age of the production of the plant is more than five years. Respondent farmers said that they were very optimistic in the future the annual crops would provide income for them. According to Mantra (2011), that part of the proceeds obtained with agroforestry/agroforestry systems will be obtained several years or after the waiting period. Therefore farmers or initiators must be able to convince them to get results in the short term if they want to invest in the long term.

Besides that, if we look at the high level of farmer acceptance of the raskin program for labor-intensive patterns of dry land agroforestry systems in Timor Tengah Utara District, the impact on the application of agroforestry technology is quite good. The application of good agroforestry technology causes the success rate of growing plants to be good, where the maintenance of annual crops by farmers will be done well. As a result, the maintenance of good annual crops will make the plants grow and bear fruit so that they can increase farmers' income.

Constraints faced by Farmers in the Implementation of the Raskin Program Solid Work Patterns for Dry Land Agroforestry Systems

The constraints referred to in this study are the constraints faced by farmers in implementing the raskisn program of labor-intensive patterns of agroforestry systems on dry land at the level of farming both technical and non-technical. The technical constraints of cultivation are constraints related to crop cultivation by farmers participating in the Raskin work-intensive pattern of agroforestry system food on dry land. While the non-technical constraints or socio-economic constraints are constraints related to economic social conditions experienced by farmers participating in the raskin agroforestry system food-intensive patterns on dry land. The constraints in question can be seen in Table 9.

Table 9. Constraints faced by Farmers in the Implementation of the Raskin Program Food-Intensive Pattern of Agroforestry Systems in Dry Land in North Central Timor Regency

No.	Constraints	**Amount (People)**	**(%)**
I.	Cultivation Technical Constraints		
	1. Pest Attack Plant diseases	85	57
	2. Lack of availability of production facilities	29	19
II.	Non Technical Constraints		
	1. Lack of capital	112	75
	2. Limited land	70	47
	3. Long distance from the market	31	21
	4. Low price	20	13
	5. The occurrence of natural disasters / winds	19	13
	6.Lack of counseling	15	10
	7. Animal attacks occur	12	8

Source: Primary Data processed

In Table 9, it can be seen that the obstacles are technical cultivation constraints and non-technical constraints. The technical constraints of cultivation are plant disease pests, the absence of superior seeds and the availability of production facilities. While non-technical constraints in the form of distance from the market, limited land, limited capital, cattle attacks, natural disasters/wind, low prices and lack of counseling.

a. Cultivation Technical Constraints

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The biggest technical obstacle was the attack of pests, which were experienced by 85 respondents (57%) and the constraints of lack of availability of production facilities in locations such as fertilizers, seeds and medicines experienced by 29 respondents (19%).

Pest attack is an obstacle because many respondents experienced a locust attack on maize, shoot death on cashew plants and attacks of diplodia which attacked citrus plants. For diplodia disease, it has become a common attack on citrus plants, because when an orange tree is fruiting it first breaks directly into the stem, then dries and then dies. Indeed, so far there have been efforts from the Agriculture and Plantation Service of North Central Timor Regency by replacing dead oranges and spraying but have not produced results, especially in the West Miomaffo Subdistrict, namely in Manusasi Village. This also makes the level of application of farmers' technology not yet maximal especially in the TUB plant care component.

The lack of available production facilities and the absence of superior seeds is also an obstacle for farmers because it is caused by the absence of production facilities such as fertilizers and agricultural medicines at the location and the absence of superior seeds of corn and beans for some farmers as recommended. Production facilities such as fertilizers and medicines are often not available on site and are rare when farmers need them. Many farmers have tried alternative natural fertilizers such as compost but the results have not been maximized.

The superior seeds referred to by farmers are superior seeds of corn which are dry-resistant and in accordance with local climate conditions, namely composite corn seeds such as lamuru corn seeds, srikandi seeds and others. While available and distributed by the government are hybrid corn seeds that are greedy and need special technical treatment. Thus farmers have difficulty in increasing production. The same thing happens with legume seeds, most farmers need short-lived, dry-resistant, high-yielding legumes such as Merak or Vilma varieties that are 55 to 60 days old, while those available are seeds imported from Java which not in accordance with local conditions.

b. Non Technical Constraints

Non-technical constraints are constraints that are socio-economic in farmers. The biggest non-technical obstacle experienced by farmers is the lack of capital experienced by 112 farmers (75%), constraints to limited land experienced by 70 farmers (47%), constraints on the distance from the market experienced by 31 farmers (21%). While the constraints of natural disasters/winds were experienced by 19 farmers (13%) and price constraints were experienced by 20 farmers (13%). Another obstacle is the lack of counseling experienced by 15 farmers (10%).

Lack of capital is an obstacle because of the difficulty of farmers' access to financial institutions, so many farmers find it difficult to increase production, especially in the context of providing production facilities such as fertilizers and medicines. In farming farmers, many use their own limited capital so that farming is done in accordance with existing capital. Respondent farmers said that there were indeed loans from financial institutions such as banks but they had difficulty accessing because they were constrained by strict requirements and administration for example they had to make a business plan. Meanwhile, according to Mantra (2011), said that if an agroforestry/agroforestry system is introduced or implemented, investment is needed initially, such as: plant material, land conservation, and fertilizer. To alleviate farmers, investments needed to facilitate the implementation of agroforestry/agroforestry systems need to be provided through loans with low interest rates. Thus investment as initial capital through credit is needed by farmers in implementing agroforestry systems. Respondent farmers said that they hoped that in the future the program would be accompanied by capital assistance with low interest.

Limited land is an obstacle for some farmers because farmers have gardens around the forest area such as in Tuntun Village and Manusasi Village. This has led to an extension effort that will be made by farmers to meet the objectives of the Raskin program of labor intensive patterns of one-year dry land agroforestry systems of 0.25 ha (25 acres) to open up new plantation land into permanent plantation land. All of this also causes the average land area of the farmers participating in the program to be only 85.88 acres (0.8588ha), even though the program has been running for 4 years from 2011 to 2014, so the area of farmers should be 100 acres (1 ha). While according to Suproyo (1979) said that narrow land tenure is one factor that causes low production and income that will be received by farmers. Farmers who have a large area of arable land will get more income. Thus respondent farmers whose land is limited suggest that for those whose land is limited there is no need to expand the land (extensification) but they intensify the existing land area because they already have permanent gardens and production can be increased so that income can increase.

The distance from the market is an obstacle because a considerable distance from the market makes it difficult for farmers to market their agricultural products. The existing markets are Pasar Baru Kefamenanu in

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Kefamenanu City, Eban Market in Miomaffo West District, and Oelolok Market in Insana District. To get a good and suitable price, the farmer must bring the results for sale in Kefamenanu City or to Atambua City, Belu Regency. Finally, many farmers sell a lot of products at locations with low prices such as candlenut, cashew and oranges which are sold wholesale in trees at low prices. Many respondent farmers said that if they could bring their produce directly to Atambua then they could get a pretty good price.

Cattle attacks and natural disasters/winds are an obstacle because many farmer gardens are not fenced or the fence is damaged during the rainy season while livestock raising is carried out by the community traditionally by releasing livestock in the fields. To overcome this, the local village government has carried out public fences such as in the Village of Tuntun and Desa Nansean, but this fence needs close supervision and needs repairs every year. Natural disasters/winds are also an obstacle for every farmer because of the climatic conditions in which during the first rain there were strong winds which caused many crops to be damaged, especially farmers' corn plants. This happens a lot in Nansean Village, Insana District and Manusasi Village, West Miomaffo District.

Low prices are an obstacle because many farmers complain that at the time of harvest the price of agricultural products falls like when harvesting candlenut, cashew and oranges. This is caused by many middlemen who enter the village so that farmers sell at low prices through a wholesale system. Farmers who are members of farmer groups have done joint marketing through farmer groups but this has not gone well because of constraints on the marketing institutions' capital. In addition, many farmers' agricultural products are sold in the form of logs, so the price offered is also low. Respondent farmers said that they needed processing results so that they could increase the price of their products such as cashew fruit which was changed to cashew nuts. Thus it will increase the price of the product because there is an increase in the value added of the product.

The lack of counseling was an obstacle because the program counseling was only done once by the technical team with the help of PPL and NGO assistants. For effective extension activities, at least a month should be held for counseling in farmer groups. According to Mantra (2011), several phases of preparation and implementation of agroforestry/agroforestry systems require good management and must be combined with adequate counseling of surrounding communities and appropriate forms of involvement. This also relates to the level of acceptance in the form of farmers' perceptions of the benefits of the technical team and the low coordination team. Many respondent farmers said that they were not familiar with the technical team. The technical team should try to overcome technical problems, both technical cultivation at the farm level so that the program can run smoothly. Respondent farmers said that so far the NGO companion had accompanied but the technical information from the technical team was very important especially regarding the distribution of rice and other technical matters related to the program.

IV. CONCLUSION

Based on the scope of work at the scope of this study, the conclusions of this study are: 1) Farmers' acceptance of the labor-intensive raskin pattern program for dry land agroforestry systems in Timor Tengah Utara District includes High acceptance criteria with an average score of 29 (73%) from maximum score of 40; 2) The technology implementation of farmers participating in the Raskin labor-intensive pattern of dry land agroforestry systems in Timor Tengah Utara Regency is included in the Good Enough criteria with an average score of 26 (79%) from a maximum score of 33; 3) Old aspects of formal education, outpouring of work time (CWK) and farmer income have a relationship (significant) with the level of application of technology; 4) Aspects of the number of productive family members, outpouring of work time (CWK) and farmer income have a relationship (significant) with the success rate of growing annual crops; 5) Farmers participating in the Raskin program for labor intensive dry land agroforestry systems in Timor Tengah Utara Regency are in the category of Near Poor with Capita Income (IC) of Rp. 486,059 (186%); 6) The average income of farmers participating in the Raskin program in the pattern of dry land agroforestry systems in Timor Tengah Utara Regency is Rp. 3,194,019.- per arable land area (85.88 acres) or Rp. 3,719,547.- per hectare and costs incurred in the amount of Rp. 1,536,019.- per arable land area or Rp. 1,788,565.- per hectare; 7) Work time outpouring (CWK) of farmers participating in the Raskin work-intensive pattern of dryland agroforestry system food is 130.92 HKO per cultivated land area or 152.45 HKO per hectare; 8) Constraints faced by farmers in the Raskin program of labor intensive patterns of dry land agroforestry systems in Timor Tengah Utara Regency are in the form of cultivation technical constraints, including pest attacks, lack of available production facilities on site, and lack of superior seeds and constraints non technical in the form of lack of capital, limited land, long distance from the market, natural/wind disasters, low prices, and lack of counseling.

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Ground Water Level Estimation for Dörtyol region in HATAY

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***Abstract*— *Accurate and reliable estimation of groundwater level is important for the development and management of water resources. In this study, models of adaptive neuro-fuzzy inference system (ANFIS) with multiple linear regression (MLR) method and its performance in predicting groundwater level were investigated. As a field of application, it was applied for General Directorate of State Hydraulic Works (DSİ) 5512 well of Dörtyol region of Hatay province. In the study, 147-month data sets between 2000 and 2015, including hydrological parameters such as Precipitation (P), average air temperature (T), relative humidity (RH), wind speed (W) groundwater level (GWL) time series, predict the groundwater level used. The determinant coefficient (R^2), mean square error (MSE) and mean absolute error (MAE) were used as the statistical performance evaluation criteria. As a result of this study, MLR and ANFIS models performed well for GWL estimation. In particular, the ANFIS model yielded better results than the MLR model.*

Keywords*— *Estimation, groundwater level, Dörtyol, MLR, ANFIS.

I. INTRODUCTION

Groundwater is vital for humanity, it is an important for fresh water source worldwide. It is usually found more easily than other sources of drinking water and is cleaner. More than 2 billion people are dependent on groundwater for drinking water resources. It is therefore essential to develop sustainable water resource management measures to ensure the supply of drinking water in a region. Accurate and reliable estimates of groundwater levels play a decisive role as it provides basic information about the groundwater conditions of an aquifer. Common practice in modeling groundwater variables is the application of numerical models that use physical relationships to define a particular area. Such models require a lot of data and their development, implementation and maintenance is time consuming and expensive. However, artificial intelligence methods are an alternative, data-driven approach commonly used to estimate water resource variables over the last decade. ANFIS, which is one of the artificial intelligence methods, can assign all possible rules according to the structure created for the problem. ANFIS's ability to form a rule or allow for the creation of a rule means that it can benefit from expert opinions. For this reason, it is possible to obtain better results according to the error criterion as it provides the opportunity to benefit from expert opinions on artificial neural networks in many estimation problems. it becomes a valuable tool for complex scenarios, which are difficult to define by methods..

Recently, artificial intelligence methods have begun to be frequently used in modeling the rainfall-runoff [1-2], suspended sediment [3-6], dam reservoir level [7-10], density flow plunging [11], dam reservoir volume [12-15], sand bar crest [16], evaporation [17-18], and groundwater level [19-25].

In the two hydrometer stations in Hajighoshan and Tamar on the Gorgan River, Vafakhah [26] compared ANNs, ANFIS and ARMA model forflow estimations using flow 1 day, 2 days and 3 days-time series. The results showed that ANNs are superior to ANFIS and ARMA for current flow estimations 1 day, 2 days and 3 days ago. Nourani et al. [27]used feed-forward neural network (FFNN), Automatic Regressive Moving Average (ARIMAX) models for the prediction of GWL in the plain Ardabil of northwestern Iran. Unes et al. [28], predicted evapotranspiration (ET) in their study. They used ANFIS model has better performance than the empirical formulas for the estimation of daily ET.

The aim of this study is to investigate the monthly ground water level (GWL) fluctuation estimation by using Multiple Linear Regression (MLR) and Adaptive network fuzzy inference system (ANFIS) models.

II. STUDY AREA

In this study, the groundwater level of Dortyol region of Hatay province was examined. Monthly groundwater level data obtained from General Directorate of State Hydraulic Works (DSI) data and monthly total rainfall (R) recorded by Antakya Meteorology Station, monthly average temperature (T), monthly average wind speed (WS) and monthly relative humidity (RH) data were used

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to determine ground water level (GWL).

Figure 1 shows the location of the selected probe well on the map in this study.

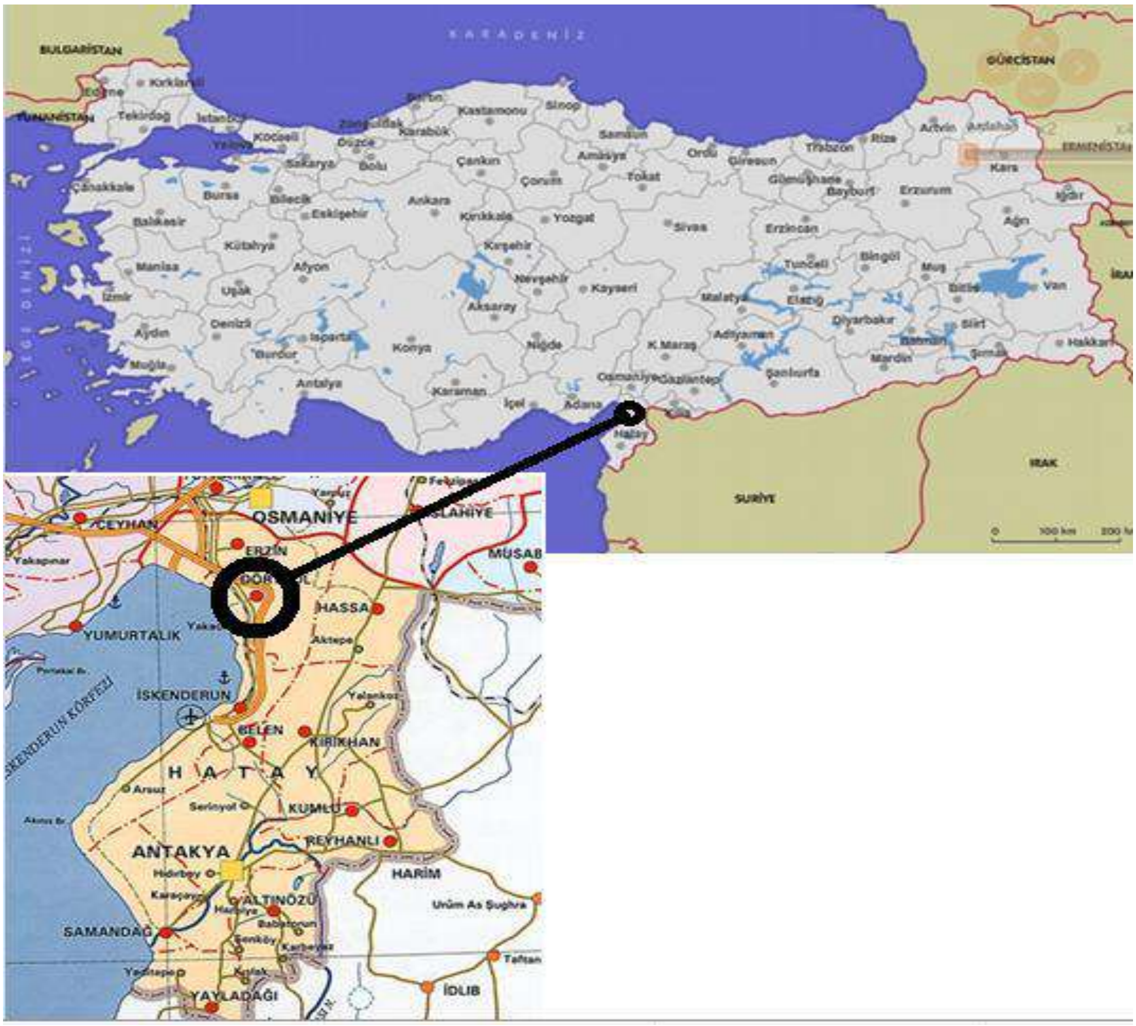

Fig. 1: Dortyol GWL station used in the study

III. METHODOLOGY

In this paper, Multiple Linear Regression (MLR) and Adaptive network based fuzzy inference system (ANFIS) models were used. In the all models, monthlyPrecipitation (P), average air temperature (T), relative humidity (RH), wind speed (W) groundwater level (GWL) time series were used for the Ground Water Level Estimations. All data obtained from Dortyol region in HatayProvincein the Turkey.

3.1. Multi-Linear Regression (MLR) Model

An MLR model is a method used to model the linear relationship between a dependent variable and one or more independent variables. The dependent variable is sometimes called a prediction, and arguments are called predictors. With these models, it is considered that the effects associated with a limited time period at some point can be approximated with the equality value.

The general equation of the MLR model is expressed as follows:

$$y=a+b_1x_1+b_2x_2+...............+(b_mx_m) \qquad \textbf{(1)}$$

In this equation, y represents the expected value of y when independent parameters (x1 = x1), (x2 = x2) ...., (xm = xm)

3.2. Adaptive Neuro Fuzzy Inference System (ANFIS)

Adaptive Neuro Fuzzy Inference System (ANFIS) is a hybrid artificial intelligence method that uses the ability of parallel neural network to calculate and learn artificial neural networks and the inference of fuzzy logic. The NF model developed in 1993 by Jang [29] uses the fuzzy inference model and Hybrid learning algorithm. Adaptive networks consist of directly connected nodes. Each node represents a processing unit. The connections between the nodes indicate an undetermined interest (weight) between them. All or part of the nodes can be adaptive.NF is a universal approximation methodology and is capable of

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approximating any real continuous function on a compact set to any degree of accuracy.NF with first-order Sugeno fuzzy model which used in this study. For more information, researchers can access Jang [20].

IV. MODEL RESULTS AND ANALYZE

4.1. Model Results

To see the relationship between created MLR model and observed values distribution graph are drawn in Figure 2 and scatter chart of this model was drawn in Figure 3.

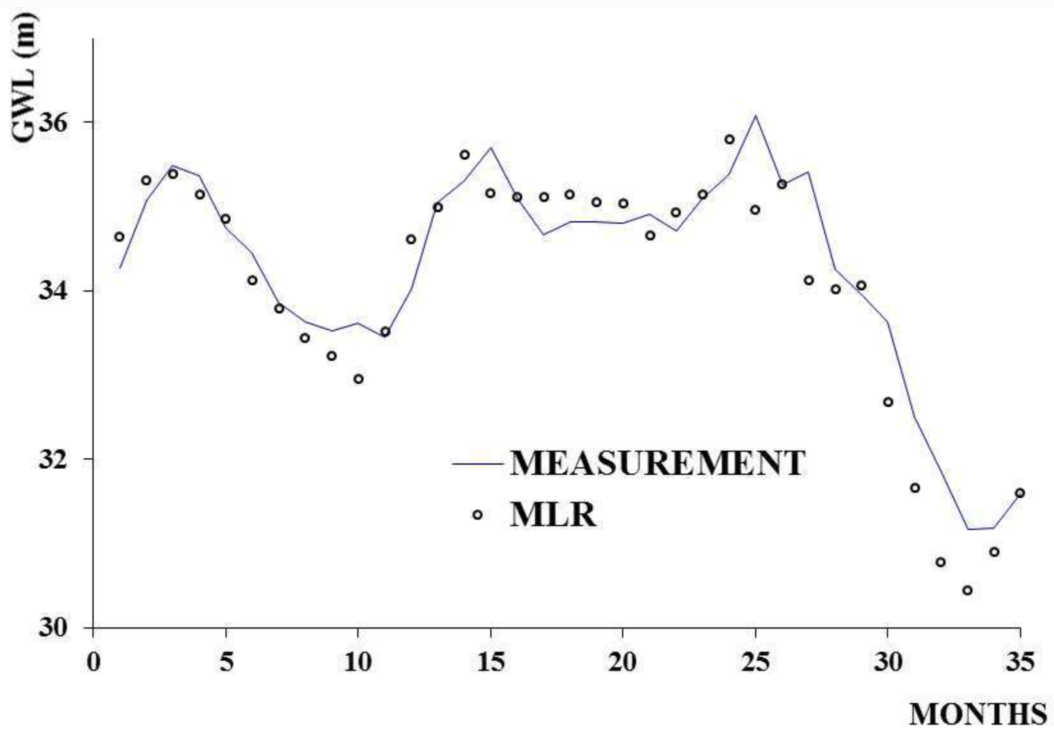

Fig.2: Distribution of MLR model

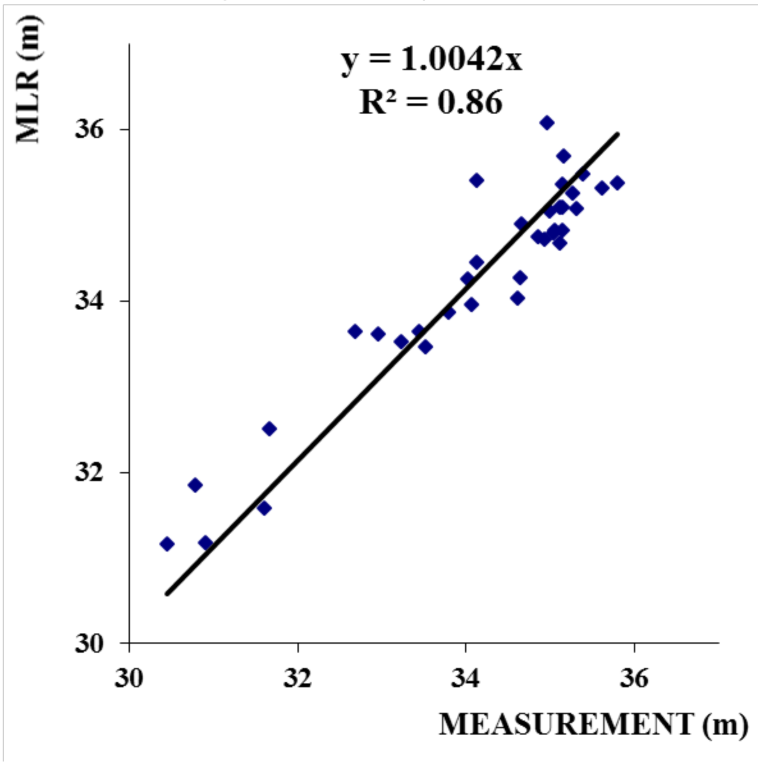

Fig.3: Scatter chart of MLR model

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Figure 2. shows that distribution of NF model test results are quite close to observed values of groundwater level for the study area.As it is seen in Figure 3, determination coefficient is calculated as 0.86 for test set of MLR method. In distribution and scatter charts, values are close to the actual values. Distribution of ANFIS method results and scatter chart is given with Figure 4. and Figure 5., respectively.

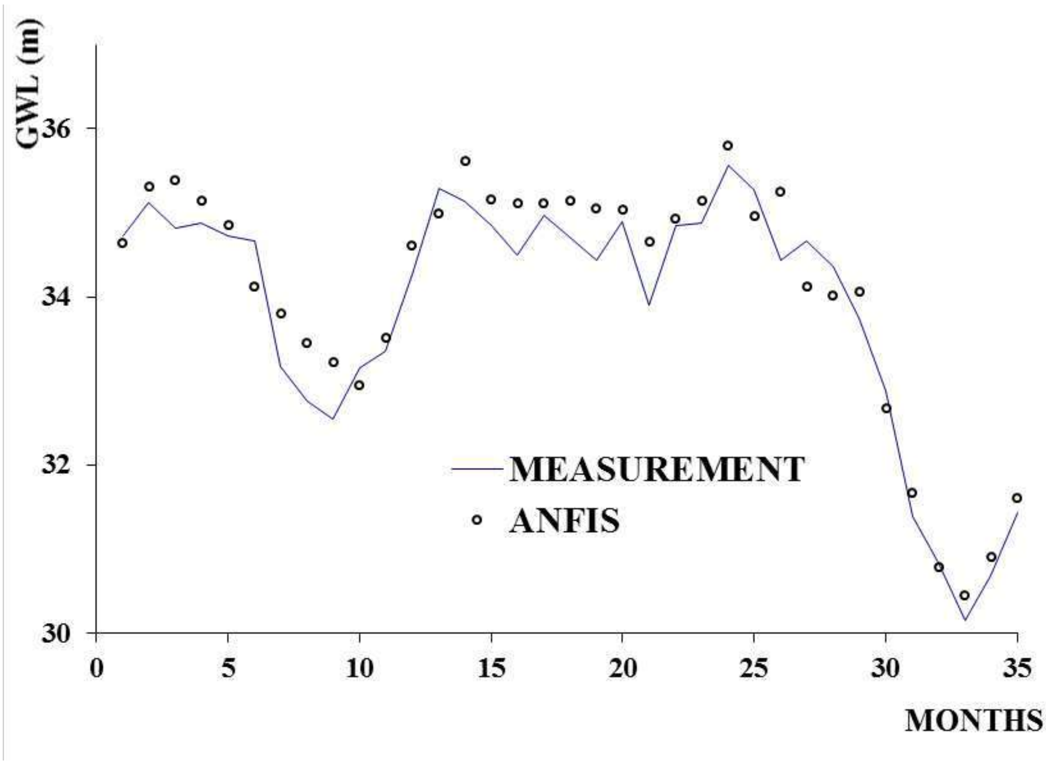

Fig.4: Distribution of ANFIS model

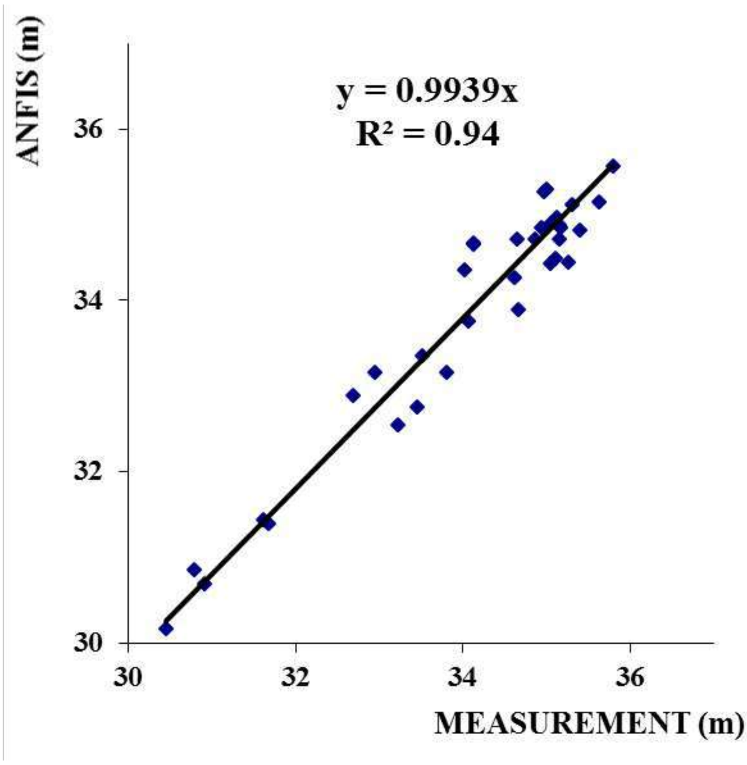

Fig.5: Scatter chart of ANFIS model

Results of ANFIS model show that the determination coefficient is high and the groundwater level estimationsarecloser to the actual values shown in Figure 4. Determination coefficient is calculated as 0.94 for ANFIS results as it is seen in Figure 5.

3.2. Model Analyze

Models were created using 151 data. 70% of the data were trained as training and 30% as test data. Model results were evaluated according to the coefficient of determination (R^2), mean squared error (MSE) and mean absolute error (MAE). Mean squared error (MSE) and mean absolute error (MAE)measures the magnitude of the error. MSE and MAE are used to diagnose the possibility of errors. MSE, MAE can take values from zero to infinite. Low values mean it is more useful.

$$MAE = \frac{1}{n}\sum_{j=1}^{n}\left|GWL_{Measure} - GWL_{predict}\right| \quad (2)$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(GWL_{Measure} - GWL_{predict}\right)^2 \quad (3)$$

Here, n is the number of data and GWL means the monthly average groundwater level (m).

The result parameters of the MSE, MAE and R^2 obtained from the test data will be shown in tabular form. The results will be used to compare estimates and performance.The statistical results of the models are given in Table 1.

Table.1: Comparison of MLR and ANFIS model performances

MODEL NAMES	MODEL INPUTS	MSE(m^2)	MAE(m)	R^2
MLR	$P_{(t)}, T_{(t)}, RH_{(t)}, WS_{(t)}, GWL_{(t+1)}$	0.25	0.44	0.86
ANFIS	$P_{(t)}, T_{(t)}, RH_{(t)}, WS_{(t)}, GWL_{(t+1)}$	0.17	0.35	0.94

MSE: Mean squared error, MAE: Mean absolute error R^2: Determination coefficient.
P (t): monthly total precipitation, T (t): monthly average Temperature, RH (t): monthly Relative Humidity, WS (t): monthly average Wind Speed, GWL (t + 1): monthly groundwater level

According to Table 1, when MSE, MAE and R^2statistical criteria were compared, all models were good. All models are evaluated separately; MLR (0.25 - 0.44-0.86) and ANFIS (0.17 - 0.35 - 0.94) models were found to perform well. Nevertheless, it is observed that the ANFIS model has a low error rate with high correlation.

In addition, the MLR model are close to ANFIS prediction performance. When the results were examined, MLR and ANFIS models were found to perform better in GWL estimations.

V. CONCLUSION

In this study, multiple linear regression (MLR) and Adaptive network based fuzzy inference system (ANFIS) models' performanceson groundwater level is investigated. As a field of application, the province of Hatay was applied to the GWL well of the Dörtyol region. 147-month data sets between 2000 and 2015, including hydrological parameters such as precipitation (P), mean air temperature (T), relative humidity (RH), wind speed (WS) groundwater level (GWL) time series, to estimate the groundwater level as input data. The determinative coefficient (R^2), mean square error (MSE), mean absolute error (MAE) were used as the statistical performance evaluation criteria. As a result of this study, MLR and ANFIS models performed well for GWL estimation. In particular, the ANFIS model has shown better results than the MLR model.

ACKNOWLEDGEMENTS

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Vermicomposting Kitchen, Municipal Market and Tea Factory Waste using *EiseniaFetida* Earthworms

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***Abstract*—** *Population increase, urbanization, industrialization and agricultural activities result in accumulation of solid waste. This waste requires sustainable management through techniques such as vermicomposting. The study aimed at determining the rate of vermicomposting of kitchen, municipal market and tea factory waste using Eisenia fetida earthworm species at the University of Embu, Kenya. The study was arranged in completely randomized design replicated thrice. Data was collected on decomposition rate, carbondioxide evolution, earthworm count, nutrient content of vermicompostedwaste and days to vermicompost maturity. SAS version 9.4softwarewas used for statistical data analysis. Treatment means were separated using least significant difference (l.s.d.) at p≤0.05 probability level. Kitchen waste vermicompost had the fastest decomposition rate of 0.6 kg/day. Carbondioxide evolution analysis was done weekly whereby a value of 0 µgCO$_2$/g vermicompost/m^2/day was recorded at week 15 when the vermicomposts had stabilized. Kitchen and market waste vermicompostshad the highest earthworm countof 169 and 153, respectively.The nutrient contents of the three vermicomposts were not significantly different. The study concluded that kitchen waste had the highest vermicomposting rate as well as earthworm count. Therefore, the study recommends that kitchen waste and market waste can be used where the aim of vermicomposting is earthworm production.*

Keywords*— *Nutrients, earthworms, decomposition, stability.

I. INTRODUCTION

Global waste production is predicted to increase from the current 2 billion tonnes per year to 3.4 billion tonnes in 2050 (Kaza*et al.*, 2018). Embu municipality produces approximately 9,344 tonnesof waste per year, out of which only 15% is collected and transported to the dumpsite (Nicholas, 2013). The waste in Embu municipality was found to compose 53% organic/biodegradable waste (Nicholas, 2013).This necessitates adoption of technologies to manage these wastes. Vermicomposting refers to the use of earthworms to convert biodegradable waste into high quality manure (Parekh and Mehta, 2015).Vermicompostprovides macro and micro-nutrients to plants.

Vermicomposted urban green waste has been found to contain: 2.0-3.0% Nitrogen (N), 16000-23000mg/kg Phosphorus (P), and 19000-26000mg/kgPotassium (K) (Sinha *et al*., 2009). In Malaysia, vermicomposted wastes were found to contain 0.87 % to 1.9 % N, 2300 -4600 mg/kg P, 4000-27400 mg/kg K and 168800 - 321400 mg/kg C (Jamaludin&Mahmood, 2008). In Kenya, manure based-vermicompost was found to contain 1.9% N, 3000 mg/kg P and 27000 mg/kg K (Savala, 2007). However, the nutrient content depends on the quality of organic waste(Kumar *et al*., 2018). Vermicompost takes about two months to mature, for instance, that of agricultural waste (Nagavallema*et al*., 2004).Vermicompost is applied to high value crops as a source of plant nutrients. For instance, agro-based waste vermicompost has been utilized for greenhouse kale productionin the Central highlands of Kenya (Karuku et *al*., 2016).

Vermicomposting is solid waste management technique which converts organic wastes into organic fertilizer (Rosman*et al*., 2017).Vermicomposting is thus efficient environmentally, economically and socially, making it a global waste reduction technique (Parekh & Mehta, 2015).Suitable earthworm species for vermicomposting include: *Eisenia Andrei*, *Eiseniafetida* and *Lumbricusrubellis* (Dominguez & Edwards, 2010).The vermicomposting earthworms are characterized by high organic matter consumption rates, high reproduction rates, high environmental stress tolerance, rapid hatching, growthand maturation(Malińskaet al., 2017).

Tea is the major cash crop in Kenya, contributingabout 11% economic growth in the agricultural sector and supportingapproximately 5 million livelihoods (Kaiyaga, 2015). Tea waste produced in Rukuriri Tea factory totals to 9.125 tonnes a year (Rukuriri Factory Tea Waste Records, 2018). This waste is a potentially valuable resource that can be utilized for preparation of vermicompost, a soil amendment.Organic wastes generated during tea processing include refuse tea, shade tree lopping's, tea pruning's and weeds (Hitinayake *et al.*, 2018). Green tea leaves were used for vermicomposting in the study.The University of Embu Kitchen generates 365 tonnesof waste annually (Mochache, 2016). This is a big resource that can be vermicomposted to supply nutrients for sustainable crop production.Embu town market generates approximately 2.6 tonneswasteannually(Environment and Natural Resource Department, Embu County Government, 2017).The 53% organic waste generated in Embu town market can be transformed into vermicompost for increasing crop productivity.

The basis of formulating this study was to prepare and analyze vermicompost from three organic wastes: kitchen waste, municipal market waste and tea factory waste. The study therefore aims at determining the rate of vermicomposting of kitchen, market and tea factory wastes using *Eisenia fetida* earthworm species.

II. MATERIALS AND METHODS

Study Area

The study site was the University of Embu, Embu County, Kenya. The site is located3 km from Embu Town along the Embu-Meru highway. The site lies at an elevation of 1, 350 metres (Kenya Information Guide, 2015). Embu has a bi-modal rainfall pattern receiving long rains between March and June and short rains between October and December (Embu County Government, 2013).The University of Embu receives an average annual rainfall of 1232 mm and has a mean annual temperature of 18.7 °C (Jaetzold*et al.*, 2006).HumicNitisolswith moderate to high fertility characterize the study site(Verde *et al.*, 2013).

Vermicompost preparation

Each vermicompost type was prepared in three plastic 120 litres capacity bins.Kitchen waste was obtained from the University of Embu kitchen, market wastefrom Embu Town Market and tea waste from Rukuriri Tea Factory in Embu. Earthworms were obtained from a commercial worm grower in Juja, Kenya and transported in a bucket containing worm casts and organic residues on top as earthworm feed.The experiment was conducted at room temperaturein a dark room. 100g banana leaves were placed as the worm bedding, 2.5 kilograms(1250 earthworms) placed on the bedding, followed by 2kg cattle manure then 1kg of waste (from the kitchen, market and tea factory). A three months pre-composting was done to preserve worm mortality and ensure multiplication. Vermicomposted kitchen waste comprised carrots peelings and cabbage and kales leaves. Vermicomposted market waste comprised banana, potato and fruit peelings. Vermicomposted tea waste comprised the green leaves. A cover of 100gdry banana leaves was added at the top. The vermicompost was kept at 60-70% moisture content by adding a litre of water once per week. The duration of the experiment was seventeen weeks for the first season and fifteen weeks for the second season.This was because in both seasons, stabilization took place by the fifteenth week whereby no further carbondioxide evolution was observed.

Study Design

The study was carried out for two seasons, July 2018 and November 2018 for the first season and November2018 and February2019 for the second season. The vermicomposting vessels were arranged in completely randomized design replicated three times. The treatments were: vermicomposted kitchen waste, vermicomposted market waste and vermicomposted tea factory waste.

Data Collection

Data collection was done on carbon dioxide evolution, earthworm count, decomposition rate and days to vermicompost maturity and nutrient content (N (%), P(mg/kg), K (mg/kg) and C %) of the three vermicomposts.

Carbondioxide Evolution

Carbon dioxide evolution (µgCO_2/g vermicompost/m^2/day) was done weekly(as from the second week of the setup) following the procedure modifiedby Strotmann *et al.* (2004). Sodium hydroxide (20 ml) was placed in a vial which was suspended on the vermicomposting bin to collect carbondioxide overnight for a period of 24 hours and afterwards analyzed by titrating with Hydrochloric acid (1M) to obtain the carbon dioxide evolved from the experiment.

Earthworm Count

Earthworm sampling was done weekly on the different vermicomposts using the method ofBouché and Gardner

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(1984). This was done by isolating, hand sorting and physical counting of the earthworms to determine their numbers once per week.

Nutrient Analysis

Nitrogen (%), Phosphorus (mg/kg), Potassium (mg/kg) and Carbon (mg/kg) were determined in the vermicomposts. Total nitrogen was analyzedfollowing the procedure of Page *et al*. (1982).The elements P and K were analysed following the procedures of Mehlich*et al*. (1962). Potassium was determined with a flame photometer and Phosphorus spectrophotometrically.Total organic carbon (TOC) analysis was analysedfollowing the procedure of Anderson & Ingram (1993).

Decomposition Rate

Decomposition rate was determined using the equation of (Rovira&Rovira, 2010):

-k = dX/dtX, where-k -decomposition rate constant, dX -change in initial litter mass, dt -change in time and X -initial litter mass

Data Analysis

SAS software version 9.4 was used for statistical data analysis (SAS, 2013). Data was subjected to two-way analysis of variance using SAS GLM code of the model CRD, to determine the difference inearthworm count and carbondioxideevolution at the beginning and end of the study. Statistically significant($p \leq 0.05$)treatments meanswere separated using l.s.d.

III. RESULTS AND DISCUSSION

Vermicomposting rate of the wastes

Maturity indicates suitability of vermicompost for plant growth (Majlessi*et al*., 2012).The vermicompost from the three organic wastes took 107 days to maturein the first season. In the second season, the three vermicomposts took 98 days for kitchen waste, 105 days for market and tea waste to reach maturity; this was because of the differences in the C: N ratio of the materials. Nurhidayati (2018) reported that compost reached maturity when the C: N ratio was <20. According to Alidadi *et al*. (2016) the adequate time for municipal solid waste vermicompost maturation was reported to be 75 days. Similarly, Aynehband*et al*. (2017) vermicomposted cereal wastes in 90 days, whereas Gopal *et al*. (2018) vermicomposted coconut wastes for 80 days. The differences in the vermicomposting rates in the different studies may be related to temperature differences. The higher the environmental temperatures the higher the decomposition rates consequently the faster the maturity.

In the first season kitchen waste was found to have the highest decomposition rate at 0.51 kg/day, tea waste followed at 0.48 kg/day and market waste had the lowest decomposition rate at 0.45 kg/day. In the second season kitchen waste still maintained a higher decomposition rate at 0.6 kg/day as compared to tea waste 0.54 kg/day and market waste 0.53 kg/day. The different vermicomposting rates of the residues may be related to residue quality as suggested by Aynehband*et al*. (2017).Wardle *et al*.(2009) and Moore *et al*.(2011)found that chemical composition differences in residues such as, lignin content, organic Carbon content, C: N ratio and lignin affected decomposition rates.Residues with a high C: Nratios have slower decomposition rates and those with high N content have high rates of decomposition (Ali,2011).

Earthworm Count

Earthworms multiply under suitable ecological conditions as well as suitable food, temperature and oxygen (Chanda *et al*., 2013). In season one, kitchen waste had a significantly higher ($p \leq 0.05$)earthworm count of 572earthworms per kilogram of vermicompost compared to market waste vermicompost at 364earthworms and tea waste vermicompost at 352earthworms at the end of the study. In the second season, significant differences ($p \leq 0.05$)were also observed at the end of the study, whereby kitchen and market waste vermicomposts had a significantly higher ($p \leq 0.05$)earthworm count of 676 and 612 earthworms respectively, per kilogram of vermicompost, compared to tea waste vermicompost at 432 earthworms.

In both seasons of the present study the initial number of earthworms was low but at the end of the study the number of earthworms had increased by over 50% for each type of vermicompost.In season one;tea waste recorded the highest earthworm increase at283%, followed by kitchen waste at 211% and market waste followed at 63%. In season two, kitchen waste vermicompost recorded the highest increase in earthworm count at273%, followed by market waste vermicompost at 248% increaseand tea waste vermicompost at 212%. High numbers of earthworms were recorded in kitchen waste as it is a rich inorganic feeding material ideal for earthworm growth and reproduction (Albasha, 2015).

This corresponds to the findings of Lalander*et al*.(2015), who foundan increase of 65 %,Abu Bakar *et al*. (2014)who found a 92 % increase,Mathivanan*et al*., (2017)who found a 77 – 94% increase andGopal *et al*. (2018) who found a 300 fold increase in earthworm numbers on vermicomposting.The results of the present study indicating differences in earthworm numbers based on the type of

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residue agree with those of Aynehband*et al*. (2017) who found differences in earthworm number and activity on using different wastes.

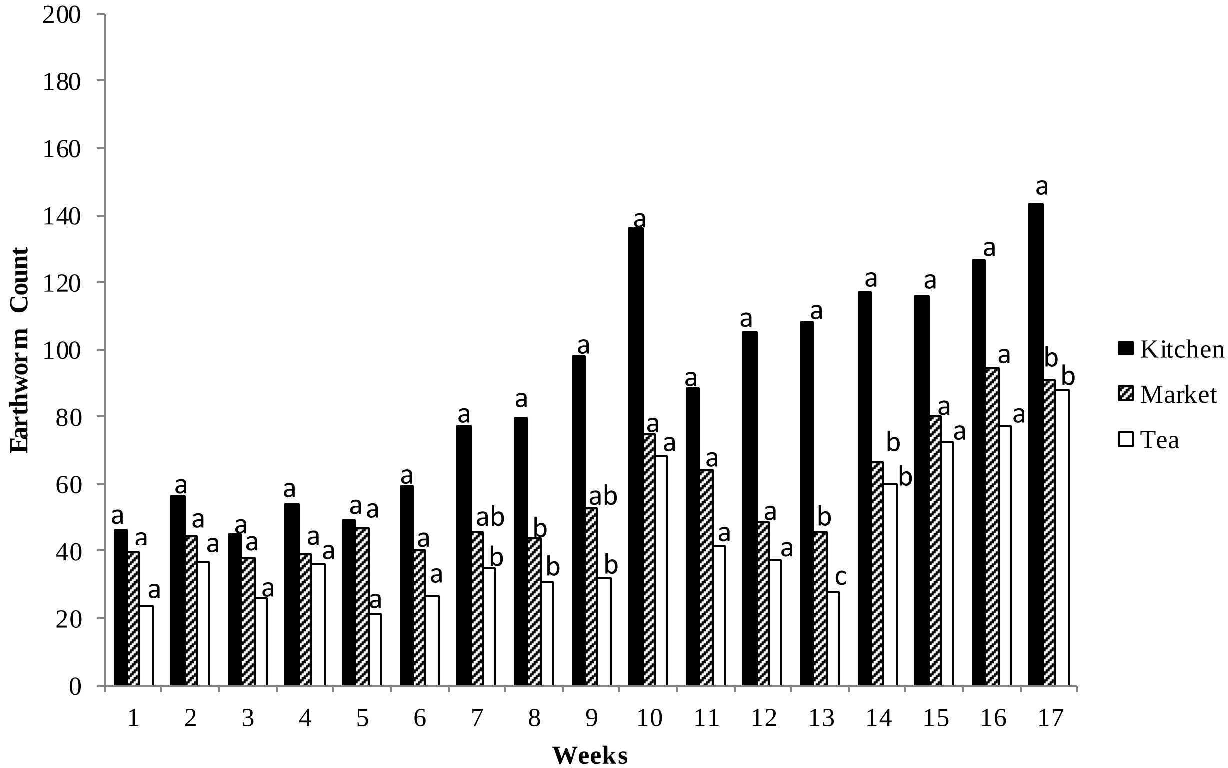

Fig.1: Season 1 Earthworm Count

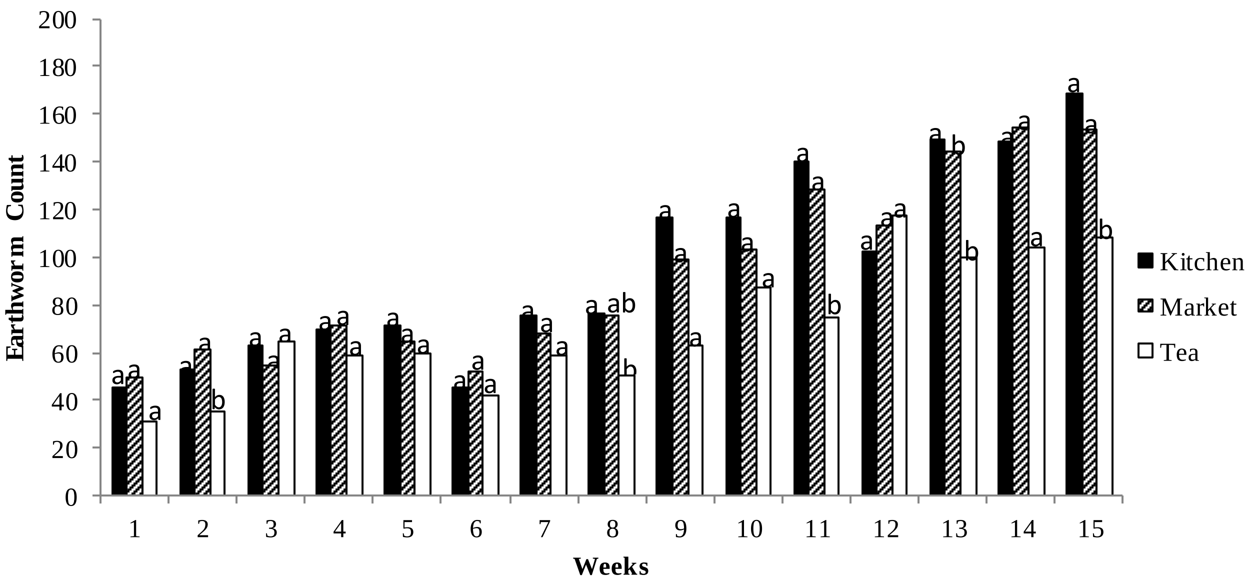

Fig.2: Season 2 Earthworm Count

Carbon Dioxide Evolution

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In the first season, there was no significant difference in carbondioxide evolution by the seventeenth week ($p \geq 0.05$). However, significant differences ($p \geq 0.05$) were observed in weeks 1 and 11, whereby in week 1, tea wastevermicompost had a significantly higher carbondioxide evolution (12767 µgCO_2/g vermicompost/m^2/day) compared to kitchen waste vermicompostat 5133 µgCO_2/g vermicompost/m^2/day and market waste vermicompost at 2033 µgCO_2/g vermicompost/m^2/day. In week 11, market waste vermicompost had a significantly higher carbondioxide evolution of 5668 compared to kitchen waste vermicompost at 800 µgCO_2/g vermicompost/m^2/day and tea waste vermicompost at 0µgCO_2/g vermicompost/m^2/day. By the end of the second season, there was no significant difference in the carbondioxide evolution of the treatments ($p \leq 0.05$). In the second season, significant differences in the carbondioxide evolution were observed in weeks 1 and 5, whereby kitchen waste vermicompost had a significantly higher carbondioxide evolution at 14567µgCO_2/g vermicompost/m^2/day and 10967µgCO_2/g vermicompost/m^2/day ($p \geq 0.05$) compared to market and tea waste vermicomposts at 10500µgCO_2/g vermicompost/m^2/day and 8833 µgCO_2/g vermicompost/m^2/day respectively.The three vermicompost types stabilized at week 15 whereby a value of 0 µgCO_2/g vermicompost/m^2/day was recorded (Figure 3, 4).

This was done to indicate vermicompost stability. In both seasons as indicated by Figures 3 and 4, high values of carbondioxide evolution were observed among the kitchen, municipal market and tea vermicompost at the beginning of the experiment, but values gradually reduced until they were constant towards the end of the experiment, thus indicating that vermicompost had stabilized. This corresponded to the findings of Nayak *et al*.(2013) whereby lower values of carbondioxide evolution indicated more vermicompost stabilization. Increased carbondioxide evolution rates indicated higher earthworm and microbial respiration rate as well as aerobic biological activity (Kalamdhad*et al*., 2008; Sonawane, 2016). Lower carbondioxide evolution values were as a result of reduced metabolic activity which results in decreased respiration rate of microbes and earthworms (Nayak *et al*., 2013).

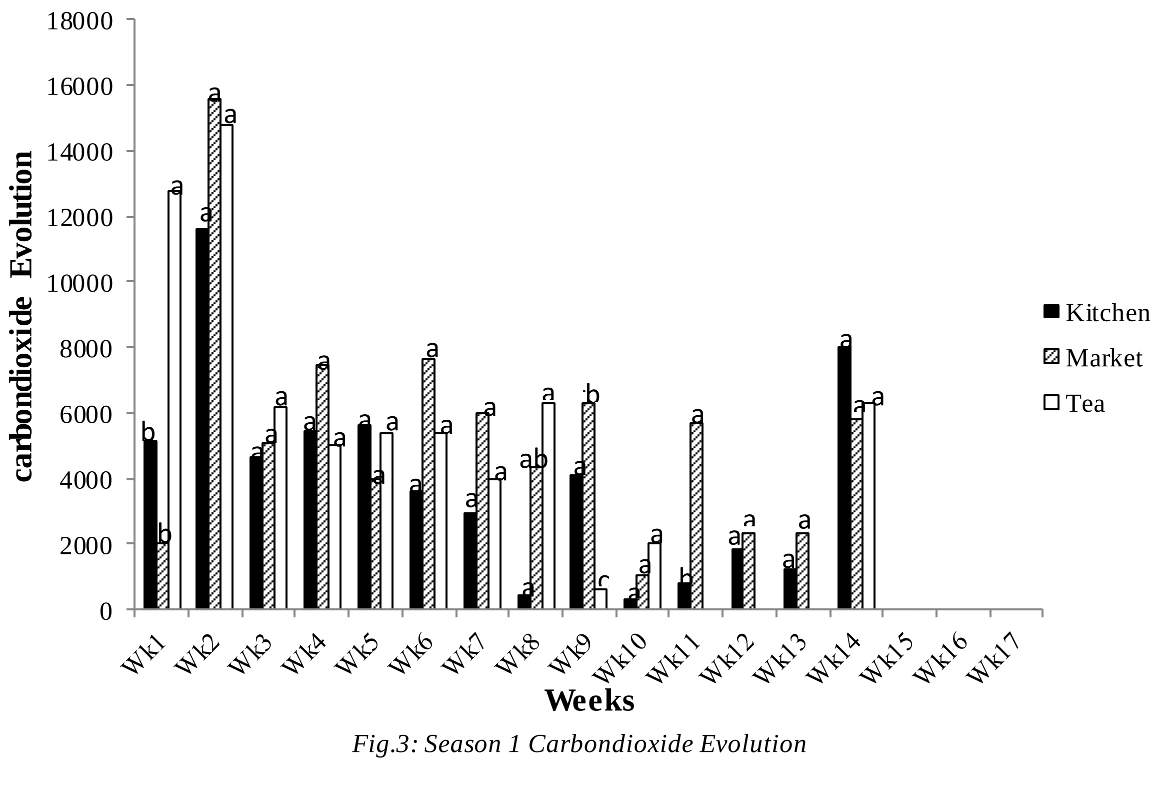

Fig.3: Season 1 Carbondioxide Evolution

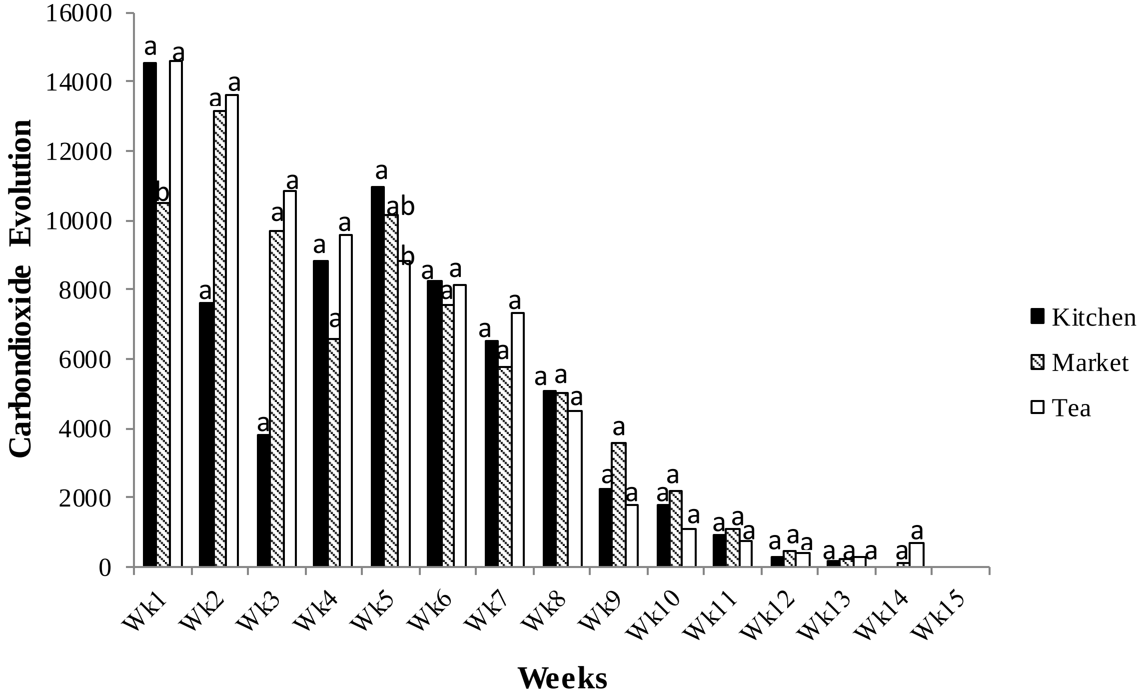

Fig.4: Season 2 Carbondioxide Evolution

Vermicompost Nutrient Analysis

Table 1 indicates the statistical analyses of the nutrient content of the vermicomposts.

Table 1 Vermicompost nutrient content

Treatment	**Nitrogen (%)**	**Phosphorus (mg/Kg)**	**Potassium (mg/Kg)**	**Organic Carbon (mg/kg)**
Kitchen	0.5067[a]	8067[a]	27433[a]	150000[a]
Market	0.8167[a]	7667[a]	19700[a]	153330[a]
Tea	0.7767[a]	7600[a]	24167[a]	150670[a]
P-value	0.1066	0.9354	0.1382	0.9499
l.s.d	0.3199	0.3362	0.8025	2.5525

Though the nutrient contents were statistically similar ($p \geq 0.05$)among the treatments, (Table 1), N content was higher in market waste than tea and kitchen waste vermicomposts by 5.1% and 64% respectively. Phosphorus content was higher inkitchen waste vermicompost than market and tea waste vermicomposts by 5.2% and 6.1% respectively. Kitchen waste vermicompost had the higherK content than tea and market waste vermicomposts by 13.5% and 39.3% respectively. Organic carbon content was higher in market waste vermicompost than tea and kitchen waste vermicomposts by 1.8% and 2.22% respectively.

An *et al*. (2014)found vermicomposted kitchen waste (food scraps) containing 0.5% available N, 2400 mg/kg available P, 3000 mg/kg available K, 0.6% Mg and 0.2% Ca. Additionally, Wani and Rao, (2013) found vermicomposted tea waste containing an organic carbon content of 133,000 mg/kg. The results of the present study on the N content of kitchen wastes agree with those of An *et al*. (2014).The higher P and K contents in the present study may be due to the quality of the kitchen wastes used. The nutrient content of municipal solid waste was found by Pattnaik and Reddy (2009) to be 0.5% N, 3000mg/kg P, 2000mg/kg K and 796 000mg/kg organic C.The N, P, K contents of the present study are much higher than those of Pattnaik and Reddy (2009). This could be due to differences in the quality of the market residues in the present study. Vermicomposted tea waste is reported to contain 0.9% N, 6000 mg/kg P, 51000mg/kg K, and 259000mg/kg C (Abbiramy*et al*., 2015). The tea waste vermicompost N and P contents in the present study are similar to those of (Abbiramy et *al*.,

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2015)but the K contents are lower. This may be due to differences in the quality of the leaves.

IV. CONCLUSION

The study determined the decomposition rates of vermicomposted kitchen, municipal and tea factory waste. Kitchen waste had a higher vermicomposting rate compared to tea and market waste. Kitchen waste and market waste vermicomposts had higher earthworm count compared to tea waste vermicompost. Stability of the vermicomposted waste was determined by carrying out carbondioxide evolution, whereby low carbondioxide evolved indicated stability of the vermicompost. Kitchen wastes and market wastes can be used to rear earthworms for use in vermicomposting or livestock feed. This study therefore recommends kitchen and market wastes where the aim of vermicomposting is earthworm production.Market, kitchen and tea waste can be used as soil amendments as they have statistically similar nutrient contents ($p \geq 0.05$).

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Pesticides Abuse and Health Implications in Ghana: A Review

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Abstract— *It is estimated that 25% of the globally produced pesticides are used in developing countries and African farmers use only 4%. However, more than 90% of the global minimum of 300,000 death cases related to pesticides poisoning annually are believed to occur in developing countries. Efforts have been made worldwide to restrict or ban the use of persistent pesticides while regulating the use of other pesticides to ensure environmental and food safety. However, most pesticide dealers and users of many developing countries including Ghana have not come to terms with the associated safety practices that ensure a safe use to keep the integrity of our environment. Pesticide residues have been detected in food, water, water sediment, air, blood and human breastmilk because of pesticide abuses. This paper reviews some abuse practices during handling and storage, application, or disposal; associated exposures; public health risks associated with the abuse practices; as well as issues of monitoring and enforcement. Some instances of casualties are also discussed.*
Keyword— ***pesticide abuse, pesticide application, pesticide disposal, pesticide handling, pesticide residue.***

I. INTRODUCTION

Global pesticides use was estimated at 2.26 megatonnes in 2001 and 2.4 megatonnes in 2006 and 2007. While 2.5 megatonnes is estimated to be used yearly in recent times, 40% is estimated to be herbicides, 17% insecticides and 10% fungicides (WHO, 2008; NPAS, 2012). According to WHO (2008), the use of pesticides has increased in developing countries with Africa, South and Central America, Asia and Eastern Mediterranean being the fastest growing pesticides markets. It is estimated that 25% of the global pesticides produced are used in developing countries and African farmers use only 4% (WHO, 2008; NPAS, 2012). In Ghana, it is estimated that pesticides worth approximately 100 million dollars are imported annually (NPAS, 2012).

Though the amount of pesticides used in the developing world is relatively low, developing countries have higher pesticides risk due to abnormal exposure to pesticides resulting from abuse/misuse. Weaker links between healthcare as well as regulatory systems and pesticide dealers and users increases the impact and occurrences of the abuses (WHO, 2008). About 1-5 million pesticide poisoning cases are estimated to occur annually in the world, causing about 20,000 fatalities in agricultural workers, and a chunk of this is recorded in developing countries (WHO, 2004). Also, about 99% of the global minimum of 300,000 death cases related to pesticides poisoning annually are believed to occur in developing countries (WHO, 2008). Most authors attribute these happenings to pesticides use but a critical analysis of issues points to the fact that pesticides misuse is the issue.

Rachel Carson in 1962 wrote the Silent Spring to raise public awareness on the effects of harmful pesticide use on our health and environment. Efforts have been made to restrict or barn the use of persistent pesticides while regulating the use of other pesticides to ensure environmental and food safety. However, most pesticide dealers and users of many developing countries have not come to terms with the associated safety practices that ensure a safe use to keep the integrity of our environment. Regulatory bodies have challenges that do not allow them to work efficiently on their mandate.

This paper is a review of some of the abuse/misuse practices, associated exposures, monitoring and law enforcement, and public health issues associated with pesticide abuses with some instances of casualties discussed.

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II. COMMON ABUSE PRACTICES

Some recorded and observed pesticides abuse/misuse practices have been discussed. These practices may be classified broadly under three main headings namely: pesticide handling and storage, application and pre – harvest observation, or disposal.

2.1 Pesticide handling and Storage

In many parts of Ghana and other Sub Saharan African countries, pesticide handling is one of the most important factors that makes its use dangerous (Clarke *et al*., 1997). Most pesticide sellers and farmers in Ghana are ignorant on proper handling of pesticides. Some pesticides sellers display their products in direct hot sunny conditions while others sit in pesticide stores and display rooms all day inhaling strong fumes of pesticides. Most sellers and farmers alike neither put on protective clothing nor wash after handling pesticides. Farmers store used or unused pesticides together with food in inappropriate places. According to Okoffo *et al*. (2016), 52% of cocoa farmers in the formerly Brong Ahafo Region store pesticides in their homes (bedrooms, storerooms, porches or on roofs). Clarke *et al*. (1997) also found out that almost 50% of farmers stored their pesticides in their homes with a lot of them storing them in their bedrooms. Mattah *et al*. (2015) also reported that 86% of farmers in the Greater Accra Region stored their pesticides at home. Considering data available since the 90s, it is obvious that not much has changed regarding how and where farmers store used or unused pesticides. In most communities, there have been numerous but unreported cases of children accidentally drinking pesticides because they were stored at home and in places that could be easily reached by children; and or the pesticides were not stored in their original containers (especially in water bottles). In 2010, it was reported in the Upper East Region that 15 farmers died of suspected pesticides poisoning as a result of poor storage of pesticides in their homes, the pesticides leaked into their food stock (NPAS, 2012). Applicators have confirmed experiencing symptoms indicative of the effect of poor handling of pesticides. Report on a survey conducted by the National Presbyterian Agricultural Services (NPAS, 2012) in 14 villages in the Upper East Region of Ghana indicated that a quarter or a third of 183 applicators confirmed inhaling or experiencing chemical spillage on their bodies respectively due to poor handling. Obviously, if the country can reduce risks of pesticide abuse and their effects on users, handling and storage are key areas to look at.

2.2 Abuses in pesticides application

In Ghana, most cocoa farmers and about 87% of vegetable farmers use pesticides (Okoffo *et al*., 2016; Dinham, 2003). Pesticides are also used extensively in the production of other commodities like cowpea, maize, rice, cassava, fruits etc. They are also used for pest control in the homes (Mattah *et al*., 2015). There are several avoidable pesticides application practices that result in greater risk to humans, other mammals, and the environment at large. Most farmers and applicators of pesticides (labourers) do not use adequate Personal Protective Equipment (PPEs). In a survey by Okoffo *et al*. (2016), it was revealed that while 45% of cocoa farmers partially used PPEs, 20% do not use PPEs at all during pesticides application. Most of these farmers used handkerchiefs or face towels in place of respirators, a practice not unique to only cocoa farmers. According to Mattah *et al*. (2015), less than 30% of farmers generally use full PPEs. Dari *et al*. (2016) confirmed 80% of tomato farmers in the northern part of Ghana do not use PPEs during pesticides application. Also, in a survey in the Ashanti and Brong Ahafo Regions, it was confirmed that less than 20% of applicators used full PPEs (Afari-Sefa, 2015). These suggest that about 70% or more of farmers generally do not dress appropriately during pesticides application exposing part(s) of their bodies as entry points for pesticides. The phenomenon has been attributed to the fact that users complain of the discomfort they go through in using PPEs because of the hot and harsh weather condition in the tropics. To others, PPEs are expensive or unavailable; and yet with some others, they don't see the relevance or are ignorant of the benefits of using PPEs (Clarke *et al*., 1997). These are issues governments and researchers must find solutions to, because pesticide use has come to stay. Research may be needed to look for innovative ways of progressively providing comfortable but affordable and readily available PPE set for farmers in the tropics. A comprehensive plan may be needed to reach almost all farmers in the country to expose them to among other things the need to use protection during pesticide application. There exists also the use of nozzles meant for applying fungicides/insecticides for herbicide application and vice versa. Most farmers and applicators alike don't know much about drift, its effect and how to minimize it. Pesticides are applied close to water bodies while the maximum number of times a pesticide can be applied in the growing season on the same field is exceeded (Asante and Ntow, 2009). Some farmers still apply banned, expired, overdose, or pesticides not registered by the EPA. Some also use pesticides on crops they are not registered for (example is the use of pesticides strictly supplied for use in cocoa on tomatoes), or unapproved mixture (cocktail) of pesticides (NPAS, 2012; Horna *et al*., 2007; Dari *et al*., 2016) and stir pesticides with bare hands (Okoffo *et al*., 2016). A survey conducted in tomato growing communities of the Asante Akyem Agogo District of Ghana confirmed the use of non-recommended pesticides in tomato production (Obiri - Danso *et al*., 2011). Also, some farmers apply pesticides too close to harvesting time ignorant of or not considering pre – harvest intervals, in order to sell "good looking" vegetables at the request of market women (market queens) and consumers who are mostly ignorant of the effect of its consumption (Adu-Kwateng *et al*., 2016; Dari *et at*., 2016; Ntow *et al*., 2006). Such fruits or vegetables are just washed with water after harvesting and sent to the market. This practice renders a greater portion of farm produce contaminated before consumption. Also, one of the most ignored precautions on pesticide application is time of re-entry after pesticide application.

2.3 Disposal

Disposal of expired pesticides, left over solutions, water from cleaned equipment and most importantly empty pesticide containers constitute the major disposal practices that lead to misuse (Okoffo *et al*., 2016; NPAS, 2012; Mattah *et al*., 2015; Afari-Sefa *et al*., 2015). With empty/used containers, most farmers dump them in the farms or bush, some bury in the farm, a few burns them while others re-use for household purposes (to keep water and other food items such as palm oil, salt or sugar among others) after washing them (Okoffo *et al*., 2016; NPAS, 2012; Mattah *et al*., 2015; Afari-Sefa *et al*., 2015). Most farmers dispose left over pesticides solutions and water from washed equipment in their fields while a few dispose them in a designated area on their farms (Okoffo *et al*., 2016; Afari-Sefa *et al*., 2015). The inappropriate disposal of pesticides, solution, cleaning water and containers has resulted in the accumulation of pesticide residues in the soil, underground and surface water.

III. ABUSE PRACTICES AND ASSOCIATED EXPOSURES

The exposure/poisoning of farmers, applicators or consumers occur in Ghana mainly through oral ingestion, dermal contact, and/or inhalation. Oral ingestion occurs accidentally, especially in children through abuse practices like storage of used or unused pesticides in the home or in used food or water containers. Others accidentally consume pesticides when they take sugar, salt, oil, water and other foods kept in used pesticide containers. Farmers and applicators again get exposed because they eat, drink water or smoke with an unwashed hand after pesticide handling or application. Consumers also get exposed largely by consuming farm produce that have pesticide residues (like fruit and vegetables), especially when farmers do not obey pre-harvest interval, spray more than the recommended dosage or exceeding the number of times a pesticide can be applied in a season and/or when pesticides are applied on crops they are not recommended for. This is very worrying situation for consumers because most of the farm produce on the local market are not tested for their pesticide residue levels before or after getting to the market. High levels of pesticide residues have been detected on cabbage, onion, cucumber, lettuce, tomatoes, okra, pepper garden eggs and pawpaw from various market centers in experimental surveys (Dari*et al*., 2016; Hanson *et al*., 2007; Hussain *et al*., 2002, Bempah *et al*., 2011; Botwe *et al*., 2012a; Ntow *et al*., 2001; Darko and Akoto, 2008, Osei-Fosu, 2014). Due to the non-observance of minimum allowable distance between water bodies and spray area, disposal abuses as well as run-offs from farms (especially on vegetable and rice farms which are close to water bodies), most water bodies are contaminated with pesticide residues which are being taken mostly by Ghanaians in rural farming communities where there is no access to treated water (Fosu – Mensah *et al*. 2016a, b and c). Botwe *et al*. (2012b) in an experiment in Ghanaian communities where pesticide abuse is rampant recorded significant levels of pesticide residues in both water and water sediments detrimental to human health. Fosu – Mensah *et al*. (2016a; 2016b; 2016c) also detected pesticide residues above the WHO Maximum Residue Limits (MRL) in soils and water samples in cocoa farms in the country.

Most farmers or applicators in Ghana have and continue to experience dermal contact with pesticides as reported by various research works (Attabila *et al*., 2017; Clark *et al*., 1997). Dermal contact is by far the largest pathway through which pesticides enter the body of farmers and applicators. Non-use or partial use of PPEs exposes body parts of farmers and applicators to spillage during handling or loading, or leakages from knapsack sprayers during application. Others get drenched in the very solution they spray because the nozzles height may be too high or the right pattern of spraying was not followed. In most situations, the applicator's clothe collects the sprayed solution from the crop or weed during spraying while they walk through the sprayed area.

Pesticides inhalation also continues to be a challenge in Ghana. The non-use of respirators or nose masks during pesticide handling, loading and application predisposes a lot of applicators to the effects of inhaling pesticides. Farmers usually complain of difficulty in breathing when using respirators. The problem with using handkerchiefs as nose mask is that they are able to absorb the solution when there is drift, increasing risk of inhalation. Some applicators also use nozzles that encourage drift resulting in pesticide mists in the air. Most farms in the peri-urban areas do not have wind/drift break borders that would save the immediate environment from some amount of drift exposing close by populations to pesticide poising. Most chemical sellers inhale chemical fumes while they sit and even eat in their store or showrooms. Some apply pesticides that are not meant for domestic use in their homes while some others do not allow aeration in their rooms after application of pesticides. Worrying health impacts similar to those seen with pesticides misuse in the agricultural sector are being recorded in homes especially in the control of bedbugs and rodents. In July of 2016 in Kumasi, two siblings died after allegedly inhaling a substance (insecticide) that was used to control bed bugs (Ghana Web, 2016).

IV. PUBLIC HEALTH ISSUES

Farmers and applicators are not the only individuals affected by the harms caused by the misuse of pesticides in Ghana but consumers too. Medical officers believe that some medical conditions and deaths among Ghanaians perceived to be "natural" may be related to pesticide abuse, partly because poisonings are hard to diagnose (NPAS, 2012). Pesticide poisoning may be similar to other disorders like pneumonia, asthma, heatstroke, low blood sugar or intestinal infections (Oudejans, 1991). Pesticide poisoning may be connected to a variety of human health challenges from headaches to cancer, reproductive issues, endocrine disruption, nervous system issues, skin and eye irritation, dizziness, fatigue, systemic poisoning and others which can be severe or fatal occasionally (Sarwar, 2015; Oudejans, 1991). While acute health challenges may occur immediately or a few minutes, hours or days after exposure, chronic health effects occur long after even minimal exposure to pesticides.

Overall, pesticide exposures to farmers, applicators and consumers result in accumulation of pesticide residues and they suffer the effects. For these practices, Ntow *et al.* (2008) revealed 88% of farmers' blood serum and 75% of human breastmilk contained high levels of pesticide residues. Daily intake of DDTs and HCHs for infants from human breastmilk were above the threshold compared with the guidelines as proposed by health Canada. Numerous other studies have also detected levels of pesticide contamination on farmers in Ghana, reviling that some farmers have accumulated pesticides residue at levels above the tolerable daily intake limit in their blood and breastmilk. This adversely affect their health as well the health and development of their lactating babies (NPAS, 2012; Ntow, 2001; Ntow *et al*., 2008; Asante and Ntow, 2009). In 2008, Ntow reported on a work carried out in 2005 which revealed that farmers with these high levels of pesticide residue had been exposed to pesticides for between 1 – 26 year(s) (Ntow *et al*., 2008). This implies that children belonging to farmer households who were fed with contaminated milk would be at least 25 years or below by the time of the research. Thirteen years on in 2018 makes children belonging to farmer households who were fed with such contaminated breast milk 38 years or below, and may be susceptible to the repercussions of the contamination. Some pesticide residues that have been detected in food, water, water sediment, air, blood and human breastmilk at significant levels are listed in Table 1. Table 2 also summarizes the possible symptom(s) seen with poisoning from some active ingredients of pesticides registered in the country.

V. PESTICIDE USE AND HEALTHCARE IN GHANA

In Ghana, one limitation that has made the recognition of pesticide poisoning a challenge is the fact that medical practitioners have mostly been left out of pesticide misuse issues as well as the fight against its effects on humans. Generally, there is a weak linkage between the occupation of individuals, the clinical symptoms they report at healthcare facilities and the diagnosis and treatment they receive, especially in the rural areas. Training health workers in the safe handling, identification and management of pesticide poisoning at all health posts will be of great help to the country. Involving healthcare practitioners in pesticide abuse issues and organizing special sensitization programmes for them would strengthen their ability to perfectly link farmers' history on pesticide use with clinical symptoms they present at health care facilities to ensure proper diagnosis and treatment. This would help to reduce the effect of exposures they (Farmers) undergo.

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Table 1: list of some pesticides detected in food and water in Ghana and their levels

Pesticide	Commodity	Level Detected	Standard Measure	Reference
Alpha-endosulfan	Water	Up to 0.018 µg/L	Australian guideline value 0.05 µg/L	Kuranchie-Mensah *et al*., 2012
Alpha-endosulfan	Water	Up to 0.03 µg/L	WHO MRL 0.01 µg/L	Fosu Mensah *et al*., 2016a
Endosulfan sulfate	Water	Up to 0.185 µg/L	Australian guideline value 0.05 µg/L	Kuranchie-Mensah *et al*., 2012
Endosulfan sulfate	Water	Up to 0.04µg/L	WHO MRL 0.01 µg/L	Fosu Mensah *et al*., 2016a
Heptachlor	Water	Up to 0.04µg/L	WHO MRL 0.03 µg/L	Fosu Mensah *et al*., 2016a
Delta-HCH	Water	Up to 0.08µg/L	Australian guideline value 0.05 µg/L	Kuranchie-Mensah *et al*., 2012
DDT	Breastmilk	0.6 - 58.2 µg/kg	Health Canada TDI = 20 µg/kg	Ntow *et al*., 2008
HCHs	Breastmilk	0.5 - 29.6 µg/kg	Health Canada TDI= 0.3 µg/kg	Ntow *et al*., 2008
HCB	Breastmilk	0.04-2.4 µg/kg	Health Canada TDI=0.3 µg/kg	Ntow *et al*., 2008
Malathion	Tomatoes	0.120 mg/kg	WHO MRL 0.1 mg/kg	Darko and Akoto 2008
Malathion	Eggplant	0.298 mg/kg	WHO MRL 0.1 mg/kg	Darko and Akoto 2008
Malathion	Pepper	0.143 mg/kg	WHO MRL 0.1 mg/kg	Darko and Akoto 2008
Malathion	Cucumber	0.03 – 0.04 mg/kg	EU MRL 0.02 mg/kg	Osei-Fosu *et al*., 2014
Chlorpyrifos	Cucumber	0.05 -0.07 mg/kg	EU MRL 0.05 mg/kg	Osei-Fosu *et al*., 2014
Chlorpyrifos	Lettuce	1.20 -1.27 mg/kg	EU MRL 0.05 mg/kg	Osei-Fosu *et al*., 2014
Chlorpyrifos	Water	0.06 µg/L	EU MRL 0.05 µg/L	Fosu Mensah *et al*., 2016b
Mythyl – Chlorpyrifos	Tomatoes	Up to 0.006mg/kg/day	WHO/FAO ADI 0.001mg/kg/day	Botwe *et al*., 2012a
Ethyl chlorpyrifos	Tomatoes	Up to 0.008mg/kg/day	WHO/FAO ADI 0.001mg/kg/day	Botwe *et al*., 2012a
Ethyl chlorpyrifos	Eggplant	Up to 0.005mg/kg/day	WHO/FAO ADI 0.001mg/kg/day	Botwe *et al*., 2012a
Fenitrothion	Cucumber	0.13-0.5 mg/kg	EU MRL 0.01 mg/kg	Osei-Fosu *et al*., 2014
Dimethoate	Cabbage	0.13–0.15 mg/kg	EU MRL 0.02 mg/kg	Osei-Fosu *et al*., 2014
Dimethoate	Cucumber	0.02 – 0.14 mg/kg	EU MRL 0.02 mg/kg	Osei-Fosu *et al*., 2014
Dimethoate	Lettuce	3.00 -3.10 mg/kg	EU MRL 0.02 mg/kg	Osei-Fosu *et al*., 2014
Diazinon	Lettuce	0.05 – 0.08 mg/kg	EU MRL 0.01 mg/kg	Osei-Fosu *et al*., 2014
Diazinon	Water	0.06 µg/L	WHO MRL 0.05 µg/L	Fosu Mensah *et al*., 2016b
Dichlorvos	Eggplant	0.007 mg/kg/day	WHO/FAO ADI 0.004mg/kg/day	Botwe *et al*., 2012a
Deltamethrin	Water	0.03-0.07 µg/L	WHO MRL 0.05 µg/L	Fosu Mensah *et al*., 2016c
Lambda – cyhalothrin	Lettuce	0.02 -0.90 mg/kg	EU MRL 0.02 mg/kg	Osei-Fosu *et al*., 2014

TDI = Health Canada Tolerable Daily Intake, ADI = Acceptable Daily Intake

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Table 2. Some active ingredients of pesticides registered in the country and their poisoning symptoms

Group	Active ingredient used in Ghana	Symptoms	Reference
Pyrethriods	Permethrin, Cypermethrin, Fenvalerate, Lambda-cyhalothrin	Fine tremor, reflex hyperexcitability, salivation, choreoathetosis, abnormal facial sensation, dizziness, headache, fatigue, vomiting, diarrhea and irritability to sound and touch, pulmonary edema, muscle fasiculations, seizures and coma	Roberts and Reigart, 2013; Goel and Aggarwal, 2007
Organophosphates (OPs)	Chlorpyrifos, diazinon, fenithrothion, dimethoate, malathion, methyl parathion (baned),	***Early symptoms***: headache, nausea, dizziness, sweating, lacrimation, salivation and rhinorrhea. ***Worsening symptoms***: muscle twitching, weakness, tremor, incoordination, vomiting, abdominal cramps and diarrhea. Blurred/dark vision, restlessness, memory loss, confusion and depression, bizarre behavior resembling alcohol intoxication. ***In children***: bradycardia, muscular fasciculations, lacrimation, sweating, Seizures, lethargy and coma.	Roberts and Reigart, 2013; Goel and Aggarwal, 2007
Carbamates	Carbofuran, Carbendazim,	Malaise, muscle weakness, dizziness, constricted pupils, salivation, slobbering, profuse sweating, incoordination, muscle twitching and slurred speech. Headache, nausea, vomiting, abdominal pain, and diarrhea. Transient hyperbilirubinemia may occur. Acute pancreatitis, coma, seizures, hypotonicity, hypertension and cardiorespiratory depression.	Roberts and Reigart, 2013; Goel and Aggarwal, 2007
Dipyridilium or Bipyridyl	Paraquat	Localized injury to tissues of contact skin, hands may become dry and fissured, horizontal ridging of the fingernails. Chronic exposure causes loss of fingernails. ***Ingestion:*** Severe inflammation, burns and ulceration of the tongue, oral mucosa and throat, corrosive injury to the gastrointestinal tract, renal tubular necrosis, hepatic necrosis and pulmonary fibrosis, haemorrhagic pulmonary oedema or acute respiratory distress syndrome (ARDS). ***Moderate poisoning***: Vomiting, diarrhoea and dysphagia, followed by mild renal tubular damage with respiratory symptoms (cough, breathlessness and pulmonary opacities), death. ***Severe poisoning***: Ulceration and multi-organ dysfunction. Respiratory problems, renal failure, metabolic acidosis, hepatocellular damage and death.	Roberts and Reigart, 2013; Goel and Aggarwal, 2007
Chlorophenoxyacetic herbicides	2,4-D (2,4dichlorophenoxyacetic acid)	***Ingestion***: Burning, nausea, vomiting, facial flushing and profuse sweating. In large quantities: headache, dizziness, muscle weakness, depression, coma, rhabdomyolysis and respiratory distress. Renal injury produces oliguria and proteinuria.	Roberts and Reigart, 2013; Goel and Aggarwal, 2007
Phosphonate herbicides	Glyphosate	Mouth and throat pain, nausea, vomiting, diarrhea and abdominal discomfort. **Oral exposures**: Tachypnea, dysrhythmias, hypotension, non-cardiogenic pulmonary edema, hypovolemic shock, oliguria and respiratory failure. Seizures and depression.	Roberts and Reigart, 2013

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VI. WEAK MONITORING AND ENFORCEMENT SYSTEMS

There are excellent mechanisms in place to ensure the regulation of pesticide sale and use in the country. As part of its objectives, the Environmental Protection Agency of Ghana is responsible for the implementation of environmental policies and ensure that planning is integrated and consistent with the country's desire for effective and long-term maintenance of environmental quality. It also ensures that there is an environmentally sound and efficient use of both renewable and nonrenewable resources in the process of national development. It also has to guide development to prevent, reduce, and as far as possible, eliminate pollution and actions that lower the quality of life. Again, it has to apply legal processes in a fair, equitable manner to ensure responsible environmental behaviour in the country, among others. However, the body lacks the human resource to embark on regular monitoring of pesticide sale and use. The Ministry of Food and Agriculture (MoFA) has the Plant Protection and Regulatory Services Directorate (PPRSD) and is mandated through the Pesticide and Fertilizer Regulatory Division Act 803 (2010) to compliment the Ghana EPA to supervise and train pesticide inspectors, register and inspect pesticide dealers and provide information materials and training on pesticides, among others, for retailers and farmers. Also, to tackle illegal pesticides trade, the Ghana Revenue Authority's Customs Division is mandated under Act 791 (2009) to regulate all imports into Ghana including chemicals (Kwakye *et al*. 2018). Under the auspices of the Ghana EPA, the customs division examines documents and certificates issued by the Ghana EPA to validate the claim of the bearer regarding a particular importation. The law (Act 791) gives customs officers the jurisdiction to search for certain persons, premises and baggage and seize prohibited items, including pesticides (Kwakye *et al*. 2018). The Food and Drugs Authority of Ghana as part of its mandate must ensure the safety and wholesomeness of food; and also ensures that household chemicals (including pesticides) are safe and effective. There seem to be no or weak systematic routine test on foods available in the Ghanaian market for pesticide residue by the appropriate governmental regulatory body (NPAS, 2012). Even though testing for pesticide residues on export crops such as cocoa is routine and stringent, such stringency is seemingly not applied when it comes to food produced and consumed locally. This is partly because there seems to be a lack of clarity over the exact governmental agency responsible for conducting such tests. Farmers involved in export – oriented cash crop production are usually trained in Good Agricultural Practices (GAP) and safe use of pesticides but those who produce for the domestic market are usually left out. Under Act 528 (1996), incorporated in Section II of the EPA Act 490 of 1994, it is required that all stakeholders including importers, dealers, applicators and transporters are licensed. The Act states that importation, formulation and manufacturing, distribution or sale, and commercial application and transportation are the key activities under pesticide management; and lists bulk storage and disposal of pesticide waste and containers as other activities (EPA, 2012). Currently, there exist unregistered pesticide dealers (NPAS, 2012), unregistered and banned pesticides are in the hands of farmers and on the shelves of dealers (Afari-Sefa *et al*., 2015; Dari *et al*., 2016), unregistered applicators, transporters and distributors, and improper disposal of pesticide waste and containers. Kwakye *et al*. (2018) after a survey on the pesticides policy implementation in Ghana concluded that the implementation has not adequately dealt with non-state actors like pesticide importers, dealers' and farmers with respect to the choice of particular pesticides for a given problem, technical knowledge on field diagnosis of pests and diseases, professionally dispensing pesticides to farmers, the use of PPEs. State actors on the pesticides policy implementation also have challenge with availability of pesticide user manuals to be effectively used by pesticide dealers, lack of accredited laboratory to test the quality of pesticide products, the lack of financial benefits and bonuses, inadequate inspectors assigned to dealers and users of pesticides, and inadequate transportation facilities to easily access pesticide dealers and users (Kwakye *et al*. 2018).

VII. PESTICIDES REGISTERED IN GHANA FROM 2010 – 2015

Ghana's Environmental Protection Act requires that pesticides are classified as for general use, restricted use, suspended or banned. A pesticide may be classified as suspended or restricted if its application may have unreasonable effects on humans, animals or the environment. Ghana's number of registered pesticides continues to rise and currently has almost 540 registered pesticides and 32 banned pesticides (Fig. 1.0). All banned pesticides are within the insecticide groups, which also constitute the largest group of pesticides registered for use in the country, followed by herbicides then fungicides. Plant growth regulators, molluscicides, rodenticides, nematicides and adjuvants constitute the other few pesticides registered for use in Ghana.

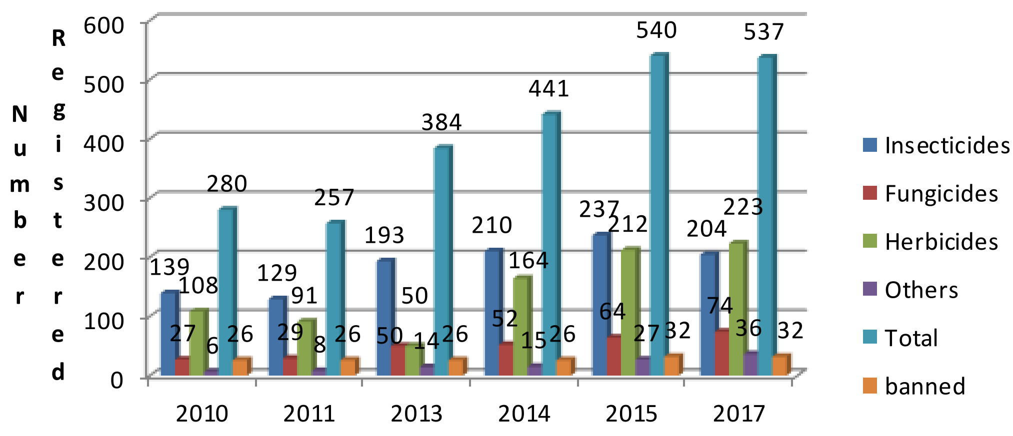

Fig.1. Ghana's pesticides registration trends from 2010 - 2017

VIII. CONCLUSION

It is evidently clear that pesticide abuse is common in Ghana and occurs from pesticides handling through usage to disposal. To minimize abuse and its consequential effects on human and the environment, there is the need for regular public awareness creation, strong linkage between pesticide use and healthcare, a reduced reliance on pesticides use through adoption of integrated pest management (IPM) systems. Research is needed in developing inexpensive PPEs suitable to for use by tropical farmers as well as innovative ways of collecting pesticide wastes to prevent pollution. It is also prudent that national agencies responsible for the monitoring and enforcement of legislation on pesticide sale and use are empowered to ensure a strict adherence to the rules and make the environment safe for now and generations to come.

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Mycorrhizal Inoculation to Increase Yield of Soybean Direct-Seeded Following Rice of Different Growing Techniques in Vertisol Soil, Lombok, Indonesia

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***Abstract*— *This study aimed to examine the impact of cultivation techniques and organic fertilization of rice, and arbuscular mycorrhizal fungi (AMF) inoculation of zero-tillage soybean on yield of soybean direct-seeded following rice in two year sequences of rice-soybean cropping (2010 and 2011) in vertisol soil taken from Central Lombok, Indonesia. The pot experiments were conducted in a glasshouse, with two treatment factors for rice crops, i.e. rice cultivation techniques (T1= Conventional, T2= SRI (System of Rice Intensification) without AMF, and T3= SRI with AMF inoculation in nursery), and organic fertilization (O1= without organic (NPK only), O2= organic manure + NPK at full recommended doses in the first or half the doses in the second year, and O3= organic manure + NPK at half the recommended doses in the first or without NPK in the second year), and AMF inoculation of soybean plants (M0= without, and M1= with AMF inoculation). The results indicated that AMF inoculation of zero-tillage soybean direct-seeded following rice crops in vertisol soil more significantly increased grain yields of soybean grown following conventional rice (with an average increase of up to 35.6%), compared with following SRI-rice (only 10.6 - 18.8% increase), indicating a need for AMF inoculation of soybean plants in rotation with conventional rice in vertisol soil. Different cultivation techniques and organic fertilization of rice plants also significantly affected yield of the soybean direct-seeded immediately after harvest of the preceding rice, which indicates positive impacts of organic fertilization of rice on grain yield of soybean following rice.*

***Keywords*— soybean, rice, arbuscular mycorrhiza, vertisols, Bokashi.**

I. INTRODUCTION

Vertisol soil in southern areas of Central Lombok is hard and cracked when dry because of its high content of clay particles in addition to low content of organic matter (Kusnarta *et al*., 2017). In the areas, soybean is mostly planted without tillage in the dry seasons (dry season 1 or dry season 2) after harvest of paddy rice. Since it is grown in the dry season, the farmers generally do not fertilize their soybean crops, so that the yields are normally low. Although with complete NPK fertilization, from a field experiment in southern Lombok reported by Adisarwanto *et al*. (1992), soybean yield was also relatively low, i.e. 1.29 ton/ha in Sengkol and 1.48 ton/ha in Keruak, and there was no significant effect of fertilization.

When conditions of vertisol soils were hard and dry in the dry season, root growth will also be hampered and nutrient uptake from the soil will also be restricted. Under these conditions, it is very likely that soybean plants require a good symbiosis with arbuscular mycorrhizal fungi (AMF), because their tiny external hyphae can explore much larger volume of soil and help their host plants to take up more nutrients and water compared with the roots (Smith and Read, 2008). However, the establishment of arbuscular mycorrhizal (AM) symbiosis naturally by soybean grown after paddy rice is possibly hampered by the generally very low population of AMF after paddy rice crops, which are normally irrigated with flooded irrigation system, as has been reported by several researchers (Ilag *et al*., 1987; Wangiyana *et al*., 2006).

Another obstacle for soybean production is its very high N requirement for seed production, because of its high protein contents of the grains. Among the 24-seed plants examined by Sinclair and de Wit (1975), nitrogen requirement of soybean plants during the seed-filling phase is in the category of the highest, and N requirement of a soybean plant far exceeds the supplying capability of the root uptake, so that soybean crop is classified as a self-destructive plant, because it often has to remobilize N contents of the leaves to the growing seeds. Therefore, seed-coating with inoculants of *Rhizobium* sp at the time of planting is the standard procedure for cultivating soybean, especially in rotation with irrigated rice crops.

The use of soybean seed plus (which has been inoculated with *Rhizobium* BTCC-B 64) of the Indonesian Institute of Science (LIPI) was reported to be able to increase soybean grain yields to more than 3 tons/ha, and even in some places could reach 4.5 tons/ha (Malik, 2008).

Besides establishing symbiosis with *Rhizobium* sp, soybean crop is also capable of establishing symbiosis with arbuscular mycorrhizal fungi (AMF), and the soybean plants are categorized as having a high level of dependency on symbiosis with AMF (Anderson and Ingram, 1993). With *Rhizobium* sp and AMF, soybean plants establish a tripartite symbiosis (Meghvansi and Mahna, 2009; Subramanian *et al*., 2011), and the presence of AMF in the tripartite symbiosis is more profitable than simply symbiosis with *Rhizobium* bacteria (Ruiz-Lozano *et al*., 2001; Antunes *et al*., 2006a,b,c). However, some researchers also reported that different varieties of soybean may show different responses to infection by AMF (Nwoko and Sanginga, 1999; Powell *et al*., 2007). A different soybean variety may also show different responses to different AMF species (Antunes *et al*., 2006c).

This study aimed to examine the effect of AMF inoculation of zero-tillage soybean direct-seeded immediately after harvest of paddy rice crop grown with different cultivation techniques (between conventional and SRI techniques) and organic fertilization of the rice crops on grain yield of the soybean crop following rice in vertisol soil taken from vertisol riceland in Southern Lombok, Indonesia. The experiment was conducted in two consecutive years (2010 and 2011), which was twice planting of soybean, respectively after the rice harvest.

II. MATERIALS AND METHOD

Design of the experiments

The experiments were conducted in the glasshouse of the Faculty of Agriculture, University of Mataram, using soil samples taken from vertisol ricefield in Mujur village, Central Lombok (Indonesia). After being air-dried and sieved using test sieve of 2 mm opening, the soil samples were used to fill the pots (12 kg/pot) for growing the rice and soybean crops in these experiments. The soybean crop direct-seeded following harvest of rice crop in this case was used to examine the impact of cultivation techniques of the rice crop. Therefore, there were treatment factors applied to the rice crop preceding the soybean crop, and there was also a treatment factor applied to the soybean crop direct-seeded following the rice crop. The successive cropping of rice-soybean was carried out in 2010 and 2011, with the same treatments, except for reduced doses of inorganic fertilizer (N, P, K) in 2011, and the data reported here were results of observations made on the soybean plants.

The experiment was arranged according to the Completely Randomized Design, with 3 treatment factors (2 treatment factors applied to the rice plants and 1 treatment factor applied to soybean plants), with the following details:

1. The first factor, namely rice cultivation techniques (T), consisted of three treatment levels, i.e. T1 (Conventional techniques); T2 (SRI technique without AMF inoculation); and T3 (SRI technique with AMF inoculation in the nursery).
2. The second factor, namely organic fertilization (O) on rice, consisted of three treatment levels, i.e. O1 (without organic fertilizer or only fertilized with full recommended doses of NPK); O2 (organic fertilizer + full doses of NPK); and O3 (organic fertilizer + half doses of NPK). The doses of NPK in the second year (2011) were reduced to half doses on the O2 and without NPK on the O3 treatment.
3. The third factor, namely AMF inoculation (M) of soybean, consisted of two treatment levels, i.e. M0 (without AMF inoculation) and M1 (with AMF inoculants applied in the planting hole together with organic fertilizer).

Thus, the number of treatment combinations for soybean plants was 3x3x2 or 18 treatment combinations, each of which was made in three replications.

Implementation of the experiments

The experiments were started in 2010, beginning with preparation of growing media, continued with successive planting of rice-soybean in 2010 and in 2011. The organic fertilizer used in the experiments was “Bokashi” fertilizer, i.e. EM4 fermented cattle manure, with application dose of 10 ton/ha (or 62.5 g/pot). The recommended NPK fertilizer doses were 300 kg Urea, 150 kg SP36 and 100 kg KCl per ha. Rice seedlings of “Silugonggo” variety were prepared differently with different growth duration in the nursery, i.e. 9 days in the dry nursery for SRI technique and 25 days in flooded nursery for conventional technique. The growing media for rice nursery were prepared by mixing air-dried vertisol soil, river sand and rice husk ash of the same volume. AMF inoculation for rice seedlings was done by spreading “Technofert” biofertilizer on the nursery media which was then covered with rice husk ash, then pre-germinated rice seeds were spread on it. Technofert was supplied by the Institute of Biotechnology Research (BPPT), Serpong, Indonesia. No NPK fertilizers were applied to the nurseries but rice seedlings were sprayed with Urea fertilizer (5 g/L) every 5 days for wet and 3 days for dry nursery since 5 days after seeding (DAS).

Rice planting I (in 2010) was done by transplanting seedlings from the nurseries to the pots on the same day. The soil in the pots was puddled 2 days before transplanting by mixing the soil with water, then left thin standing water for two days. Just before transplanting, basic fertilization was done with 62.5 g Bokashi, 0.625 g Urea (1/3 dose), 0.938 g SP-36 and 0.625 g KCl per pot, which were mixed with the mud of 8 cm diameter and 5 cm depth on the center of the soil surface in the pot. Transplanting was done by planting 3 seedlings for conventional and 1 seedling for SRI technique in each planting hole in the center as the main plant and another planting hole of 10 cm apart as a reserve for a maximum of 10 days. The rest of the Urea was applied at 30 and 50 days after transplanting (DAT), each with 0.625 g/pot. The SRI rice plants were intermittent irrigated during vegetative growth stages by applying thin flooding every 7 days, and maintained in thin (2 cm) flooded condition during the reproductive growth stages, while the conventional rice plants were maintained in flooded condition (5-10 cm) from transplanting to the reproductive growth stages. The rice plants were harvested at 93 DAT.

Soybean planting I (in 2010) was done by dibbling soybean seeds ("Grobogan" variety) next to the rice stubble immediately after harvest of the rice crop without tillage, after seed-coating the seeds with "Rizoplus" (*Rhizobium* inoculants). For the M1 treatment, the planting hole was first filled 5 g "Technofert" in the bottom, which was then covered with "Bokashi" of 62.5 g/pot, and Rhizoplus-coated soybean seeds were then placed on it and covered with soil. Each pot after planting the soybean seeds was covered with pieces of rice straw harvested from that pot. NPK fertilization was done at 7 DAS by dibbling Phonska fertilizer (15-15-15) of 1.25 g/pot 5 cm beside the planting hole at 5 cm depth. Watering was done as necessary, and soybean plants were harvested at 76 DAS.

Rice planting II (in 2011) was done after puddling the soil and applying the basic fertilizers as in 2010 rice planting, except for the NPK doses, which were half dose for the O2 and zero for the O3 treatment. Puddling was done after fallowing for a month after harvest of 2010 soybean plants, which was then flooded with water for a week and puddled. The procedures for preparing the nursery and growing the rice plants were the same as those applied in 2010, except for the rice variety, which was "Inpari 13" in 2011.

Soybean planting II (in 2011) was also done by dibbling soybean seeds without tillage immediately after harvest of the 2011 rice. All the procedures applied were the same as those for growing the 2010 soybean plants.

Observation variables and data analysis

Observation variables for the soybean plants were plant dry weight, grain number, and dry grain yield per pot, and weight of 25 dry grains, both for 2010 and 2011 soybeans. Data were analyzed with analysis of variance (ANOVA) and the Tukey's HSD test at 5% level of significance using the statistical software CoStat for Windows ver. 6.303. The graph is displayed in the form of a bar chart using the values of Mean ± SE (standard error) based on Riley (2001).

III. RESULTS AND DISCUSSION

The results showed that AMF inoculation of soybeans direct-seeded following paddy rice significantly increased soybean seed yields, both in 2010 and 2011, as shown in Table 1. The increase in soybean yield due to AMF inoculation of soybeans is presented in more detailed in Figure 1 for soybean yields in 2010, and Figure 2 for soybean yields in 2011, on each combination of rice cultivation techniques and organic fertilization treatment on rice plants preceding soybean planting.

In addition to the AMF inoculation treatment that was applied directly to soybean plants, the two treatment factors applied to rice plants preceding the soybean plants, namely rice cultivation techniques (T) and organic fertilization (O) on rice plants, also affected grain yields of soybean direct-seeded following the rice crop. This indicates significant residual effects of rice growing techniques on yield of the subsequent soybean crop. There were also some significant interaction effects on soybean grain yields between AMF inoculation of soybeans and the treatments applied to the preceding rice plants, but the patterns of the interactions were different between year I (2010) and year II (2011). In year I, significant interactions occurred between AMF inoculation of soybeans and cultivation techniques of the preceding rice, whereas in year II, significant interactions occurred between AMF inoculation of soybeans and organic fertilization in the preceding rice plants (Table 1).

Based on the interaction effects on yield of soybeans following rice crop in 2010 (Figure 1), it appears that the significant effects of AMF inoculation of soybean on the soybean yield were more common on soybean plants direct-seeded following conventional paddy rice compared with following SRI rice, especially those with AMF inoculation in the nursery. This is also evident from the significant interactions between rice cultivation techniques and AMF inoculation of soybeans (Table 1), which is also graphically illustrated in Figure 3.

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Table 1. Summary of ANOVA results of the effects of cultivation techniques and organic fertilization of rice plants and AMF inoculation of soybean direct-seeded following rice both on 2010 and 2011 soybean crops

Observation variables per year	Main effects			Interaction effects			
Experiment year I (2010):	**Tech**	**Org**	**Myco**	**TxO**	**TxM**	**OxM**	**TxOxM**
Soybean plant dry weight per pot	*	***	***	***	*	ns	ns
Soybean grain number per pot	***	***	*	ns	*	ns	ns
Soybean dry grain yield per pot	***	**	***	ns	*	ns	ns
Weight of 25 dry grains	ns	ns	***	ns	ns	ns	ns
Experiment year II (2011):	**Tech**	**Org**	**Myco**	**TxO**	**TxM**	**OxM**	**TxOxM**
Soybean plant dry weight per pot	***	***	***	***	ns	*	ns
Soybean grain number per pot	***	**	***	ns	ns	ns	ns
Soybean dry grain yield per pot	***	*	***	ns	ns	*	ns
Weight of 25 dry grains	ns	***	***	***	**	ns	**

Remarks: Tech (T)= rice cultivation technique; Org (O)= organic fertilization of rice; Myco (M)= mycoriza (AMF) inoculation of soybean; ns= non-significant (at p-value 0.05); *, **, *** = significant at p-value < 0.05, p-value < 0.01, and p-value < 0.001, respectively

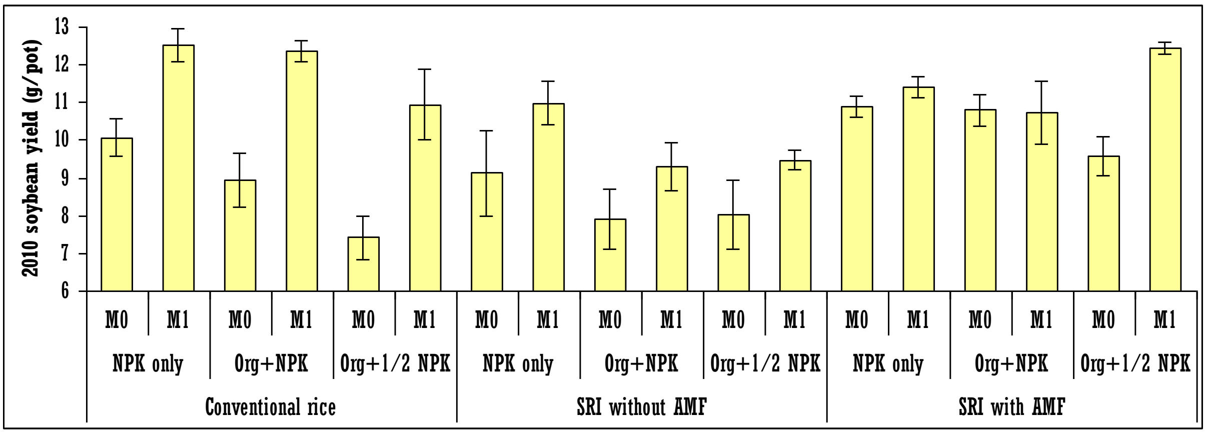

Fig.1. Average (Mean ± SE) grain yield (g/pot) of soybean in year I (2010) as affected by AMF inoculation of soybean and residual effects of cultivation techniques and organic fertilization on rice plants preceding the soybean crop

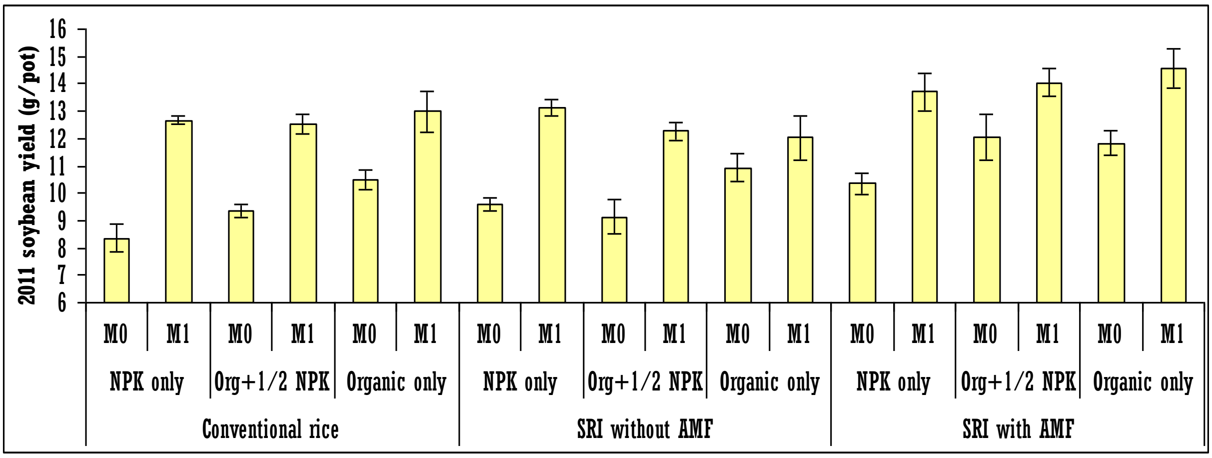

Fig.2. Average (Mean ± SE) grain yield (g/pot) of soybean in year II (2011) as affected by AMF inoculation of soybean and residual effects of cultivation techniques and organic fertilization on rice plants preceding the soybean crop

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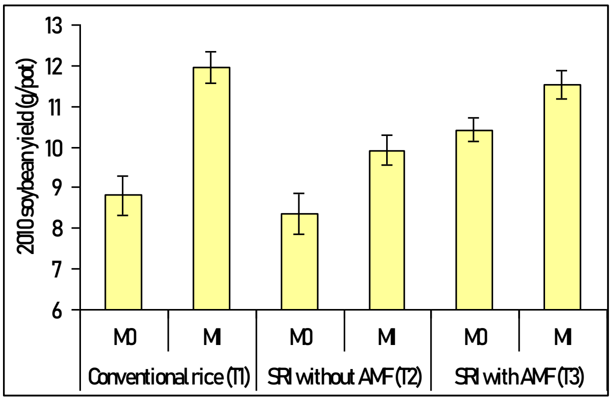

Fig.3. Average (Mean ± SE) grain yield (g/pot) of soybean in year I (2010) as affected by interaction between AMF inoculation of soybean and cultivation techniques of the rice crop preceding the 2010 soybean (conventional; SRI without; or SRI with AMF inoculation in the rice nursery)

From Figure 3 it can be seen that AMF inoculation of soybeans increased soybean yield, which was higher in soybeans direct-seeded following conventional paddy rice compared with following SRI technique of growing rice which seedlings were inoculated with AMF in the nursery. As described in the methods that rice plants in conventional techniques were grown with a flooded irrigation system whereas in the SRI techniques, they were irrigated intermittently between thin flooding and dry conditions with a 7 day watering interval. Thus the conditions of the growing media were more aerobic in SRI techniques compared with in conventional techniques. These aerobic conditions in the SRI techniques seem to be more conducive for better development of AMF propagules in the root systems and rhizosphere of the rice plants under SRI than under flooded conditions. This better development of AMF in the SRI can be inferred from the higher grain yield of uninoculated soybean (M0) direct-seeded following SRI-rice having AMF inoculation in the nursery (T3M0) compared with following conventional (T1M0) or SRI-rice having no AMF inoculation in the nursery (T2M0).

Because of the high potentials in highly reducing AMF population by inundated conditions in conventional rice (Ilag *et al*., 1987; Wangiyana *et al*., 2006), which was flooded since the nursery, then the uninoculated soybean plants (M0) direct-seeded following conventional rice did not have sufficient infective propagules of AMF to establish optimum AMF symbiosis in the uninoculated soybean plants grown following conventional rice, just like the "post flood syndrome" that was stated by Ellis (1998). Due to its high dependency on AMF symbiosis (Anderson and Ingram, 1993), then the uninoculated soybean plants, which were direct-seeded following dried conventional rice soil of vertisol type, would not be able to take up sufficient nutrients and water to support high growth and grain yield. On the other hand, AMF inoculated soybean plants (M1) direct-seeded following the conventional rice (T1), because of the direct AMF inoculation, would be able to immediately establish a good AM symbiosis, hence AMF functions in helping their hosts (in this case, the soybean plants) to take up more nutrients and water (Smith and Read, 2008; Smith and Smith, 2011), even in compacted soils (Miransari *et al*., 2009), would be well established since the beginning of the soybean growth stages. Therefore, AMF inoculation on soybean direct-seeded following conventional rice would be more advantageous than following SRI-rice having AMF inoculation in the nursery (T3), as shown in Figure 3.

In year II (2011), unlike in Figure I, it can be seen from Figure 2 that the difference in soybean grain yield between AMF inoculation (M1) and without inoculation (M0) is higher in soybean direct-seeded following rice plants receiving no organic than following those receiving organic fertilization. This indicates a change in the effect of organic fertilization of rice plants on yields of the subsequent soybean between year I and year II, which had a more significant impact on the soybean crops after two years of organic fertilization to rice plants. Figure 4 also shows those differences, which also shows a significant interaction between AMF inoculation of soybeans and organic fertilization of the rice plants preceding the soybean plants. This possibly occurred as a result of the positive influence of organic fertilization on the development of AMF in the soil, as reported by several other researchers (Joner, 2000; Gryndler *et al*., 2001, 2006). As also shown in Figure 4, after 2 years, grain yields were also higher in soybean plants direct-seeded following rice receiving organic fertilization for two years, compared with those following rice receiving no organic fertilization, although the soybean plants were not inoculated with AMF at planting. Thus, organic fertilization for two years in rice plants had a positive impact on AMF symbiosis with soybean plants grown following rice crops (Figure 4).

It can also be seen from Table 2 that, based on the main effect, in year I (2010), grain yield of soybean direct-seeded following rice receiving organic fertilizer but only half doses of NPK fertilizers (T3) were lower than following rice receiving full doses of NPK although without organic fertilization (T1). In contrast, in year II, i.e. after two years of organic fertilization of rice, grain yields were on average higher in soybean following rice receiving full organic fertilization (T3) compared with following rice that received only full doses of NPK fertilizers.

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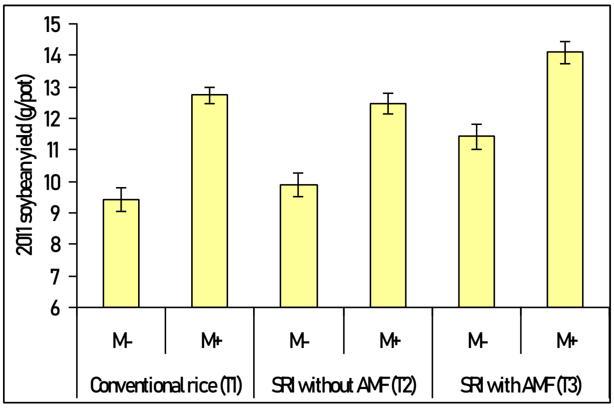

Fig.4. Average (Mean ± SE) grain yield (g/pot) of soybean in year II (2011) as affected by interaction between AMF inoculation of soybean and organic fertilization on the rice crop preceding the 2011 soybean

In addition, there appears to be an increase in soybean grain yield from year I to year II. The increase was more common in soybean plants inoculated with AMF compared with no AMF inoculation, as shown in Table 3. However, the increase in soybean seed yield was also related to the positive impact of organic fertilization of rice plants on yields of soybean plants grown following rice, which possibly happen through positive impacts of organic fertilization on AMF development. On the other hand, it seem that grain yields declined from year I to year II in soybean plants following rice receiving no organic fertilizer, especially following rice plants cultivated under flooded systems or conventional rice (T1). This also indicates a negative impact of rice flooding on the development of AMF infective propagules in the soil (Ilag *et al*., 1987; Ellis, 1998; Wangiyana *et al*., 2006).

Table 2. Mean plant dry weight, grain number, dry grain yield, and weight of 25 dry grains of soybean for each levels of the treatment factor in 2010 and 2011 planting

Treatment factors and year:	**Mean values of yield component data**			
Results of 2010 treatments	**Plant dry weight (g/pot)**	**Grain number/pot**	**Grain yield (g/pot)**	**Weight of 25 dry grains (g)**
T1= Conventional rice	18.88 ab	55.78 a	10.38 a	4.64 a [1)]
T2= SRI without AMF	17.62 b	50.67 b	9.14 b	4.50 a
T3= SRI with AMF inoculation	19.60 a	60.11 a	10.98 a	4.59 a
Tukey's HSD 5%	1.57	4.57	0.88	0.30
O1= No organic (NPK only)	17.23 b	61.00 a	10.84 a	4.47 a
O2= Organic + 100% NPK	19.01 a	52.44 b	10.01 ab	4.75 a
O3= Organic + 50% NPK	19.86 a	53.11 b	9.65 b	4.52 a
Tukey's HSD 5%	1.57	4.57	0.88	0.30
M0= No AMF inoculation	15.97 b	53.96 b	9.20 b	4.27 b
M1= With AMF inoculation	21.42 a	57.07 a	11.13 a	4.89 a
Tukey's HSD 5%	1.06	3.09	0.60	0.20
General mean of all factors	**18.70**	**55.52**	**10.17**	**4.58**
Results of 2011 treatments	**Plant dry weight (g/pot)**	**Grain number/pot**	**Grain yield (g/pot)**	**Weight of 25 dry grains (g)**
T1= Conventional rice	16.14 a	59.33 b	11.07 b	4.60 a
T2= SRI without AMF	13.90 b	61.67 b	11.18 b	4.54 a
T3= SRI with AMF inoculation	17.62 a	68.94 a	12.76 a	4.61 a
Tukey's HSD 5%	1.95	3.90	0.75	0.08
O1= No organic (NPK only)	13.96 b	62.72 ab	11.30 b	4.49 b
O2= Organic + 100% NPK	16.41 a	60.89 b	11.57 ab	4.70 a
O3= Organic + 50% NPK	17.29 a	66.33 a	12.14 a	4.55 b
Tukey's HSD 5%	1.95	3.90	0.75	0.08
M0= No AMF inoculation	13.71 b	59.11 b	10.24 b	4.32 b
M1= With AMF inoculation	18.06 a	67.52 a	13.10 a	4.84 a
Tukey's HSD 5%	1.32	2.64	0.51	0.06
General mean of all factors	**15.89**	**63.31**	**11.67**	**4.58**

Remaks: [1)] Mean values in each column followed by the same letter are not significantly different between levels of a treatment factor in each year

Table 3. Changes in mean grain yield (g/pot) of soybean direct-seeded following rice year I (2010) and rice year II (2011)

Treatments for rice crops preceding soybean crops		AMF ino-culation on soybean	Mean (± SE) soybean grain yield (g/pot) in Year I (2010) and Year II (2011)		Grain yield differences (g/pot)
Rice cultivation technique	Organic fertilization *)		(2010 ± SE)	(2011 ± SE)	(2011-2010)
Conventional rice (T1)	No organic (NPK only)	M0	10.08 ± 0.48	8.36 ± 0.50	-1.72
		M1	12.53 ± 0.44	12.67 ± 0.17	0.13
	Organic + 100% NPK	M0	8.95 ± 0.71	9.37 ± 0.24	0.43
		M1	12.37 ± 0.27	12.52 ± 0.38	0.15
	Organic + 50% NPK	M0	7.42 ± 0.56	10.50 ± 0.37	3.09
		M1	10.95 ± 0.93	13.00 ± 0.75	2.05
SRI without AMF inoculation (T2)	No organic (NPK only)	M0	9.13 ± 1.13	9.59 ± 0.22	0.46
		M1	10.99 ± 0.59	13.10 ± 0.30	2.11
	Organic + 100% NPK	M0	7.90 ± 0.80	9.14 ± 0.66	1.25
		M1	9.30 ± 0.64	12.27 ± 0.32	2.97
	Organic + 50% NPK	M0	8.04 ± 0.92	10.93 ± 0.49	2.89
		M1	9.48 ± 0.26	12.02 ± 0.81	2.55
SRI with AMF inoculation in the nursery (T3)	No organic (NPK only)	M0	10.89 ± 0.27	10.36 ± 0.38	-0.54
		M1	11.41 ± 0.28	13.70 ± 0.67	2.29
	Organic + 100% NPK	M0	10.79 ± 0.41	12.06 ± 0.85	1.27
		M1	10.73 ± 0.82	14.05 ± 0.53	3.31
	Organic + 50% NPK	M0	9.58 ± 0.52	11.83 ± 0.46	2.25
		M1	12.44 ± 0.15	14.56 ± 0.73	2.11

*) Organic fertilization for O2 and O3 treatments were: in year I (2010), O2= organic + 100% NPK doses, and O3= organic + 50% NPK doses, while in year II (2011), O2= organic + 50% NPK doses, and O3= organic fertilization only (0% NPK)

Thus, it is suggested that in a rice-based cropping system with a cropping sequence of rice-soybean or rice-rice-soybean, especially in vertisol ricelands, rice plants should not always be flooded. In addition, application of organic fertilizers also has a positive impact on the sustainability of the production system due to better development of soil biota, especially AMF, especially if rice is cultivated under the System of Rice Intensification (SRI) using seedlings produced in a dry nursery initiated with AMF inoculation in the nursery.

IV. CONCLUSION

It can be concluded that AMF inoculation of zero tillage soybean direct-seeded following rice crops in vertisol soils more significantly increased grain yields of soybean plants grown following conventional rice, with grain yield increase of up to an average of 35.6%, compared with following SRI-rice (10.6 - 18.8%), which indicates a negative impact of conventional rice cultivation techniques on post-rice soybean. Different cultivation techniques and organic fertilization of rice plants in vertisol soils also had significant effects on grain yield of soybean direct-seeded immediately after harvest of the rice crops. However, AMF inoculation of soybean plants showed significant interaction with cultivation techniques of the preceding rice in year I, whereas in year II, with organic fertilization of the preceding rice, indicating changes in the impact of organic fertilization of rice on yield of soybeans grown following rice after two years of organic fertilization of rice in vertisol soils.

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