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FOREWORD

I am pleased to put into the hands of readers Volume-6; Issue-5: September-October 2021 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: November, 2021

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Status of Solid Wastes and its Management in the Coastal Environments of Sri Lanka

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Abstract— *The study was conducted to evaluate the status of solid wastes and waste management in the coastal environments of Sri Lanka. Fifteen coastal environments of Sri Lanka were selected as the sampling locations for the study. The research methodology comprised analyzing the types of solid wastes and the composition of each type of solid wastes in a unit area. The sources of solid wastes governing marine litter were identified. In addition, interviews were conducted with individuals in each sampling location and with five major organizations related to the coastal environment of Sri Lanka, to investigate the solid waste management methods existing in the coastal zone and their effectiveness. The study revealed that there was an average of 4.2 kg and 50 pieces of debris per a square meter of beach. The results of the study presented that tourist beaches away from the town limits show the highest density of solid wastes and coastal environments in river mouths show the next highest. Plastic was identified as the dominant material governing marine litter in coastal environments. The interviews indicated that the lack of infrastructure facilities in the country, inappropriate and illegal waste dumping, absence of proper waste management systems, and less awareness on this issue governs the accumulation of solid wastes in the coastal environments and end up as marine litter. The study concludes that there is a need for utilizing proper waste management in the coastal environments with the effective use of available infrastructure facilities. At the same time, the need of promoting plastic recycling and acknowledging the community regarding the impact of solid wastes on marine litter is much needed. The study introduces a mobile application as a communication application to enhance the effective waste management of solid wastes with the available facilities and to motivate the community to save the coastal ecosystems of Sri Lanka.*

Keywords—***Coastal environment, Marine litter, Mobile application, Plastic, Solid wastes***

I. INTRODUCTION

Sri Lanka, the pearl of the Indian ocean is an island hallowed with plenty of natural resources. The ocean surrounding is one of the major assets comprise of attractive ecosystems with lots of endemic species including coral reefs and mangroves which, provide food and shelter for marine lives as well as food and livelihoods for human beings. Recently, Marine litter has become a burning issue threatening marine lives and human populations in the world.

A study conducted on selecting 192 countries which include residents that border the Atlantic ocean, Pacific ocean, Indian ocean, Black Sea, and Mediterranean sea revealed that Sri Lanka is 5th out of the top twenty countries polluting the ocean (J. Jambeck et al., 2015). Vital economic sectors such as tourism, fishing industry, and recreational activities are related to the coastal ecosystems in the country. On the other hand with the growth of populations, most of the beaches have become residential. It is obvious, the marine litter which accumulates in the ocean from various sources gets transport long distances in the sea before they get settled in the sea due to winds and other ocean currents. As a result, it becomes a complex task to identify the relevant source of marine litter found in the ocean. But it has been identified that 80% of marine litter is getting generated from land-based sources (OSPAR UNEP, 2009).

A study on water quality of the coastal belt, western province, Sri Lanka, revealed the organic and inorganic pollution in all the sampling locations. It demarcated that ammonia concentration and dissolved oxygen levels are below the maximum permissible limits while pH value within the acceptable limit and biochemical oxygen demand was higher than the allowed threshold limit. It realizes that the wastes added to the ocean contribute to lowering the quality of seawater (Jinadasa, 2014).

Waste Management Authority, 2016, estimated municipal waste generation in Sri Lanka is about 6500 to 7000 Mt/day and collection is 3500 Mt/day which is 50% of the generated. The other 50 percent is dumped or discarded into the nearest environment. As a result of these situations, a large amount of land-based solid wastes enter the coastal and marine environments (JICA, 2016).

Solid wastes in the coastal environment significantly contribute to marine pollution. Long-term deposition of wastes in the ocean for many decades causes harm to the ecological and economical value along with the biodiversity (Gregory, 2009). As a developing country, Sri Lanka faces challenges related to environmental management due to poorly developed waste management infrastructure and extreme weather events (Jang et al., 2018). Hence, marine litter has become a vital issue related to solid waste management in the coastal environments of Sri Lanka. There are scattered numbers of government agencies responsible for solid waste management. Thus, the effectiveness of marine solid waste management is still questionable.

Jinadasa (2014) revealed the need for awareness programs about coastal pollution should be conducted among the coastal communities including fishermen and school children. The improvement of public health to increase sanitation such as providing facilities for people in the vicinity of the coastal areas should be implemented as it will have an impact on the aquatic health of the marine environment. He recommends that proper monitoring programs should be initiated for the identification of water pollutant sources, which discharge pollutants into the marine waters directly or indirectly and actions should be taken to prevent further damage to the marine environment.

The study aims to minimize the addition of solid wastes into the ocean. Therefore, the objectives of the study were, to identify the types of solid wastes and their composition in the coastal environments of Sri Lanka, to evaluate the sources of solid wastes in the marine environments, to identify the existing solid waste management methods currently adopted and their effectiveness, and finally to propose a solution for the issues related with the solid waste management in coastal environments.

II. MATERIALS AND METHOD

2.1 Coastal Zone of Sri Lanka

Sri Lanka lies in the bay of Bengal between 5º55" and 9º51" latitudes north and between 79º41" and 81º53" south east longitude near the equator with an extent of the land area of 65610 km2. Figure 1 depicts the coastal belt of Sri Lanka which is 1,620 km long divided into eight coastal sections centering the Piduruthalagala mountain range (Silva, Katupotha, Amarasinghe, Manthrithilake, & Ariyaratna, 2013).

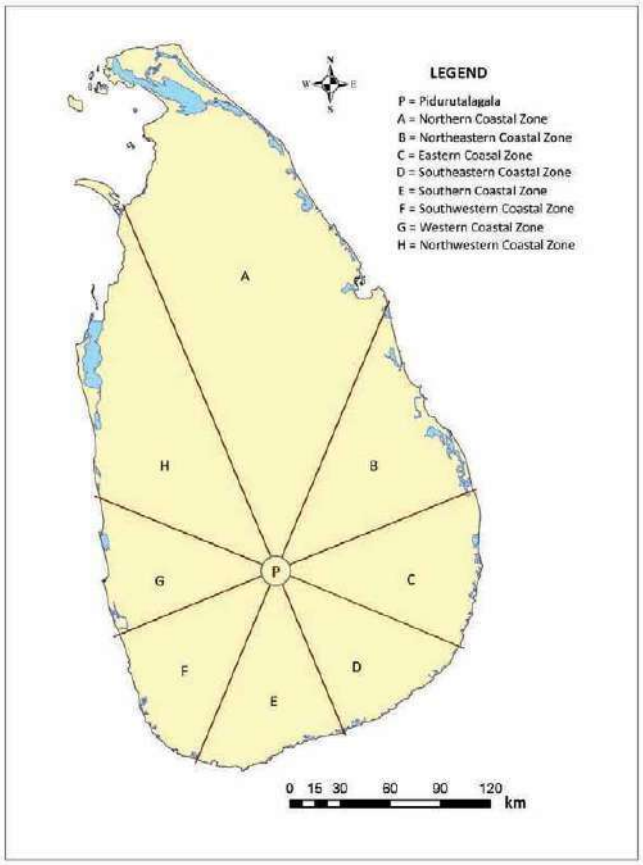

Fig. 1 – Coastal zone of Sri Lanka divided into eight coastal sections (Silva et al., 2013).

Sri Lanka owes an extent of 517,000 km^2 of the ocean from the declaration of an exclusive economic zone. The coast conservation (amendment) act, No. 49 of 2011 defines the "Coastal zone" as "The area lying within a limit of 300 m landward of the mean high water level and a limit of 2 km seaward of the mean low water level. In the case of a water body connected to the sea either permanently or periodically, the land boundary extends 2 km measured perpendicular to the straight baseline drawn between the natural entrance points. Thereof and includes the waters of such rivers, streams, and lagoons or any other body of water so connected to the sea" (Parliament of the Democratic

Socialist Republic of Sri Lanka Coast Conservation (Amendment) Act. No. 49 of 2011, 2009).

Accordingly, the coastal zone of Sri Lanka comprises 24% of the total land area of the country under 74 divisional secretariat divisions. Approximately, the coastal population is around 18.8 million which represents one-fourth of the total population of the country. (Koralagama, 2008) There are numerous shipping and fishery harbors, ports, salterns, and tourist hotels located along the coastal belt while most of the beaches have become residential due to high population. Significantly, the inhabitants of the coastal environments are mostly low-income families in the country with deprived standards of existing.

2.2 Data Collection

The study was mainly conducted by visiting selected beaches of Sri Lanka. Table 3 shown below depicts the sampling locations selected for the study, differed from each other including urbanized beaches near cities, remote beaches which are isolated from human interfere, tourist beaches, and marine environments near river mouths. A team comprised of 4 trained members were involved and the data were collected from one location per day. The data collection was begun in July 2019. In each of the coastal environments, a land area was randomly selected and surface area was measured. The solid wastes in the selected area were categorized following the type of material. The weight of each type of solid wastes was measured. At the same time, the number of items of each type of material was counted. Then the composition of solid wastes in each of the coastal environments per unit area was calculated in terms of weight and number of items. Simultaneously, sources of marine solid wastes were identified and the solid waste management method implied in each of the beaches was identified. A vocal interview was conducted with a selected responsible person in each of the marine environments to gather data on waste collection and frequency of collection.

Five major government authorities related to the coastal environments of Sri Lanka were interviewed with a questionnaire to collect information on the effectiveness of the existing solid waste management methods in the coastal zone of Sri Lanka and their suggestions to overcome this marine debris issue. The organizations interviewed were the Central Environmental Authority (CEA), Marine Environmental Protection Authority (MEPA), Moratuwa Municipal Council (MMC), Coast Conservation & Coastal Resource Management Department (CC&CRMD) and United Nations Development Program (UNDP).

From the findings of the study, a mobile application was introduced as a solution to the accumulation of solid wastes in the beaches through a communication system. It was developed using Android Studio and java coding using firebase as the database. The coding can be changed and further improvements to the application can be done.

III. RESULTS AND DISCUSSION

3.1 Sources of Solid wastes

Solid wastes in the coastal environments were comprised of domestic wastes, solid wastes from recreational activities, tourism and other industry-based wastes, smoking-related wastes, medical waste, etc. In the study, they were classified according to the type of material. The main types of solid wastes identified were categorized as plastic, glass, metal, wood, paper and polythene, and others. Solid wastes from the inland of the country, accumulated in the coastal zone came via rivers, streams, and other waterways were observed in the sampling locations. In addition, solid wastes with foreign labels and fishing vessels floated with sea waves due to winds were identified in the beaches as litter. Solid wastes scattered in the beaches due to tourism, recreational activities, and coastal-based industries were most commonly noted in the visited coastal environments. Solid wastes directly dumped into the beaches were commonly observed in the visited residential beaches. It is manifest that the sources of solid wastes in the coastal environment can be identified as land-based, coastal based and sea-based sources. Wickramaarachchi et al (2010), revealed that litter from the inland-based sources mostly governs on marine debris.

3.2 Composition of Solid Wastes

The study revealed that there was an average of 4.2 kg and 50 pieces of debris per square meter of a beach. Plastic was observed as the dominant material in marine solid wastes where 48% concerning weight and 41% concerning the number of items per unit area were observed in the

Fig. 2 – Solid wastes scattered in the coastal belt, Galle Face, Colombo

sampling locations. Table 1 shows the composition of each type of solid wastes in the overall sampling locations.

The analysis of the composition of solid wastes in the sampling locations are illustrated in Table 2. Plastic is the

leading material in solid wastes in most of the beaches concerning weight, except in Galle fort, Wennappuwa, Negombo, and Ging river valley where glass is the highest due to its high density. But concerning the number of items per unit area, plastic was recorded the highest in all the sampling locations in the study.

Table 1 – Overall percentage of each type of solid wastes collected in sampling locations in a unit area

Material	Weight	Number
Plastic	48%	41%
Glass	39%	8%
Metals	9%	10%
Woods	1%	3%
Paper and Polythene	1%	19%
Others	2%	20%

Table. 2 – Composition of each type of solid waste in the sampling locations

	Weigh per unit area						Parts per unit area					
Coastal Environment	Plastic	Glass	Metals	Woods	Paper and polythene	Others	Plastic	Glass	Metals	Woods	Paper and polythene	Others
Colombo Galle face	65%	26%	5%	0%	1%	3%	48%	5%	5%	0%	19%	24%
Galle Fort	20%	79%	0%	0%	0%	0%	40%	40%	0%	0%	20%	0%
Jungle beach	50%	39%	7%	1%	1%	2%	42%	8%	7%	4%	19%	19%
Mount Lavinia beach	50%	40%	6%	1%	1%	4%	42%	8%	6%	4%	11%	30%
Ratmalana beach	47%	40%	9%	1%	1%	2%	43%	9%	11%	5%	16%	16%
Galle fishery harbor	48%	37%	10%	0%	1%	4%	37%	7%	10%	0%	18%	28%
Wennappuwa beach	40%	46%	9%	0%	2%	3%	29%	8%	8%	0%	31%	23%
Negombo beach	37%	49%	10%	0%	1%	4%	30%	10%	10%	0%	20%	30%
Kahawa beach	49%	42%	6%	0%	0%	3%	48%	10%	7%	0%	7%	28%
Akurala beach	40%	37%	20%	0%	1%	3%	33%	8%	21%	0%	15%	23%
Balapitiya beach	45%	39%	13%	0%	2%	1%	40%	9%	14%	0%	31%	6%
Moda river mouth valley	79%	12%	5%	2%	2%	1%	57%	2%	4%	4%	28%	4%
Ging river mouth valley	25%	60%	12%	1%	1%	1%	27%	16%	16%	5%	22%	14%
Kalu river mouth valley	61%	21%	13%	2%	1%	2%	50%	4%	13%	7%	13%	13%
Kelani river mouth valley	52%	35%	9%	1%	1%	3%	43%	7%	9%	4%	16%	21%

The highest density of solid wastes per unit area (8.25 kg/m^2, where 98 items/m^2) was observed in Jungle beach which is a famous tourist beach located in a remote area away from the main city of Galle. The least was recorded in Galle fort (1.05 kg/m^2, where 5 parts/m^2) which is also one of the famous tourist beaches in the central town of Galle. Figure 3 shows the comparison of total solid wastes in terms of weights and parts observed in a unit area in each of the sampling locations. Mount Lavinia beach, one of the famous tourist beaches away from the capital city of Sri Lanka shows the next highest percentage of solid wastes.

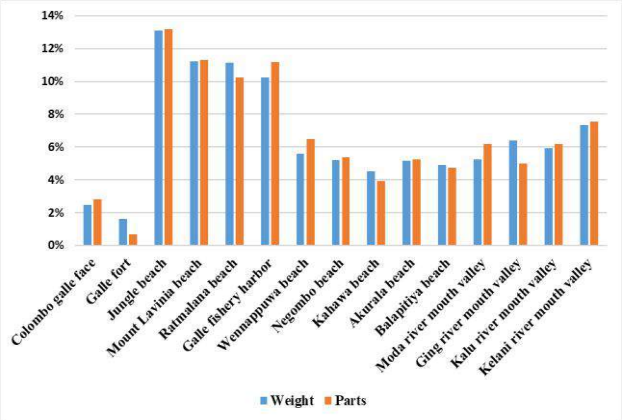

Fig. 3 – Comparison of solid wastes per unit area in each sampling location concerning weight and number of items.

Ratmalana beach is a highly residential beach with more than 50 families living in the coastal belt. Galle fishery harbor, one of the eminent centers for the fishing industry in the southern province too shows a high percentage of solid wastes when compared to others. It is vital that apart from the tourist beaches the next highest composition of solid wastes is depicted in the coastal environments near river mouths. It is prominent that most urbanized Colombo Galle Face beach and the Galle Fort beach which are highly populated with higher densities of population depict the least percentage of solid wastes per unit area. The waste generated is collected systematically with proper waste management on these beaches.

In the study, the data were collected once in a single day from each location. Hence, the composition of solid wastes obtained is an average value with the change of daily attraction of tourists and coastal-based industries. In addition, when counting the number of items from each type of material, the size of the parts was not taken into account and the microplastics deposited in the beach were neglected. Results obtained in the international coastal cleanup programs conducted in Sri Lanka in some of the selected beaches from 2008 to 2013 too reveal that 22.68% of plastic bags, 14.35% paper bags, 10.97% plastic bottles, 5.77% lids, and 5.61% food wraps have observed. Also, it has been investigated that 74.93% of recreational activities were likely to contribute to the accumulation of solid wastes in marine environments (Gunasekara, Priyadarshana, & Ranasinghe, 2013).

A study on assessment of marine debris selecting 22 beaches along the coastal belt of Sri Lanka depicts an average of 4.1 large (> 25 mm) and 158 small (5–25 mm) pieces of debris have amassed per square meter of beach and 93% of the total waste collected represent plastic in the debris. Further, the author reveals that the beaches near river mouths and tourist beaches have a greater debris accumulation. Also, the study depicts that the monitoring of marine litter will find the pathway for the possible management of solid wastes in the coastal environments (Yong Chang Jang, R R M K P Ranatunga, & Kyung Shin Kim, 2011).

3.3 Current Status of Solid Waste Management in the Coastal Environments.

From the beach visits of the sampling locations the existing solid waste management methods which have been adopted in coastal environments were able to identify. In Colombo Galle Face and Galle Fort beaches, separated garbage collectors were available. Wind resistive solid waste collector was observed in Mount Lavinia beach while awareness notice boards were available on a few of the beaches. A management method for solid wastes directly entering the ocean via waterways was absent in the visited river mouths.

Table 3 illustrates the summary of the data gathered from the beach site visits and the oral interviews done with the people in the vicinity of each sampling location regarding the existing solid waste management practices adopted and their progress. Conveniently, the proper disposal of solid waste is conducted in the marine environments located in the urbanized cities while proper maintenance of solid waste is absent in the other beaches due to a lack of infrastructure facilities. The absence of a systematic collection of the generated solid wastes finally results in the accumulation of solid wastes over years and finally becomes marine debris.

Fig. 4 – Wind resistive waste collector in Mount Lavinia

Table 3 – Summary of solid waste management in the sampling locations

Sampling location in the coastal area.	Nature of the location	Solid waste management method adopted and observations	Frequency of solid waste collection
Colombo Galle Face	Tourism and Commercial activities/ Urbanized	Separated solid wastes collection and regular removal.	Daily
Galle Fort	Tourism and Commercial activities/ Urbanized	Separated solid wastes collection and regular removal	Daily
Jungle beach	Tourism/ Remote	None. Directly dumped solid wastes.	More than six months
Mount Lavinia	Tourism / Urbanized and residential	Metal garbage collectors present and nonsystematic garbage removal.	Weekly/ Monthly
Ratmalana	Remote / Residential	None. Directly dumped solid wastes for a prolonged duration of time.	More than 6 month
Galle fishery harbor	Commercial/ Urbanized	None. Directly dumped solid wastes for a prolonged duration of time.	More than a year
Wennappu	Tourism	Nonsystematic garbage collection	Monthly
Negombo	Tourism	Nonsystematic garbage collection	Monthly
Kahawa	Remote / No human interfere	None, Scattered wastes	None
Akurala	Remote / No human interfere	None, Scattered wastes	None
Balapitiya	Remote / No human interfere	None, Scattered wastes	None
Moda river mouth valley	Urbanized	None, Solid wastes directly off to the ocean	None
Ging river mouth valley	Urbanized	None, Scattered wastes in the valley	None
Kalu river mouth valley	Urbanized	None.	No exact duration
Kelani river mouth valley	Urbanized	None.	No exact duration

3.4 Effectiveness of the Marine Solid Waste Management Methods

The interviews with the five main authorities related to the coastal environment reveal that inappropriate, illegal waste dumping and the absence of proper waste management systems cause marine debris. When regard to plastic waste management techniques in Sri Lanka, the central environment authority revealed that still, we do not have a formal procedure in using available techniques and apply them for waste management. The following were the responses from the governing authorities for the reasons behind the addition of solid wastes into the Ocean in Sri Lanka. Absence of proper waste collection and disposal system, lack of proper technology transferring procedures, lack of public awareness, poor implementation of regulatory mechanisms, lack of collecting centers for plastic waste management, lack of enough rules and regulations and poor application of existing rules.

The five authorities suggested proposals to use the 3R concept, starting with reduce, then reuse, and finally recycle to overcome the threat of plastic as marine litter which governs highest in marine solid wastes. Mainly, to reduce the usage of plastic and polythene bags, using alternatives such as cloth bags, increasing the price of plastic, implementation of laws & regulations to plastic usage was suggested. Promoting the use of plastic bags repeatedly, organizing eco fairs in every local authority, introducing reusable plastic & establish collecting centers, and

awareness was suggested to promote reuse. Use of selected yards & produce small items, placing of recycling centers island-wide, implementation of recycling plants & centers, incentives for debris collectors were suggested to promote recycling of solid wastes. Further, the need of promoting new inventions as depicted in figure 5 and apply them practically was suggested.

Fig. 5 – Proposed garbage trappers to prevent the entering of solid waste into the ocean through waterways.

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4.5 A solution to manage solid wastes in the coastal environments of Sri Lanka

It was prominent that apart from the coastal environments in the town limits of the country, solid wastes are accumulated over years in the coastal environments out of towns. It is obvious that due to lack of infrastructure facilities the collection of solid wastes for disposal is not done systematically. The ineffective management of available infrastructure facilities and lack of communication has resulted in it. Therefore, the study introduces a mobile application to track the locations of solid wastes in the coastal zone of Sri Lanka and engage the relevant authorities to collect the wastes without letting them become marine debris.

J. R. Jambeck and Johnsen (2015) introduced a global scale mobile application “Marine Debris Tracker” to track and monitor the global scale marine litter using an application. More than 400,000 items have been tracked with plastic becoming the prominent type of litter polluting the ocean. “MarPol Tracker" which was introduced in the study was targeted to use as a communicating application to use in managing the solid wastes which causes marine debris in Sri Lanka.

The introduced application can be installed on android mobile phones and it was named "MarPol Tracker" which meant Marine Pollutant Tracker. Anyone with the application can upload a photo of a polluted coastal environment with a caption. A google map of Sri Lanka allows the user to mark up the location and it is marked in the map in red color as a critical location. Then a notification is sent to all the users with the application regarding the polluted area. So beach clean-up organizers and the relevant authorities can notify the polluted locations and can engage the available infrastructure facilities effectively to remove the solid wastes from relevant locations.

Fig. 6 – The main interface of the mobile application

Figure 6 illustrates the main interface to the login of the application. The map interface was taken from google maps. Figure 7 shows the interface available to input the data.

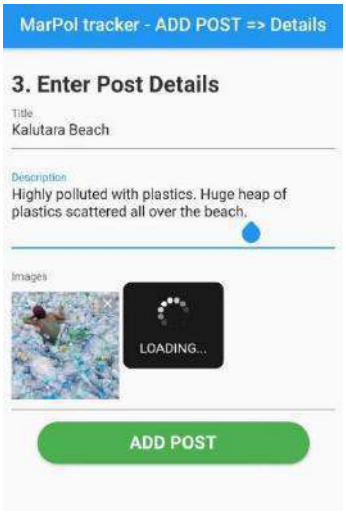

Fig. 7 – Interface to enter the location with a comment and add a photo.

After clearing the polluted environment the application allows uploading a new photo of the cleaned location with a comment. Simultaneously, the location marked previously in red turns to green in color and a notification is sent to all the users mentioning that the location was cleared. This application is a human controlling application and wise use of it is expected. It is mainly a communication application and also ii aware the community even in the inland of the country regarding the marine litter. Also, it can be

mentioned as a motivational application because it motivates to turn the critical locations depicted in red, into the green color.

Fig. 8 – Depicting the polluted locations in red and cleared locations with green.

The application can be further improved to make use not only for marine solid wastes, but also for solid waste management of the entire country.

IV. CONCLUSION

The research elaborates regarding the sources of marine solid wastes which were categorized as sea-based, coastal based and land-based. The results of the analysis showed that the highest percentage of solid wastes records from tourist beaches away from the town limits and the next highest from coastal environments near the river mouths, and the least from beaches in the town. As a developing country, due to lack of resources the solid waste management has become non-systematic. Also, the paper proves that plastic governs marine pollution and acknowledges the needed authorities and community.

This paper presents the current situation of solid waste management with lacking infrastructure facilities in the country. The concern of governing authorities will pay attention in this regard and their suggestions will be implemented from the analysis of the research. Implying 3R principles for plastic recycling as suggested by the authorities related to coastal environments will be advantageous for future generations of the country. The introduced mobile application can be used as a communication application in the effective management of solid wastes with the available resources and can be used to aware the community. It is also a motivational application that can minimize marine debris.

This paper has analyzed only a few of the coastal locations in the country and the analysis has been done on a random day of the year. Analysis of marine litter following the seasons of the year considering the populations need to be implemented in the future studies.

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Growth and Yield Response of Sweet Potato (*Ipomoea batatas*) to Organic and Inorganic Fertilizer on Degraded Soil of Southern Guinea Savanna of Nigeria

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Abstract— *The problem of soil fertility makes it difficult for some crops to attain higher yields; especially crops like sweet potato that our traditional peasant farmers believed that it does not require the application of fertilizer. Therefore, experiments were conducted during the rainy season of 2018 and 2019 titled "Growth and Yield Response of Sweet Potato (Ipomoea batatas) to Organic and Inorganic Fertilizer on Degraded Soil of Southern Guinea Savanna of Nigeria" to determine the optimal levels of both organic and inorganic fertilizer for sustainable sweet potato production. The treatments consisted of three levels of NPK fertilizer (0, 50 and 100 kg ha^{-1}); three rates of poultry manure (0, 5 and 10 t ha^{-1}). A factorial experiment laid in a Randomized Complete Block Design (RCBD) and replicated three times. The result showed NPK fertilizer applied at the rate of 100kg ha^{-1} produced the highest number of leaves/plant (178, 233); number of branches/plant (10.45, 11.98); and vine length/plant (245.43cm, 258.79cm) in both 2018 and 2019 cropping season respectively. Also, poultry manure at 10t ha^{-1} produced the highest number of leaves/pant (221, 242); number of branches/plant (11.24, 13.25); and vine length (252.45cm, 275.54cm) in both years of cropping respectively. Application of 100kg ha^{-1} of NPK fertilizer produced the highest tuber weight of 21.12 and 22.72t ha^{-1}, in both 2018 and 2019 cropping seasons respectively compared with the other rates of fertilizer application and the control. Also, Poultry manure at the rate of 10 t ha^{-1} produced the highest total weight of 21.32 and 24.19t ha^{-1} of sweet potato in both 2018 and 2019 cropping seasons respectively compared with the other rate of fertilizer application. Interaction between NPK fertilizer and poultry manure show a significant ($p<0.05$) increased on the total tuber weight in both years (2018 and 2019). For integrated nutrient management in sweet potato production 50kg ha^{-1} of NPK fertilizer and 10t ha^{-1} of poultry manure produced the best total tuber weight.*

Keywords— Growth, Yield, NPK fertilizer, poultry manure, Sweet potato.

I. INTRODUCTION

Sweet potato *(Ipomoeae batatas* (L Lam) is a perennial crop belonging to the family of Convulvulaceae with its origin from tropical America (Hahn, 1983). However, it is usually cultivated as an annual crop. Globally, it is among the important food crops in the world. It ranks second following Irish potato in the world's root and tuber crop production; also within the sub-Saharan Africa, it is the third most important root and tuber crop after cassava and yam (Hahn and Hozyo, 1998). China accounts for the highest world production of sweet potato, followed by Uganda and Nigeria in that order (FAO, 2004). Presently, 381,000 – 510,000 ha of land are subjected to sweet potato cultivation in Nigeria with an annual production figure of 3.46 million metric tons (NRCRI, 2009). Estimated yields of sweet potatoes in the research fields varied from 40 to 70 t/ha for improved varieties, while in multilocational trials yields averaged 23.5t/ha across seasons and locations (Tewe *et al.*, 2003).

Sweet potato is adaptable to tropical and subtropical climates, tolerant to drought and grows under marginal condition of low fertility and pH. The increasing potential of the crop in poverty alleviation and food security due to its high productivity per unit area and timely maturity makes sweet potato an important crop for the survival of the resource poor farmers in Nigeria (NRCRI, 2003). Sweet potato is valued for its tubers which are boiled, fried, baked or roasted for humans or boiled and fed to livestock as a source of energy. The potential of sweet potato to guarantee food security is under-estimated as its uses is often limited to a substitute food in African countries (Muktar *et al;* 2010). The tubers can also be processed into flour for bread making, starch for noodles as well as used as raw material for industrial starch and alcohol. The flour is utilized also in sweetening local beverages like *Kunu-zaki*, *burukutu*, and for fortifying baby foods and foo-foo/pounded yam in Nigeria (Tewe *et al.,* 2003). The leaves are used as vegetables in yam and cocoyam porridge and are rich in proteins, vitamins and various minerals. Sweet potato tubers are rich in vitamins A, B, and C; and minerals such as K, Na, Cl, P and Ca (Onwueme & Sinha, 1991). It can therefore be a high value-added food particularly for children and pregnant women who are more often exposed to vitamin A deficiency in sub-Saharan Africa (Degras, 2003).

The problem of soil fertility and variability in climatic condition makes it difficult for some crops to attain higher yields especially crops like sweet potato that our traditional peasant farmers believe that it does not require the application of fertilizer. However, sweet potato like any other root and tuber crops is a heavy feeder, exploiting greater volume of soil nutrients and water (Osundare, 2004). Low soil fertility is one of the constraints in production of sweet potato in Nigeria (Okpara 2000). Rapid depletion of soil nutrients and poor physical condition of the savanna soils constitute a strong limitation to crop production (Sanchez 1996). As such, these soils must be supplemented with adequate macronutrients in order to keep them productive (Aisha *et al.,* 2007). Therefore, external nutrient inputs are essential to improve and sustain the growth and yield of sweet potato; these nutrient inputs may either be from organic sources or inorganic fertilizers (Njoku *et al.,* 2001). Though inorganic fertilizers have been the conventional method of soil nutrients input in sweet potato production. These fertilizers may pose a great danger to the environment, especially if it is inappropriately applied. Also, the shortage and high cost of inorganic fertilizers have limited their uses for crop production among the peasant farmers in Nigeria. Hence, organic manures can serve as alternative to mineral fertilizers for improving soil fertility (Dauda *et al.,* 2008). Therefore, there is the need for increased dependence on the use of organic waste such as farmyard manure, crop residues and poultry manure for crop production (Ndors *et al.,* 2013). The production, marketing and utilization of sweet potato have expanded in the last decade to almost all ecological zones in Nigeria (NRCRI, 2009). In the north central Nigeria, this crop has also gained acceptability and currently there is increased in cultivation by small farm holders as a source of income. However, there is a dearth of documented information regarding the soil nutritional requirement of the crop and other agronomic practices that may be of help to these farmers for increasing the yield of the crop in this zone. This research therefore, is aimed at determining the optimal levels of both organic and inorganic fertilizer for sustainable sweet potato production in southern guinea savanna of Nigeria.

II. MATERIALS AND METHOD

Field trials were conducted at the College of Agriculture Lafia, Nasarawa State, Nigeria; Teaching and Research Farm in 2018 and 2019 cropping seasons. The study area falls within southern guinea savanna agroecological zone of Nigeria, and is located between Latitude 08.33 N and Longitude 08.32 E. Rainfall usually starts from April – October and the average monthly rainfall figures ranges from 40 mm-350 mm. The months of July and August usually records heavy rainfall. The daily maximum temperature ranges from 20.0ºC – 38.5ºC and daily minimum ranges from 18.7ºC – 28.2ºC. The months of February to early April are the months that have the highest maximum temperature while the lowest maximum temperature months are recorded in December and January because of the prevailing cold harmattan wind from the northern part of the country at this period. The relative humidity rises as from April to a maximum of about 75-90 percent in July (NIMET 2019).

2.1 Treatment and Experimental Design

The treatments consisted of three levels of NPK fertilizer (0, 50 and 100 kg ha^{-1}); three rates of poultry manure (0, 5 and 10 t ha^{-1}). A factorial experiment laid in a Randomized Complete Block Design (RCBD) and replicated three times.

2.2 Field preparation and Agronomic practices

The plot size was 3 m by 4 m; 0.5 m between plots and 1 m between replicates. The land was cleared, harrowed and made into 0.75m row ridges. The local sweet potato vines were obtained locally from farmers in Lafia town. Four node cuttings of the sweet potato vines were planted on the ridge at a spacing of 35 cm; the brand of the chemical

fertilizer used was NPK 20:10:10. The plots were kept weed free by regular hoe-weeding.

2.3 Soil Analysis

Soil samples were collected from the experimental site before planting for determination of both physical and chemical properties. Also, the poultry manure was subjected to chemical analysis.

2.4 Data Collection

Two plants were tag in the middle roll of each plot and the following data were collected: vine length, number of branches and number of leaves were taken at 8 and 12 weeks after planting. While, biomass weight, number of marketable tubers, weight of marketable tubers, number of unmarketable tubers, weight of unmarketable tubers, total marketable tubers and total unmarketable tubers were recorded at harvest.

2.5 Data Analysis: The data collected were subjected to analysis of variance for using GENSTAT (2008 Ed), and where there was a significant difference; the means were separated using F-LSD at 5% probability level.

III. RESULTS AND DISCUSSION

The result of soil analysis of the experimental site before cropping in the first year showed that, the soil was very sandy (87.0%) and slightly acidic in nature (pH 6.08 in H_2O and 6.00 in 0.01MKCL); but low in nitrogen, phosphorus, potassium, organic carbon and cation exchange capacity (Table 1). The result in second year did not show much difference, however, there were little reduction in the quantity of sand (80.0%), but there were increases in soil pH (6.34), nitrogen, phosphorus, potassium, organic carbon and cation exchange capacity.

Table 1: Laboratory analysis of soils at 0-20cm before cropping

Properties	2018	2019
Mech. Composition		
Clay (%)	10.6	14.6
Silt ‘’	2.4	5.4
Sand ‘’	87.0	80.0
TCL (USD)	SL	SL
Chemical composition		
pH(H_2O)	6.08	6.34
pH(0.01MKCl)	6.00	6.09
Ashes(gkg^{-1})	Nd	Nd
T N(gkg^{-1})	0.90	1.02
OC(gkg^{-1})	4.40	6.80
Avail. P ($mgkg^{-1}$)	4.17	5.52
K($mgkg^{-1}$)	0.31	1.21
Mg(mol/kg)	1.12	1.78
Ca(mol/kg)	2.41	3.48
Na(mol/kg)	0.67	0.67
Al + H(acidity)	0.83	0.61
CEC(mol/kg)	5.17	6.24
%Base Saturation	81	87

The poultry manure used had N, P and K (3.14, 3.48 and 4.95%) respectively and a pH of 7.9 (slightly alkaline); the exchangeable cations were of moderate levels (Table 2). The nutrient contents of the manures were moderate to high; therefore, the quantities applied must have supplied the important nutrients such as N, P and K, which are critical for sweet potato growth and yield

Table 2. Chemical composition of the poultry manure used during the study

% Chemical properties	**2015**
Ph	7.9
N	3.14
P	3.48
K	4.95
Ca	5.52
Mg	0.45
Na	0.32

3.1 Effect of NPK fertilizer and poultry manure on vegetative growth of sweet potato

Application of NPK fertilizer and poultry manure had a significant ($p<0.05$) increased in all the growth parameters assessed in both years of cropping (Table 3). NPK fertilizer applied at the rate of 100kg ha^{-1} produced the highest number of leaves/plant (178, 233); number of branches/plant (10.45, 11.98); and vine length/plant (245.43cm, 258.79cm) in both 2018 and 2019 cropping season respectively. Also, poultry manure at 10t ha^{-1} produced the highest number of leaves/pant (221, 242); number of branches/plant (11.24, 13.25); and vine length (252.45cm, 275.54cm) in both years cropping respectively. The interaction between NPK fertilizer and poultry manure did not produced any significant effects on growth parameters of sweet potato throughout the duration of this experiment.

Table 3: Effect of NPK fertilizer and poultry manure on vegetative growth of sweet potato

Treatments	No of leaves/plant		No of branches/plant		Vine length/plant(cm)	
NPK fertilizer (kg/ha)	2018	2019	2018	2019	2018	2019
0	129	145	5.12	7.23	130.25	181.34
50	147	186	7.32	9.89	158.56	206.53
100	178	233	10.45	11.98	245.43	258.79
LSD(0.05)	21	35	2.16	2.21	25.34	27.67
Poultry manure(t/ha)						
0	118	128	5.71	8.75	170.48	213.76
5	172	185	9.42	11.13	214.67	225.32
10	221	242	11.24	13.25	252.45	275.54
LSD(0.05)	20	31	1.45	1.87	31.65	26.73
Interaction						
NPK X PM	NS	NS	NS	NS	NS	NS

PM= Poultry manure NS= Not significant

3.2 Effect of NPK fertilizer and poultry manure on biomass weight and yield parameters of sweet potato at harvest

NPK fertilizer and poultry manure rates significantly ($p<0.05$) increased the biomass weight and the tuber yield of sweet potato (Table 4). Application of 100kg ha^{-1} of NPK fertilizer produced the biggest (15.00 and 16.13 t ha^{-1}) sweet potato biomass weight in both 2018 and 2019 cropping seasons respectively compared with the other rate of fertilizer application. The control produced the smallest biomass of 12.12 and 12.24 t ha^{-1} in both 2018 and 2019 cropping seasons. Also, application of 10 t ha^{-1} of poultry manure produced the biggest (16.24 and 17.65 t ha^{-1}) sweet potato biomass weight in both 2018 and 2019 cropping seasons respectively compared with the other rate of poultry manure application. The interaction between NPK and poultry manure showed a significant effect.

Application of NPK fertilizer and poultry manure rates had a significant ($p<0.05$) increased on the weight of marketable tuber (Table 4). 100kg ha^{-1} of NPK fertilizer produced the highest tuber weight of 20.58 and 20.01t ha^{-1}, in both 2018 and 2019 cropping seasons respectively compared with the other rate of fertilizer application. Poultry manure at the rate of 10 t ha^{-1} produced the highest weight of 20.76 and 23.24t ha^{-1} of sweet potato in both 2018 and 2019 cropping seasons respectively compared with the other rate of fertilizer application.

Application of NPK fertilizer and poultry manure rates did not have any significant ($p<0.05$) increased on the weight of unmarketable tuber (Table 4). However, there was gradual decrease in the weight of unmarketable tubers when NPK and poultry manure were increased in both 2018 and 2019 cropping seasons respectively compared with the other rate of fertilizer application.

Application of NPK fertilizer and poultry manure rates had a significant ($p<0.05$) increased on the total weight of tubers (Table 4). 100kg ha^{-1} of NPK fertilizer produced the highest tuber weight of 21.12 and 22.72t ha^{-1}, in both 2018 and 2019 cropping seasons respectively compared with the other rates of fertilizer application and the control. Also, Poultry manure at the rate of 10 t ha^{-1} produced the highest total weight of 21.32 and 24.19t ha^{-1} of sweet potato in both 2018 and 2019 cropping seasons respectively compared with the other rate of fertilizer application. The interaction between NPK and poultry manure showed a significant effect.

The result on (Table 5) showed that the interaction between NPK fertilizer and poultry manure show a significant ($p<0.05$) increased on the biomass yield in both years. Application of 100kg ha^{-1} of NPK fertilizer and 10 t ha^{-1} of poultry manure produced the highest fresh biomass yield of 19.23 t ha^{-1} and 19.80 t ha^{-1} in 2018 and 2019 cropping seasons respectively. Another interaction result is on (Table 6), which showed that the interaction between NPK fertilizer and poultry manure show a significant ($p<0.05$) increased on the total tuber weight in both years (2018 and 2019). Application of 50kg ha^{-1} of NPK fertilizer and 10 t ha^{-1} of poultry manure produced the highest tuber weight of 23.08 t ha^{-1} and 24.02 t ha^{-1} in 2018 and 2019 cropping seasons respectively. This result is statistically a par with application of 100kg ha^{-1} of NPK

and 10 t ha^{-1} of poultry manure in both 2018 and 2019 cropping seasons.

Table 4: Effect of NPK fertilizer and poultry manure on biomass weight and yield parameters of sweet potato at harvest

Treatments	**Fresh biomass weight (t ha^{-1})**		**Wt of marketable tubers (t ha^{-1})**		**Wt of unmarketable tubers (t ha^{-1})**		**Total weight of tubers (t ha^{-1})**	
NPK(Kg/ha)	2018	2019	2018	2019	2018	2019	2018	2019
0	12.12	12.24	15.08	15.79	0.79	0.98	15.87	16.77
50	13.22	13.82	18.14	19.12	0.62	0.74	18.76	19.74
100	15.00	16.13	20.58	22.01	0.54	0.71	21.12	22.72
LSD(0.05)	1.04	1.11	2.02	2.54	2.14	2.42	2.04	2.25
PM(t/ha)								
0	12.05	12.25	14.03	15.09	0.74	0.97	14.77	16.05
5	14.12	15.49	16.29	19.57	0.59	0.96	16.88	20.35
10	16.24	17.65	20.76	23.24	0.56	0.95	21.32	24.19
LSD(0.05)	1.20	1.06	2.14	2.25	2.60	2.28	2.09	2.41
Interaction								
NPK X PM	*	*	NS	NS	NS	NS	*	*

PM= Poultry manure, NS= Not significant

Table 5: Interaction between NPK and poultry manure on fresh biomass weight (t ha^{-1}) of Sweet potato at harvest

	2018 Cropping season			2019 Cropping season		
Treatment	Poultry manure (t ha^{-1})			Poultry manure (t ha^{-1})		
Urea(kg^{-1})	0	5	10	0	5	10
0	12.67	14.82	16.89	12.84	14.76	16.67
50	14.90	16.45	17.27	15.45	17.62	17.76
100	17.45	17.42	19.23	17.25	17.98	19.80
LSD(0.05)	1.04			1.12		

Table 6: Interaction between NPK and poultry manure on total weight of tubers (t ha^{-1}) of Sweet potato at harvest

	2018 Cropping season			2019 Cropping season		
Treatment	Poultry manure (t ha^{-1})			Poultry manure (t ha^{-1})		
Urea(kg^{-1})	0	5	10	0	5	10
0	16.52	19.65	19.54	16.97	20.43	18.45
50	18.05	20.98	23.08	18.78	19.98	24.02
100	19.68	21.67	22.24	19.85	21.34	23.56
LSD(0.05)	1.02			1.12		

IV. DISCUSSIONS

The result in table one, showed that the soil was slightly acidic, sandy and already exhausted; which may be due to intensive and continuous cultivation and without adequate application of replenishment measures to sustain it productivity. This result agrees with finding of (Jayeoba *et al.*, 2012; Ndor and Iorkua, 2013), who reported that soils around Lafia are exhausted and slightly acidic. The significant increased recorded in growth parameters (number of leaves, number of branches and vine length) of sweet potato could be attributed to the ability of both fertilizers (NPK and poultry manure) to activate spontaneous vigorous growth which may be as a result of the high content of nitrogen in both NPK and poultry

manure which stimulated vegetative growth in both years of cropping. This corroborates the finding of Muktar *et al.*, (2010), who worked on agronomic characteristics of sweet potato grown under organic and inorganic fertilizers. Also, the significant increased recorded in the weight of sweet potato tubers in both 2018 and 2019 cropping season as a result of increased application of poultry and NPK fertilizers. This may be attributed to the fact that, after incorporation poultry manure into the soil and addition of NPK (20:10:10) fertilizer, macronutrients were readily available within the soil for plant uptake. This resulted in the synthesis of more photo-assimilates, which is used in dry matter (DM) accumulation in sweet potato tubers. This result is in line with the discovery of Yeng *et al.*, (2012); who study the growth and yield of sweet potato (*Ipomoea batatas* L.) as influenced by integrated application of chicken manure and inorganic fertilizer in Ghana. The superior performance of poultry manure in both years (2018 and 2019) compared to NPK fertilizer may not be attributed to the high quality of poultry manure (Table 2) used only; but the poultry manure was also able to improve on water retention capacity and the reduction of soil acidity so that availability of macronutrient like phosphorus will be enhance (Adam, 2005). The significant interactions between NPK fertilizer and poultry manure on biomass weight and total tuber weight was an affirmation of the fact that combined applications of both organic and inorganic manure is essential for increased growth and yield (Yeng *et al.*, 2012).

V. CONCLUSION

From this study, it can be concluded that 100kg ha^{-1} of NPK fertilizer and 10t ha^{-1} produced the highest tuber weight of (21.12 and 22.72t ha^{-1},) and (21.32 and 24.19t ha^{-1}) in both 2018 and 2019 cropping seasons. Therefore, the above rates could be the optimal fertilizer level for a good growth and yield of sweet potato. For integrated nutrient management in sweet potato production 50kg ha^{-1} of NPK fertilizer and 10t ha^{-1} of poultry manure can be used. However, further locational trials should be conducted within the zone to confirm this result.

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The Planting Media Management on Vegetative Growth of Cassava (Manihotesculentacrantz)

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Abstract— *The Media Management of Cassava (Manihotesculentacrantz), this research aims to increase the vegetative growth component of cassava clone L-18 SMEs. This research was carried out at an altitude of ± 50 m above sea level, on andosol of pH 5.20. Using Factorial Randomized Block Design (RFBD) as the environmental design with two factors, namely the first factor: tillage treatment with 3 levels: P0 = No tillage, P1 = Minimum tillage, P2 = Perfect tillage and the second factor was the treatment of compost organic matter with 4 levels: B0 = No organic matter (control), B1 = compost organic matter 5 tons/ha, B2 = compost organic matter 10 tons/ha and B3 = compost organic matter 15 tons/ha.The response variables observed and measured were increase in plant height (cm), increase in stem diameter (mm), number of leaf additions (strands), leaf stalk length (cm). The results shows that the management of planting media combined with minimal tillage with the application of 6.02-7.10 tons/ha of organic has a significant effect on the increase of plant height (cm), and the number of leaves (strands). The management of growing media without tillage with the addition of 7.94 tons/ha of organic has a significant effect on the increase of stem diameter (mm), and the addition of 15 tons/ha of organic significantly affected the increase in the number of leaves (strands) and petiole length (cm).*

Keyword— ***Tillage, Organic Materials, Clones L-18 UKM.***

I. INTRODUCTION

Indonesia is said to be an agricultural country which is rich in food and self-sufficient in food. It is very ironic that Indonesia is currently said to be food insecure, due to the condition of Indonesia's food security is still low (Budiono, 2011) therefore domestic food security must be the main key (Latif. A, 2011) and the President of the Republic of Indonesia urges in order to maintain food availability to take advantage of home yard for plant cultivation (Anonymous, 2013).

Food is a basic need for humans for their survival, so that food sufficiency is a human right that must be fulfilled (Suryana. A, 2005).According to Ginandjar. K, (2005) food sources come from biological sources to be processed and utilized by humans as staple food and additional for the necessities of life.

The 1945 of Indonesian's Constitution Article 27 paragraph 2 states, "Every citizen has the right to a decent life", so that the National food security program refers to aspects of food security for human health (Food Security Agency, 2012).

Dependence on one food commodity must be reduced (Anonymous, 2009) and can be maintained, so that there is a need for a food diversification policy and not only dependence on one food commodity, so it is necessary to continuously socialize national food diversification based on local food.

The efforts to diversify food (transfer of staple foods) can maintain the stability of food security, so that it is not too dependent on rice (Hermanto. S, 2012). Food verification outside of rice has been developed in several areas by utilizing local food crops such as cassava (MzAmirul. T, 2011) and as a staple food variation (Sudarmonowati.E, 2013).

So that Indonesia does not lack food, it must utilize the right technology and existing natural resources, so that food is still available for the needs of the population (Budiono, 2011), by improving infrastructure between food producers and food users (Latif. A, 2011).

Cassava is one of the commodities of food diversity that is not foreign to the people of Indonesia. Generally, cassava plants are found in every area. Cassava can be processed into various forms of food. In Medan, cassava has been processed into souvenirs as "Bika Cassava". The Food Security Agency of North Sumatra has coined the term in the Toba Batak language, "Manggadong", which means let's eat cassava as a staple food. The author remembers that when she was a child, her parents always gave her cassava food before eating rice, so that only a little rice was eaten, because at that time it was difficult to obtain rice food. Therefore, the people of North Sumatra are no stranger to cassava food. In the current generation, especially in rural areas, the term "Mangadong" is no longer recognizable, because many agricultural lands have been converted to land functions and even cassava is grown mostly, "poison cassava", for the manufacture of tapioca flour and cannot be processed into a source of fast food.

The area of cassava food commodities tends to decrease by 0.3% annually, and the harvested area of North Sumatra cassava is 38,611 ha with a productivity of 26.1 t/ha (Jonharnas., et al 2012). The productivity of cassava as a food ingredient is still limited due to the absence of superior clones, not optimal cultivation methods and the utilization of existing natural resources.

Cassava production can be increased by means of cultivation techniques, the use of superior clones and the maximum use of natural resources, through soil processing and the use of organic matter. Generally, soil processing in cassava cultivation is carried out twice, namely tillage by reversing the soil and followed by loosening the soil which is called perfect tillage. Soil cultivation must be adjusted to the productivity of soil fertility, namely its structure and texture. Hakim, (1986) stated that continuous land cultivation can result in damage to soil structure, so that efforts are made so that the soil is not treated too often or is sufficient with minimum tillage.

The purpose of tillage is to create space for plants to grow, so that roots can support growth and development on it (Arsana, 2007). Plants can grow optimally, if the soil is loose, the tillage layer is 25-35 cm and drained. Lack of tillage can cause roots not to develop, so plant growth is depressed and yields are low (Somaatmadja, 1991). Many "palawija" farmers apply without tillage using herbicides with the active ingredient glyphosate to eradicate weeds (Tjokrowardojo, 2001 in Mulyadi et al., 2007).

The tillage layer can become dense (difficult to process) due to low organic matter, so that the addition of organic matter to the tillage layer can improve the physical, chemical and biological fertility of the soil, to support increased vegetative growth, especially the production of cassava tubers in order to maintain and increase soil fertility. food security in North Sumatra, namely using the L-8 clone of SMEs with production potentials that can reach 25-50 kg per tree or the equivalent of 120-200 kg/ha.

II. REVIEW OF LITERATURE

The Land as Treatment

The land was divided into three replicates with a total land area of 52.5 x 12 m (630 m2). The research site was sprayed with contact grass poison and left for 2 weeks until it was completely dry, then the plot area for the combination treatment was 3 x 3 m (9 m2). Land preparation is adjusted to the tillage treatment.

No Tillage

Weeds from herbicide sprays are left to dry for 2 weeks on the land. After 2 weeks the dried weeds were removed from the soil surface and without tillage.

To minimize the bias of tillage treatment, the treatment without tillage and minimum tillage was carried out simultaneously with complete tillage in the second week. Then in the three tillage treatments, planting holes were made at the growing point measuring 20 x 20x20 cm (the size of a hoe) for placing organic compost and planting cassava cuttings of clone L-18 SMEs that have rooted according to the recommended planting distance of 150 x 150 cm.

Minimum Tillage

The weeds were first sprayed with herbicide with the active ingredient "paraquat" and allowed to dry for 2 weeks on the soil surface. Weeds that have dried are scraped off the soil surface to facilitate tillage. Soil is cultivated only once in a row of crops.

Perfect Tillage

One week after the herbicide spray, the dried weeds are removed from the soil surface to facilitate tillage. Then the first hoeing is done and left for a week. One week later, a second hoeing is carried out while loosening and smoothing the soil.

2.6. Organic Material Treatment

Organic matter is given to the available planting holes measuring 20 x 20 x 20 cm as a place for sowing compost organic matter. Compost organic matter was given according to the level of treatment, ie without compost organic matter as a comparison/control followed by compost organic matter 5 tons/ha (1.1 kg/plant), 10 tons/ha (2.2 kg/plant) and 15 tons/ha (3.3 kg/plant). Compost organic matter is given per planting hole and the planting hole is covered with excavated soil.

The Clone of Cassava Planting L-18

The transportation of cassava stem cuttings from the nursery to the research location was attempted to avoid

damage to the root cuttings of cassava clones L-18. One day after tillage and application of organic compost, cassava stem cuttings were planted. The cuttings must be planted carefully, because the grafted cassava stem cuttings have formed roots, stem shoots and leaves, so they are not damaged or broken. Cassava cuttings resulting from grafting are first selected for the number of seeds needed, namely with the criteria of the same stem cutting length, stem diameter and number of stem and leaf shoots trying to be uniform, notattacked by pests/diseases, the age of stem cuttings is the same.

Before planting the seedling cuttings, it is watered first, so that the condition of the seeds is fresh at the time of planting. The grafted cassava stem cuttings were planted in the planting hole/perpendicular growing point as deep as 10 cm from the base of the stem, and the grafting eye (stem and leaf shoots) faced the sun and then the planting hole was covered again with soil and watered.

The Plant Care and Maintenance

Care and maintenance of cassava plants is carried out starting at the age of one week after planting (WST) and continued once every two weeks after planting by observing the growth and development conditions of the plant and the weather conditions of the research location.

The Stitching

Crop embroidery was carried out at all locations of the research plot and if it was found that the cuttings of the L-18 UKM clones died, then the seedlings were replaced with the same age of seedlings. Crop embroidery is done until the age of 4 weeks after planting.

The Weed Weeding and Hoarding

Weeds (nuisance plants) that grow around the plants are controlled manually. Weed control was carried out from the age of 3 months after planting as well as the implementation of hoarding on cassava stems. Weed control and hoarding are then carried out after the plants are 24 weeks after planting.

The Sprouting/Removal of Shoots

The removal of shoots on the main stem is carried out after the plant is 16 weeks after planting and is only done once. Shoots that grow on the main stem are maintained only 3 shoots and if more than 3 shoots do remove shoots using a knife or scissors carefully so as not to damage other shoots. The former removal is smeared with liquid wax so that it is not easy to enter the source of fungal diseases.

Fertilization

The fertilization by chemical means is not carried out at the beginning of planting until the age of 12-16 weeks after planting, in order to obtain homogeneity of vegetative growth observations, because the treatment of organic compost fertilizer was given at the beginning of planting cassava seed cuttings. After the plant is 20 weeks after planting, then NPK fertilization is carried out at 15:15:15.

The Response Variable Observation

Variables observed growth components:

Increase in Plant Height (cm), Increase in Stem Diameter (mm), Increase in Number of Leaves (strands), Petiole Length (cm), One Leaf Area (cm2), Number of Bulbs Formed (bulbs).

III. THE RESEARCH METHOD

The research was conducted at the location of community-owned agricultural land in April - September 2021.The ingredients consist of: SME clone L-8 seeds, municipal waste compost organic, NPK fertilizer, “gramoxsone” herbicide, round up, fungicide and insecticide, liquid wax.

The tools consist of: hoe, rake, tape measure, ruler roller, bucket, knapsack sprayer, scales, scissors, knife, saw, plastic rope, tacks, markers, labels and other necessary stationery.

Factorial Randomized Block Design with two factors, namely: 3 levels of tillage treatment: P0 = No Tillage, P1 = Minimum Tillage, P2 = Perfect Soil Treatment and organic compost treatment consists of 4 levels: B0=Without organic (control), B1= with organic matter 5 tons/ha, B2= with organic matter 10 tons/ha, B3= with organic 15 tons/ha. Repeated 3 times with the determination of the layout of the study as an experimental unit, replications and samples were carried out randomly.

IV. THE RESULTS AND DISCUSSION

The Growth Component

Table 1. Average Plant Height Gain (cm) Cassava Clone L-18 Age 23 and 25 WAP on Planting Media Management

Olah Tanah	Bahan Organik	Pertambahan Tinggi Tanaman Pengamatan (MST) 23	25
		.... cm	
P_0 = (TOT)	B_0 (Tanpa Bahan Organik)	23.43c	45.49bc
	B_1 (5 ton/ha)	24.98abc	51.47bc
	B_2 (10 ton/ha)	26.77ab	44.24bc
	B_3 (15 ton/ha)	26.48ab	69.48ab
P_1 = (OTM)	B_0 (Tanpa Bahan Organik)	25.16abc	51.35bc
	B_1 (5 ton/ha)	25.57abc	75.98ab
	B_2 (10 ton/ha)	26.03abc	50.52bc
	B_3 (15 ton/ha)	24.80bc	45.08bc
P_2 = (OTS)	B_0 (Tanpa Bahan Organik)	26.12abc	47.16bc
	B_1 (5 ton/ha)	25.81abc	27.83c
	B_2 (10 ton/ha)	24.12bc	28.30c
	B_3 (15 ton/ha)	27.63a	90.59a

Note: The mean number followed by the same letter in each column of the same treatment is not significantly different at the 5% level based on Duncan's distance test.

The increase in plant height had a significant effect and was obtained on the management of planting media with a combination of minimum tillage and the application of 5-10 tons of organic matter/ha, namely

observations at the age of 23 WAP (27.63 cm) cm and age 25 WAP (90.59), and not significantly different from management of other growing media.

The lowest increase in plant height was obtained in the management of combined planting media without tillage and without giving organic matter at 23 WAP and at 25 WAP (23.43 cm) occurred in perfect tillage 5-10 tons/ha (P2B1 and P2B2). Observation of plant height increase can be seen in the following figure.

The relationship between the management of planting media, a combination of tillage and the addition of organic matter to the increase in plant height cassava clones L-18 SMEs aged 23 and 25 MST can be seen in Figure 1.

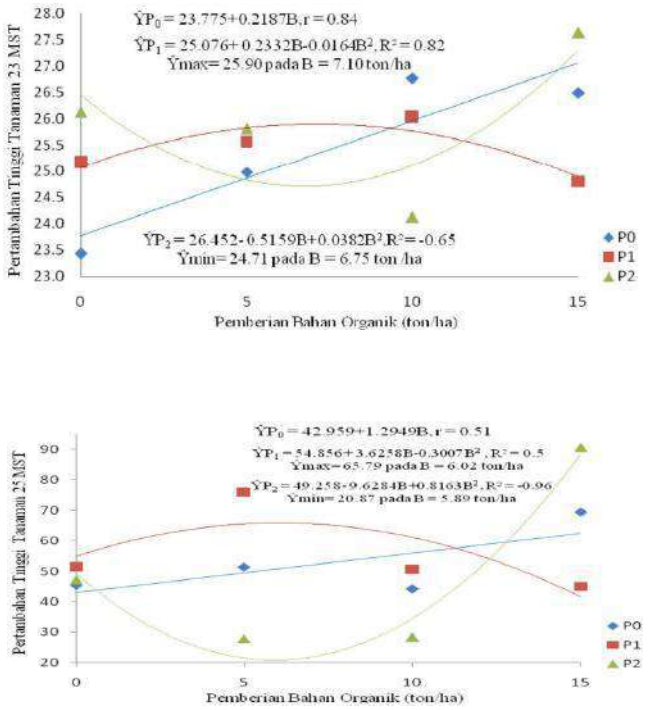


Fig.1: Plant Height Increase (cm) Cassava Clones L-18 SMEs Age 23 and 25 WAP on Planting Media Management

Figure 1 shows the maximum plant height increase at the age of 23 WAP (25.90 cm) in the management of combined minimum tillage (OTM) soil media with the application of organic matter 7.10 tons/ha and the increase in plant height continued to increase until the age of 25 WAP (65.79 cm), namely, combination of minimum tillage with an organic matter requirement of 6.02 tons/ha.

The management of perfect tillage planting media is tillage that is commonly carried out intensively, namely twice with the aim that the soil properties, the soil composition is looser (Larson, 1964) and followed by the application of organic compounds such as "humic" acid and "fulplic" acid which is maximally capable of increase the value of CEC, the soil is more loose and saves a lot of water (Titiek, 2011). Thus the plant is able to form a deeper, more flexible root system and will affect absorptionnutrients and water so that it has an impact on increasing stem diameter better.

Table 2. Average Increase in Stem Diameter (mm) Cassava Clones L-18 Age 23 and 25 WAP in Soil Media Management

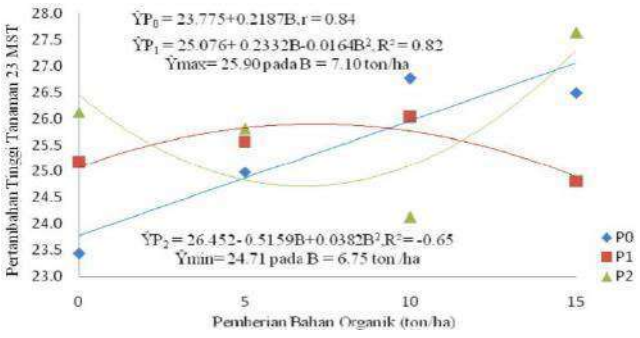
Olah Tanah	Bahan Organik	Pertambahan Diameter Batang Pengamatan (MST) 23	25
		.... mm	
P_0 = (TOT)	B_0 (Tanpa Bahan Organik)	23.43cd	45.49bc
	B_1 (5 ton/ha)	24.98bc	51.47bc
	B_2 (10 ton/ha)	25.10bc	44.24bc
	B_3 (15 ton/ha)	23.82bcd	49.48bc
P_1= (OTM)	B_0 (Tanpa Bahan Organik)	21.97d	52.02bc
	B_1 (5 ton/ha)	24.80bc	49.32bc
	B_2 (10 ton/ha)	25.16bc	28.30bc
	B_3 (15 ton/ha)	25.57abc	52.42bc
P_2= (OTS)	B_0 (Tanpa Bahan Organik)	23.79bcd	26.16c
	B_1 (5 ton/ha)	24.12bc	27.26bc
	B_2 (10 ton/ha)	25.63ab	64.83ab
	B_3 (15 ton/ha)	27.21a	97.20a

Note: The mean number followed by the same letter in each column of the same treatment is not significantly different at the 5% level based on Duncan's distance test

Table 2, The increase in stem diameter has a significant effect on the management of mixed soil media with minimum tillage with organic matter 15 tons/ha, at 23 WAP (25.57 mm), while at 25 WAP (97.20 mm) the management of mixed tillage media was perfect. with 15 tons/ha organic matter and significantly different from the management of other growing media.

The lowest stem diameter was obtained in the management of soil media at a combination of minimum tillage without organic matter at 23 WAP and at perfect tillage and without organic matter at 25 WAP.

The relationship between media management without tillage and organic matter on the increase in stem diameter of cassava plants from Clones L-18 UKM is shown in Figure 2.



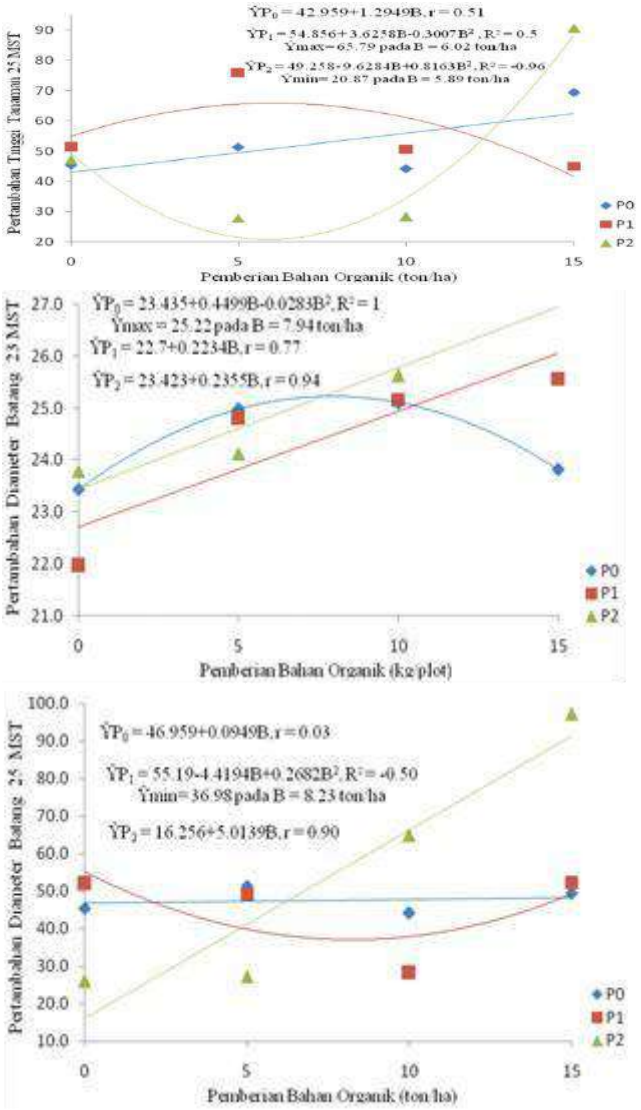


Fig.2. Increase in Stem Diameter (mm) Cassava Clones L-18 SMEs Age 23 and 25 WAP on Soil Media Management

Figure 2, The results of the graph show the management of growing media without tillage with the application of organic matter 7.94 tons/ha aged 23 WAP can maximize the increase in stem diameter (25.22 mm), and the management of growing media without minimum tillage and perfect tillage with the addition of organic matter 15 ton/ha can increase the increase in stem diameter to a certain extent.

While at the age of 25 WAP, there was a graphic change from positive quadratic to positive linear and almost horizontal, increasing the increase in stem diameter to a certain extent in the management of combined planting media without tillage and perfect tillage with the addition of 15 tons/ha of organic matter.

The increase in plant height was followed by an increase in stem diameter in the management of soil media combined with perfect tillage with the addition of 15 tons/ha of organic matter. Atman Roja, (2009) states that such soil conditions can ensure the circulation of O2 and CO2 in the soil, especially in the perfect tillage layer, so that the activity of microorganisms and root function is more optimal, due to the availability of plant nutrient needs.

Table 3. Average Number of Leaves (strands) of Cassava Clones L-18 Age 23 and 25 WAP on Soil Media Management

Olah Tanah	Bahan Organik	Pertambahan Jumlah Daun Pengamatan (MST) 23	25
		.... helai	
P_0 = (TOT)	B_0 (Tanpa Bahan Organik)	23.43cd	35.49b
	B_1 (5 ton/ha)	24.98bcd	37.47b
	B_2 (10 ton/ha)	25.10bcd	38.24b
	B_3 (15 ton/ha)	24.82bcd	38.48b
P_1 = (OTM)	B_0 (Tanpa Bahan Organik)	24.37bcd	32.68b
	B_1 (5 ton/ha)	24.47bcd	36.65b
	B_2 (10 ton/ha)	24.50bcd	40.52b
	B_3 (15 ton/ha)	27.46a	41.42b
P_2 = (OTS)	B_0 (Tanpa Bahan Organik)	23.22d	26.16b
	B_1 (5 ton/ha)	25.48bcd	27.83b
	B_2 (10 ton/ha)	25.63b	27.26b
	B_3 (15 ton/ha)	28.24a	59.30a

Note: The mean number followed by the same letter in each column of the same treatment is not significantly different at the 5% level based on Duncan's distance test

Table 3, The increase in the number of leaves had a significant effect on the management of minimum tillage plant media with the application of organic matter 15 tons/ha at 23 WAP (27.46 strands) significantly different from the management of perfect tillage plant media with the addition of 15 tons/ha organic matter and significantly different with the others. Furthermore, at the age of 25 WAP, the number of leaves increased to (59.30 strands) in the management of planting media with a combination of perfect tillage with the addition of 15 tons/ha of organic matter and significantly different from other combinations of planting media management. The lowest increase in the number of leaves at the age of 23.25 WAP was obtained in perfect tillage without organic matter.

The relationship between the combination of planting media management and the provision of organic matter on the number of leaves of the cassava plant clone L-18 UKM can be seen in Figure 3.

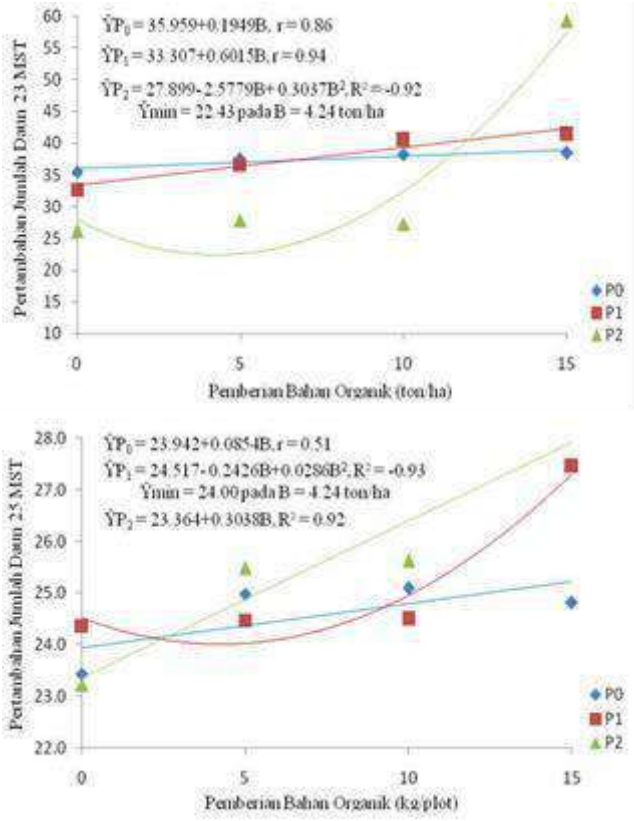


Fig.3: Increase in the number of leaves (strands) of cassava clones L-18 UKM Age 23 and 25 WAP on Soil Media Management

Figure 3 shows an increase in the number of leaves increased in the management of combined planting media without tillage, minimum tillage with the addition of 15 tons of organic matter/ha and perfect tillage, the increase in the number of leaves was low (22.43) strands with the addition of organic matter 4.24 tons/ha aged 23 MST. The management of planting media without tillage and perfect tillage with the addition of 15 tons/ha of organic matter at the age of 25 WAP increased the number of leaves to a certain extent (positive linear) and a negative quadratic (decreased) occurred in the increase in the number of leaves, namely (24.00 strands) in tillage. minimum soil with organic matter application of 4.24 tons/ha.

Minimum and perfect tillage with the addition of 15 tons/ha of organic matter can improve soil physical and chemical properties to obtain optimum plant growth and development with soil carrying capacity. (YuliWidyastuti and SugengSugiarso, 2003).

The availability of nutrients and water in the planting medium will increase the component of increasing the number of leaves. The higher the number of leaves, the higher the sunlight reception and the increased photosynthesis. Increased photosynthesis followed by increased respiration will cause metabolic processes to take place better and will support plant growth and development (Ruiz-Lozano et al, 2000 in Girsang, 1999).

Table 4. Average Leaf Stem Length (cm) Cassava Clone L-18 Age 23 WAP on Soil Media Management

Olah Tanah	Bahan Organik	Panjang Tangkai Daun Pengamatan (MST) 23
		.... cm ....
P_0 = (TOT)	B_0 (Tanpa Bahan Organik)	45.49b
	B_1 (5 ton/ha)	51.47b
	B_2 (10 ton/ha)	44.24b
	B_3 (15 ton/ha)	49.48b
P_1 = (OTM)	B_0 (Tanpa Bahan Organik)	54.68b
	B_1 (5 ton/ha)	49.32b
	B_2 (10 ton/ha)	57.19b
	B_3 (15 ton/ha)	52.42b
P_2 = (OTS)	B_0 (Tanpa Bahan Organik)	41.16b
	B_1 (5 ton/ha)	37.83b
	B_2 (10 ton/ha)	51.63b
	B_3 (15 ton/ha)	78.93a

Note: The mean number followed by the same letter in each column of the same treatment is not significantly different at the 5% level based on Duncan's distance test

Table 4, The length of the longest petiole has a significant effect on the management of planting media with a combination of perfect tillage with the addition of 15 tons/ha organic matter at 23 WAP (78.93 cm) and significantly different from the management of other growing media. The shortest petiole length was obtained in the management of planting media with a combination of perfect tillage with the addition of 5 tons of organic matter/ha and it was not significantly different from the management of other growing media.

The relationship between the combination of planting media management and the application of organic matter to the leaf stalk length of cassava clones L-18 UKM can be seen in Figure 4.

Figure 4, Observation of 23 WAP the length of the petiole decreased or minimum (37.54 cm) in the management of planting media with a combination of perfect tillage with organic matter 3.34ton/ha, however, in the management of growing media without tillage, and minimum tillage when combined with the addition of 15 tons/ha of organic matter, the leaf stalk length still increases to a certain extent.

Sarno (2006) and Simatupang (2008), state that conservation tillage includes minimum tillage and perfect tillage is able to improve or maintain land productivity compared to no tillage followed by the provision of maximum organic matter which can increase the carrying capacity of the soil to produce plant growth (YuliWidyastuti and SugengSugiarso, 2003).

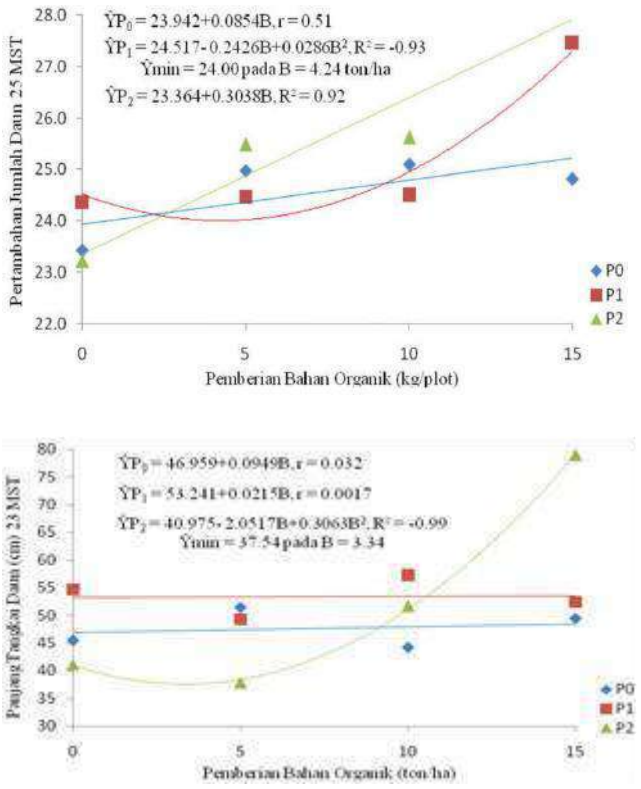


Fig.4: Average Leaf Stem Length (cm) Cassava Clone L-18 Age 23 WAP on Planting Media Management

V. CONCLUSION

1. Management of combined planting media without tillage with the addition of 7.94-ton/ha organic matter can increase the increase in stem diameter (mm), increase in number of leaves (strands), leaf stalk length (cm) of cassava plants Clones L-18 UKM with the addition of 15 tons of organic matter/ha.
2. Management of planting media combined with minimum tillage with the application of organic matter 6.02-7.10 tons/ha can increase the increase in plant height (cm), and increase in the number of leaves (m2) of cassava plants Clones L-18 SMEs.

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Identification and molecular characterization of groundnut leaf miner in Uganda

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Abstract— *Although the leaf miner attacking groundnut in Africa has been widely reported as Aproaerema modicella (Deventer), a common groundnut (Arachis hypogaea L.) and soya bean (Glycine max (L.) Merr.), a Pest in Indo-Asian countries, a proper taxonomic identification of the pest has not been completed. A survey for species diversity of the pest was conducted on groundnut, the common host crops for leafminer species in Uganda, across 100 groundnuts farmers' fields in four agro ecological zones during the 2016-2017 growing season. 80 specimens comprising 40 larvae, 25 pupae and 15 moths of what was thought to be A. modicella (all from groundnut) were collected from the ten survey sites, and their mitochondrial DNA (mtDNA) COI were sequenced and compared with those from the BOLD gene bank. Infestation by GLM was observed on all groundnut fields sampled with Eastern Uganda being the hot ecological spot especially Namutumba district. The mtDNA COI from all specimens of the pest, matched 100% with the sequences in BOLD belonging to Aproaerema simplexella PS1, a species occurring in Australia, and known as the soya bean moth in that country. There was very little genetic diversity between and within the populations from the ten sites, which suggested that the populations were maternally of the same origin. Conclusively, this study like other studies elsewhere in Africa confirm to the fact that the leafminer attacking groundnuts and other crops such as soya bean was A. simplexella PS1 (100% match on the BOLD system), native to Australia, which suggested that Australia may be the origin of the pest not Indo-Asian countries.*

Keywords— ***Arachis hypogaea, Aproaerema modicella, mitochondrial DNA.***

I. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the second most important legume after beans (Phaseolus *vulgaris)* in both nutritional and economic empowerment of communities in Uganda. It's grown primarily for high quality edible oil (36-54% on dry matter basis) and easily digestible proteins (20-50%) in its seeds (D. okello *et al*, 2010). It is cultivated worldwide in tropical, sub-tropical and warm temperature areas located between 400N to 400S with world production of 36.9 million tonnes from an area of 25.2 million ha. Africa accounts for 40% of the global area planted to groundnuts, with only 26% production. The highest average yields were observed in Southern Africa and the lowest in East Africa (ICRISAT 2012). In 2006, the average groundnut yield recorded in Sub Saharan Africa was 980 kg/ha, considerably less than the world average of 1,690 kg/ha (Bucheyeki *et al.* 2008). The production of these crops on the continent is seriously threatened by a leaf miner, widely reported as Aproaerema modicella (Deventer) (Lepidoptera: Gelechiidae) (Kenis and Cugala, 2006). First noticed as a serious pest on groundnut in 1998 in Uganda and described as groundnut

leaf miner (GLM), the pest has raised considerable alarm and concern in the groundnut production zones of Uganda (Epieru, 2004).

In Uganda, GLM is known to be present in all groundnut producing areas of the country (Epieru 2004). However, the epidemic of GLM on groundnut in Uganda is sporadic and its severity appears to vary from place to place and from year to year, making it extremely difficult to predict. As a new pest, not much information is available on its ecology and ecophysiology that might help to predict its incidence and outbreaks.

The identity of the GLM in Africa, including Uganda, has generally been assumed to be A. modicella (Subrahmanyam *et al.,* 2000; Page *et al.,* 2000; Du Plessis, 2002; Munyuli *et al.,* 2003; Epieru, 2004; Kenis and Cugala, 2006) although Shanower *et al.* (1993) has hinted that it might be a different species. Since no proper taxonomic identification has been done on this new pest with no/little information characterizing this pest in Uganda and hence this study, its more probable that adoption of the name A. modicella was based on morphological characteristics of the larvae and adults and on crop damage symptoms that are similar to those of A. modicella in addition to strong prevalence of the pest on groundnut elsewhere in the world (Du Plessis, 2002, 2003; Kenis and Cugala, 2006). Van der Walt et al. (2008) examined the gonads of the female and male larvae of the GLM specimens collected in South Africa, and concluded that they were similar to those reported for A. modicella in Asia by Shanower et al. (1993), which reinforced the assumption that the pest was A. modicella. Because of its sudden appearance, the GLM occurring in Africa is thought to be a recent invasion from the IndoAsian continent (Kenis and Cugala, 2006) where A. modicella is native and seriously infests groundnut and soybean (Shanower *et al.,* 1993). Whilst this is possible, the pest may have evolved and spread within Africa.

Whereas morphological studies have been the keystone of insect pest identification in the past, and continue to be in the present, modern molecular techniques offer complementary, faster and more precise options for species identification (Scheffer, 2000), and is especially useful in differentiating related species that share similar morphological characteristics.

In addition, molecular techniques, e.g. DNA finger printing, especially those involving the mitochondrial DNA (mtDNA), are more reliable in pinpointing or tracing the geographical origin/links of pests and their paths of spread, (Scheffer, 2000; Simmons and Scheffer, 2004). There were two objectives in the present study. The first objective was to identify the species of leafminer attacking groundnuts in Uganda.t and the second objective was to determine its inter and intra-population genetic diversity by analysing in both cases the mtDNA CO1 gene of specimens collected from widely separated sites.

II. MATERIALS AND METHODS

Sampling

Groundnut leaf miner samples (both adult and larvae) were collected from ten agro ecological zones in Uganda. All larvae were preserved in 70% ethanol then brought to the *icipe* Molecular Pathology Lab in the Arthropod Pathology Unit for processing. Morphological characteristics were documented using a Leica EZD stereomicroscope (Leica Microsystems (UK) Ltd) then the samples were immediately preserved in 95% ethanol and stored at – 20 °C for DNA extraction later.

DNA extraction, PCR and sequencing

Each individual insect sample was surface-sterilized using 3% NaOCl and rinsed three times with distilled water. Genomic DNA was extracted using the Isolate II genomic DNA Kit (Bioline, UK), following the manufacturer's instructions. The purity and concentration of the resultant extracted DNA was determined using Nanodrop 2000/2000c Spectrophotometer (Thermo Scientific). Polymerase chain reaction (PCR) was done to amplify the mitochondrial region and 2 sets of markers were used (Table 1). The PCR was carried out in a total reaction volume of 20 µL containing 5X My *Taq* Reaction Buffer (5 mM dNTPs, 15 mM $MgCl_2$, stabilizers and enhancers), 10 µmole of each primer, 0.5 mM $MgCl_2$, 0.25 µL My *Taq* DNA polymerase (Bioline, UK) and 15 ng/µL of DNA template. This reaction was set up in the Nexus Mastercycler gradient (Eppendorf). The following cycling conditions were used: initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 sec at 95 °C, 45 sec annealing and 1 min at 72 °C, then a final elongation step of 10 min at 72 °C. The target gene region was 700 base pairs.

Table 1: Primer information used in this assay

Name	Sequence5' - 3'	Target	Source	Range	Annealing Temp (°C)
LepF1	ATTCAACCAATCATAAAGATATTGG	COI	Hajibabaei *et al*., 2006	Insects	52
LepR1	TAAACTTCTGGATGTCCAAAAAATCA	COI			
LCO1490	GGTCAACAAATCATAAAGATATTGG	COI	Folmer *et al*., 1994	Insects	50.6
HCO2198	TAAACTTCAGGGTGACCAAAAAATA	COI			

The amplified PCR products were resolved through a 1.2% agarose gel. DNA bands on the gel were analyzed and documented using KETA GL imaging system trans-illuminator (Wealtec Corp). Successively amplified products were excised and purified using Isolate II PCR and Gel Kit (Bioline, UK) following the manufacturer's instructions. The purified samples were shipped to Macrogen Inc Europe Laboratory, the Netherlands, for bi-directional sequencing.

The successful sequences were assembled and edited using Chromas Lite Version 2.1.1 (Thompson *et al*., 1997) and Geneious Version 8 (http://www.geneious.com) (Kearse *et al*., 2012). The primer sequences were identified and removed from the consensus sequences generated from both the forward and reverse reads. Phylogenetic and molecular evolutionary analyses for all the sets of sequences was inferred using MEGA 6, version 6.06 (Tamura *et al*., 2013) by the Maximum Likelihood method. The reliability of the tree was assessed using 1000 bootstrap replications.

DNA amplification and sequencing

DNA amplification by PCR was performed with the primers Ron and Nancy. The PCR conditions were as follows: 1x KAPA Robust Ready Mix (KAPA Biotech), 1x Enhancer A, 0.4 µM of each primer and 20 ng DNA. The PCR was performed in a verity PCR-cycler (Applied Biosystems) with the following conditions: 95°C for 5 min followed by 40 cycles of 95°C at 30 s, 55°C at 60 s and 72°C for 90 s and a final extension of 72°C for 10 min. Post-PCR purification was done using the NucleoFast Purification System (Separations). Sequencing was performed with each primer and Big Dye Terminator V1.3 (Applied Biosystems) followed by electrophoresis on the 3730xl DNA Analyser (Applied Biosystems). Sequences were analyzed using the Sequencing Analysis Version 5.3.1 software (Applied Biosystems). Editing of DNA sequences DNA sequences were manually edited (for base calling errors) pruned and aligned by ClustalW using the Bio Edit Sequence Alignment Editor (Hall, 1999) to create consensus sequences which were saved in the fasta format in MEGA5 (Hall, 1999).

Additionally, all consensus sequences were entered in BOLD to positively identify species. All specimen were identified to be from the same species, except two sample, which was identified to be from a different species, and was therefore used as an outgroup in the analysis. Additionally, the sequences were also exposed to Multiple Sequence Alignment by ClustalW (http://www.genome.jp/tools/clustalw/) to verify level of similarity between samples.

III. RESULTS

Summary of the identities of the processed samples

For conclusive identification of the species, similarity analysis was done. Similarity searches were conducted by querying the consensus sequences via BLAST at the GenBank database hosted by National Centre of Biotechnology Information (NCBI). BLAST (Basic Local Alignment Search Tool) algorithm finds regions of local similarity between sequences, in which consensus sequences were compared to reference sequences in the GenBank database. In addition to this, query was also done in BOLD (Barcode of Life Database).

Table 2.

Phyllum	Class	Order	Family	Genus	Species	Specimen similarity (%)
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100

Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	98
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	Tuta absoluta	90
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	95
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	Tuta absoluta	90
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	97
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100

Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	89
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	99
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100
Antropoda	Insecta	Lepidoptera	Aproaerema	Gelechiidae	simplexella	100

Identification by mtDNA (CO1) Based on comparisons with published sequences from the BOLD genebank, two samples were identified as possibly Tuta absoluta (Lepidoptera: Gelechiidae) (90% match), but the remaining samples (78) were identified as Aproaerma simplexella PS1 (Walker) (Lepidoptera: Gelechiidae) (89 to 100% match).There was very little genetic diversity within and between the specimens from the ten surveyed sites (Table 1).

IV. DISCUSSION

There has been a general assumption that the GLM occurring on groundnut in Africa has its origins in Asia,

with all reports from the African continent assuming the name A. modicella (Deventer) for the pest (Du Plessis, 2002, 2003). Contrary to this assumption, the mtDNA CO1 sequences of the GLM specimens examined in this study matched 100% with those of A. simplexella PS1 (previously Stomopteryx subsecivella (Ziller) (Bailey, 2007). This particular strain of A. simplexella is thought to be native to Australia where it is reported to be a pest for soybean (Common, 1990; Bailey, 2007). The evidence obtained from DNA analysis in the present study therefore suggests that the GLM in Uganda may have links with Australia. This is further supported by the fact that all GLM specimens taken from the ten widely separated sites in Uganda were identified as A. simplexella PS1, with A. modicella not listed in the most closely related species (Table 1). This infers that all infestations of GLM in Africa may be caused by the former, and not the latter species. Based on morphological characteristics, Shanower *et al.* (1993) suggested that the species found in Africa may be different from that found in India or Indonesia, describing the GLM in India as Anacampsis nerteria (Meyr.) (Meyrick, 1906), the one in Africa as Stomopteryx subsecivella and another in India-Indonesia as A. modicella (Deventer). It is thus clear that a large degree of uncertainty has always existed as to the correct classification of GLM in Africa. No attempt has however been made to discriminate between the species genetically. Previous to our DNA analysis, A. simplexella PS1 was known to be present only in Australia with recent identification in South Africa.

V. CONCLUSION

Mitochondrial DNA COI analysis identified GLM in Uganda as A. simplexella PS1 (100% match on the BOLD system), native to Australia, which suggested that Australia may be the origin of the pest. It is most likely that GLM being reported on groundnut in other parts of Africa is also A. simplexella PS1. Secondly, the 100% match on the BOLD system indicated that there was very little genetic diversity between and within the populations, suggesting that the pest might be from the same origin and could be a recent introduction to Uganda. Given that the sequences of GLM in Uganda matched those of A. simplexella PS1 and that the damage symptoms of the pest on groundnut are similar to those of A. modicella found in Asia, there is a need to determine if the two species are indeed genetically different. This has a bearing on the development and use of groundnut lines that are resistant to GLM, in countries where it is a problem. For the purpose of formulating strategies for managing the pest, there is also a need to determine its correct identity, its host range as well as it's in between season survival tactics in Africa.

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Medicinal plant: *Garcinia spp.*

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Abstract*— *Garcinia is a tropical fruit tree with promising pharmacological properties. This review presents an overview of the bioactive compounds derivative from Garcinia fruits and their biological activities for promoting human health as food and medicine.

Keywords*— Medicinal plant, Garcinia spp., Therapeutic properties, phytochemical properties.

I. INTRODUCTION

Plants are considered as the great reservoir of structurally diverse bioactive molecules such as phenolics, terpenoids, carotenoids, anthocyanins and flavonoids which are having therapeutic values and are useful in the treatment of various ailments. Now-a-days these bioactive molecules are widely used in the food, pharmaceutical and cosmetics industries (Hosakatte *et al*., 2018).

Garcinia is a polygamous tropical tree or shrub under Clusiaceae family. It consists of 250 species, out of which about 30 species are indigenous to India. *Garcinia pedunculata* (Amlavethasa), *G. cowa*, and *G.Morella* (Indian gamboge) are grown in North-Eastern parts of India and Andaman Islands (Negi *et al*.,2008; Sharma and Devi,2015; Murthy *et al*., 2020). *Garcinia* are rich source of nutrients, minerals, vitamins, and dietary fibers. It has the folklore claims such as rejuvenator, cardio tonic, asthma, obesity and arthritis. The mature fruit is eaten cooked or raw and also for pickle preparation.

Garcinia pedunculata is an evergreen tree. The tree is endemic to the south eastern regions of Asia such as parts of Myanmar and North-Eastern parts of India. The tree has a fluted trunk with short spreading branches. Leaves are lanceolate with prominent mid ribs. Male flowers are light green in sparsely flowered panicles, the female flowers are solitary. The fruit is round with a diameter ranging between 8cm and 12cm. It has a juicy interior with edible arils. The mature *G. pedunculata* fruit is greenish yellow and is consumed as a vegetable.

II. MEDICINAL PROPERTIES OF GARCINIA

The fruits of *Garcinia* have been used since ancient times in traditional medicinal practices. These species provide a rich natural source of bioactive compounds with relevant therapeutic properties and anti-inflammatory effects, for the treatment of skin disorders, wounds, ulcers, dysentery, pain, infections, fever, cough, bronchitis, asthma, rheumatoid arthritis, obesity and having antioxidant, antiaflatoxigenic anti-inflammatory, leishmanicidal, and antiprotozoal activities (Joseph *et al*.,2005; Ali *et al*.,2017 ;Espirito *et al*.,2020).

III. PHYTOCHEMICAL PROPERTIES OF SOME SPECIES OF GARCINIA

Garcinia are rich sources of fiber, total phenols, and natural antioxidants with high amount of ascorbic acids .Extracts of the pericarp, epicarp, and seeds of *Garcinia* have demonstrated the phytochemicals such as pedunculol, garcinol, cambogin 3 and hydroxyl citric acid. Bennet and Lee (1989) and Rao *et al*.(1974;1980) have isolated the bioactive compounds namely benzoquinones, triterpenes and anthocyanins. Garcinol, being rich in derivates of poly-isoprenylated benzophenones, polyphenols, bioflavonoids (kolaviron, volkensiflavone, fukugetin) and xanthones (Sarma *et al*.,2016). Xanthones are the major class of phenolic compounds in *Garcinia* species, followed by benzophenones and biflavonoids. Xanthones have demonstrated effects against human cervical cancer, lung cancer cells, and hepatocellular carcinomas (Vo *et al*., 2015). These compounds have been associated with biological activities such as free-radical scavenging,

antiulcer effects, cytotoxicity, inhibition of nitric oxide synthase, chemoprevention of cancer, induction of apoptosis, anti-HIV, and trypanocidal effects (Hung *et al*.,2015; Fu *et al*.,2017). These compounds exhibiting a wide range of pharmacological activities such as antimicrobial, antioxidant, antitumour-promoting, cytotoxic, etc. (Jayaprakasha *et al*., 2006; Mundugaru *et al*., 2014;Adegoke *et al*.,1998; Asano *et al*.,1996; Bakana *et al*., 1987; Iinuma *et al*., 1996;Mackeen *et al*., 2000; Fu *et al*.,2014; Minami *et al*.,1994;1995;1996; Islam *et al*.,2015 ;Paul *et al*.,2017).Crude extracts as well as partially purified compounds from different parts of some species of *Garcinia* plants have shown potential antibacterial activities against *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*.

Some prominent *Garcinia* species are known to have good medicinal value and fruit extract has traditionally very well known for treatments of various diseases (Deore *et al*., 2011).

G. pedunculata was estimated to contain moisture 88.20%, protein 0.50%, *β* carotene 45.00mg/100 g, vitamins (thiamine 0.03, riboflavin 0.02, and ascorbic acid142.83mg/100 g, resp.), minerals (sodium 1.80, potassium106.00, calcium 18.00, magnesium 23.00, iron 0.08, zinc0.15, copper 0.12, and phosphorus 17.00mg/100 g), phenolics(19.45mg gallic acid/100 g), and flavonoids (18.33mg rutin/g).The dried fruit rinds and pericarp of *G. pedunculata* have been reported to contain some benzophenones, pedenculol, hydroxy citric acid, garcinol, and cambogin , some of which are strong antioxidants (Sahu *et al*., 1989; Mudoi *et al*.,2012; Ravi *et al*.,2014; Sarma *et al*.,2015; Mundugaru *et al*.,2019). This fruit extract is reported to possess a variety of pharmacological benefits including antimicrobial, anti-inflammatory, hepatoprotective, and cardioprotective properties (Kagyung *et al*., 2010; Mundugaru et al.,2014,2016; Ali *et al*., 2017). The aqueous extract of *Garcinia pedunculata*, exhibited significant neuroprotection against $AlCl_3$ induced neurotoxicity (Mundugaru *et al*., 2016, 2017).

In traditional system of medicine the leaves of *G. lancifolia* are used as stomachic and diuretic.The acidic fruits are used to prepare juice, pickle and curries. *G. lancifolia* is used as stomachic, diuretic and its fruit is used to cure dysentery and diarrhoea. The bark of *G. lanceifolia* has also been reported to contain prominent antibacterial and anthelmintic potential (Chowdhury and Handique, 2012;Bora *et al*.,2014a; 2014b). The phytochemical analysis of different extracts of *G. lancifolia* leaf, stem and fruit revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids and cardiac glycosides. The high phenolic content was observed in the methanol extract of leaf followed by methanol extract of stem and dichloromethane extract of leaf.

Antimicrobial and free radical scavenging xanthones from the latex of *G. cowa* (Mahabusarakam *et al*., 2005; Na Pattalung *et al*.,1994; Auranwiwat *et al*.,2014), and antimalarial xanthones (Likhitwitayawuid *et al*.,1998 ;) from the stem bark of *G. cowa* have been reported.

A polyisoprenylated benzophenone known as garcinol isolated from stem bark of *G. huillensis* has been shown to possess chemotherapeutical activity against Gram-positive and Gram negative cocci .

Alpha-mangostin, rubraxanthone and xanthochymol isolated from *G. mangostana*, *G. diocia* and *G. subelliptica*, respectively, showed strong antibacterial activity (Iinuma *et al*., 1996).

Crude extracts of leaves, fruits, root, stem and trunk bark of *G. atroviridis* exhibited antibacterial (Mackeen *et al*., 2000).

IV. INDUSTRIAL RELEVANCE

This traditional medicines are assuming greater important because of its effective, safer, locally available and no side effects and more reliable medicine than synthetically produced drugs. *Garcinia* extracts can be utilized as nutraceuticals and as food biopreservatives which could be developed into value added products or medicine (Acuna *et al*., 2012; Biswas *et al*., 2017). To produce potentially more active and safer drugs the plant-derived compounds should be isolated which could improve the economy of pharmaceutical industries.

V. CONCLUSION

Though the fruits of *G. cowa* and *G. pedunculata* are underutilized, recent year the interests in research activities in the fields of chemistry and pharmacology has arisen in exploiting on the fruit species. The advanced technology for isolation of the bioactive compounds from plants is very important as it could help in structural modifications of the synthetic products from the fruits. Based on the mechanism and mode of action of these plants it is confirmed the curative and therapeutic effectiveness of the plant. Hence, much research effort on crop improvement and physiologically active components is needed.

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The Effect of Some *Verbascum* Plant Extracts on Cytoplasmic Membrane of Multidrug Resistant Bacteria by Flow Cytometry

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Abstract**—***Verbascum species have been the focus of antioxidant and antimicrobial studies thanks to their secondary metabolites, especially saponins. While disk diffusion and dilution methods are generally used within the scope of antimicrobial studies, the Flow cytometry method is not well known. The ability of six Verbascum species to increase the permeability of multidrug resistant bacterial cells was conducted by flow cytometric assay on Listeria innocua and Escherichia coli. Fluorescence based flow cytometry is a technique for measuring characteristics of cells or biological particles and using flourescent dye as fluorogenic substrate provides. Propidium iodide (PI) is a fluorescent molecule and it can be used to stain cells. The SYTO dyes can be used to stain nucleic acids in both live and dead cells, as well as in Gram-positive and Gram-negative bacteria.*

Keywords**— Verbascum, flow cytometry, antibacterial, Lischeria Innocua, Escherichia Coli. Propidium iodide, SYTO.**

I. INTRODUCTION

The natural compounds also known as secondary found in medicinal plants belong to various chemical structures and some of these compounds have anticancer, antioxidant, and antimicrobial activity. However, little is known about the antibacterial drug resistance mechanisms of these compounds.**.** The *Verbascum* genus is known as "Mullein" has more than 2500 species in the world. This plant species in Turkey has about 233 units and 196 of these types indicate an endemic feature. Verbascum is a plant species used for a variety of medical studies in Turkey. Leaves and flowers of Verbascum species are used as an expectorant and diuretic as sweaty [1]. The antibacterial antioxidant, anticancer, antimalarial and cytotoxic activities of Verbascum species have been the subject of many studies. [1–5]. Mirjalili et al reported the antibacterial and antioxidant properties of *Verbascum* species belonging to various regions of Iran [4]. Soltaninejad and Akhgar have obtained a report on these components by isolating some secondary metabolites from a *Verbascum* genus. Biologically active components of plants of the *Verbascum* genus are saponins, iridoid, and phenylethanoid glycosides, neolignan and monoterpene glucosides, phenolic and fatty acids, spermine alkaloids and steroids, and flavonoids [6–10]. Since verbascum seeds contain saponin, they show a poisonous effect. For this reason, it is used especially in fishing on the Black Sea coast. In the same study, antimicrobial properties of three *Verbascum* species (*Verbascum Olympicum* Boiss., *Verbascum Prussianum* Boiss. And *Verbascum Bombyciferum* Bois) were reported. [11]. The antioxidant and phenolic structure of the extract of *Verbascum Glomeratum Linneus* species

was observed by HPLC [12]. Anticarcinogenic and antioxidant properties of Verbascum thapsus L. species have been reported in the literatüre. *Verbascum thapsus L*. leaf was extracted with different solvents, and its antibacterial activity was investigated. In addition, in these studies, it was stated that microbial biofilms are also very important in ensuring that microorganisms live in a complex structure. [13-16]. It has been reported that the leaves of Verbascum Thapsus have antibacterial properties against Escherichia Coli, Yersinia Pestis, Bacillus Cereus, Pseudomonas Aeruginosa, Listeria Monocytogenes and Staphylococcus Aureus bacteria. Similarly, it has been reported in the literature that the roots of Verbascum Undulatum also exhibit antibacterial properties. [17-18]. CuO nanoparticles synthesized from Verbascum thapsus leaves showed both photocatalytic and antibacterial activity against Gram-positive Staphylococcus aureus and drug-resistant Gram-negative Escherichia coli bacteria. Such approaches also help to develop new biofilm strategies from plants. [19-20]. The pharmacological properties of Verbascum species have been revealed in many studies such as those described above and took their place in the literature. In conclusion, Verbascum species have an important place in the treatment of diseases due to their antioxidant and antibacterial properties [16,21-23]. The pharmacological properties of *Verbascum* species have been revealed in many studies such as those described above and took their place in the literature. In conclusion, *Verbascum* species have an important place in the treatment of diseases due to their antioxidant, antibacterial and biofilm activity properties [3,16,21-26,33-40]. In this study, the antibacterial effects of six Verbascum species (*Verbascum tripolitanum, Verbascum sinuatum, Verbascum caesareum, Verbascum gaillardotti, Verbascum pinetorum* and *Verbascum antiochium*) against *Listeria innocua* and *Escherichia coli* bacteria were investigated by Flow cytometry.

II. MATERIALS AND METHOD

Plant Material

Verbascum plant samples (*Verbascum tripolitanum, Verbascum sinuatum, Verbascum caesareum, Verbascum gaillardotti, Verbascum pinetorum,* and *Verbascum antiochium)* were collected by Dr. Yelda Guzel. The voucher specimen is stored in the fungarium at the Biology Department of Mustafa Kemal University. *Verbascum tripolitanum* was collected from Yayladağ road, Şenköy pasture,in Hatay (Turkey). *Verbascum sinuatum L* was collected from Aşağıokçular, Antakya in Hatay (Turkey). *Verbascum caesareum* was collected from Musa Mountain, Çevlik-Arsuz, Antakya in Hatay (Turkey). *Verbascum antiochium* and *Verbascum gaillardotti* were collected from near St. Pierre Church,Antakya in Hatay (Turkey). *Verbascum pinetorum* was collected from Samandağ, Antakya in Hatay (Turkey).

Preparation of the methanol extracts

The plant sample, weighing about 100 g was extracted with methanol at 40-45ºC for 2 hours (3 times). The filtrates were combined and concentrated in vacuo at 45ºC. Finally, the extracts were then lyophilized and kept in the dark at 4ºC until tested.

Experimental Stage

Initially optimum conditions were determined. *Verbascum* concentrations were arranged 6 mg/ml-3mg/ml-1,5mg/ml in 10% dimethylsulfoxide. 200 µl of the amount of extract for added in bacterial culture. PI (propidium iodide) or Fluorophores SYTO 9 concentration was applied at 10% in PBS buffer in *Listeria Innocua* bacteria, while PI was applied as 10% SYTO undiluted in *Escherichia coli*. In the experiments for *Listeria Innocua* bacteria, 2.5 µL PI (diluted) and 2.5 µl SYTO (diluted) were applied in a 500 µl bacteria culture. Operations were carried out in a 1-hour time unit at 70° C. The incubation process is for positive control. In the negative control, 200 µl of 10% dimethylsulfoxide was added. This application was applied in a period of 25°C. 2.5 µl PI (diluted) and 2.5 µl SYTO (undiluted) was applied in 500 µL bacteria culture for *Escherichia coli*. The incubation process was applied in one hour at 70°C. This process is for positive control. In the negative control, 200 µl 10% dimethylsulfoxide was added. The incubation was carried out at 25°C for 1 hour. All these trials were performed by incubating 6 mg/ml-3mg/ml-1,5mg/ml 10% Dimethylsulfoxide at 25°C for 1 hour for *Listeria Innocua* and *Escherichia coli* bacteria.

III. RESULTS AND DISCUSSION

Taking positive and negative controls of six species of *Verbascum* plants *(Verbascum Tripolitanum, Verbascum Sinuatum L, Verbascum Caesareum, Verbascum Antiochium, Verbascum Gaillardotti, and Verbascum Pinetorum)* against *Listeria Innocua* and *Escherichia Coli* bacteria, their antibacterial efficacy was demonstrated. After exposure incubated at 25°C for one hour, the herb extracts mediated staining of PI and SYTO 13 combined system were brighter and more uniform when the cell was treated at the increasing concentration. The negative and positive control of *Verbascum* species against *Listeria Innocua* bacteria is shown in figures (1, 3, 5, 7, 9 and 11) and the antibacterial activity of the extracts against

Listeria Innocua bacteria is shown as the percentage of dead cells in figures (2, 4, 6, 8, 10 and 12). As understood in all figures, all Verbascum species show antbacterial activity. When the results are examined, the right side shows live cells, while the left side shows dead cells. With reference to these figures, the lowest concentration of *Verbascum* species reveals that the number of viable cells is greater than the higher concentration (Figures 2-24, even-numbered figures). When the extract concentration in the bacterial culture reduced, the number of living cells increased.

Lischeria Innocua tests

Verbascum tripolitanum

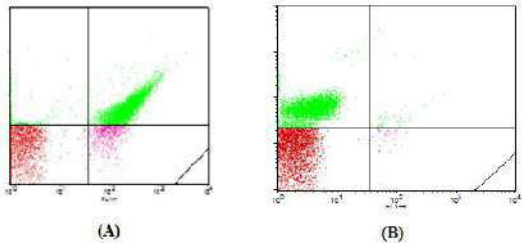

Fig.1: Negative (A) and positive (B) control of Listeria innocua

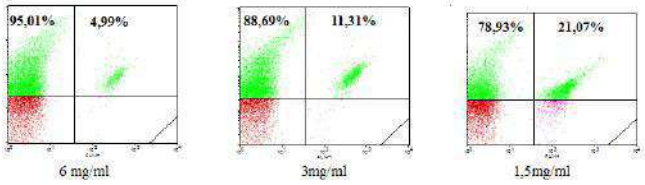

Fig.2: Percentage of dead cell in Listeria innocua bacterial culture.

According to Figure 2, when the drug concentration is 6 mg/mL, it is understood that 95.01% of the living *Listeria innocua* cells die and this ratio decreases as the concentration decreases. This ratio is 78.93% while the concentration is 1,5 mg/mL.

Verbascum sinuatum L

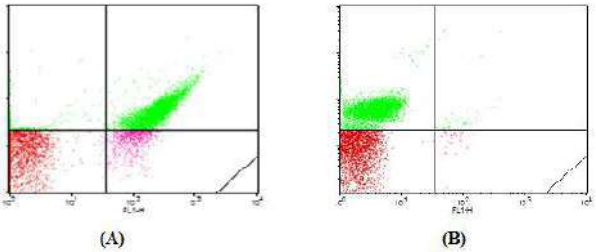

Fig.3: Negative (A) and positive (B) control of Listeria innocua

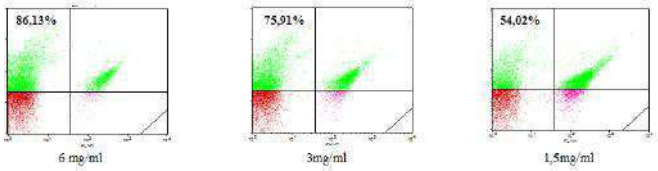

Fig.4: Percentage of dead cell in Listeria innocua bacterial culture.

According to Figure 4, when the drug concentration is 6 mg/mL, it is understood that 86,13% of the living *Listeria innocua* cells die and this ratio is 54,02% while the concentration is 1.5 mg/mL.

Verbascum caesareum

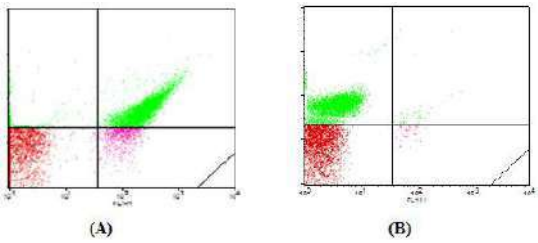

Fig.5: Negative (A) and positive (B) control of Listeria innocua.

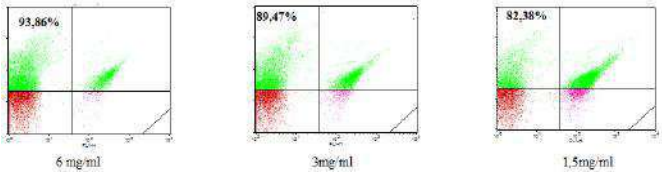

Fig. 6: Percentage of dead cell in Listeria innocua bacterial culture.

According to Figure 6, when the drug concentration is 6 mg/mL, it is understood that 93,86% of the living *Listeria innocua* cells die and this ratio is 82,38% while the concentration is 1.5 mg/mL.

Verbascum antiochium

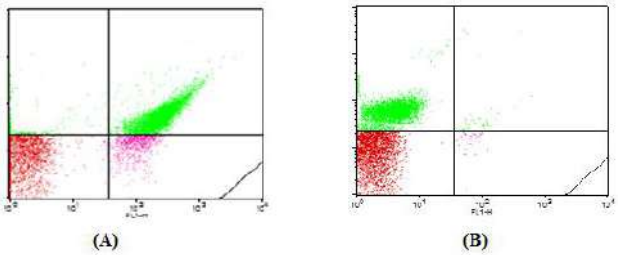

Fig.7: Negative (A) and positive (B) control of Listeria innocua

According to Figure 8, when the drug concentration is 6 mg/mL, it is understood that 93,15% of the living *Listeria*

innocua cells die and this ratio is 70,97% while the concentration is 1.5 mg/mL.

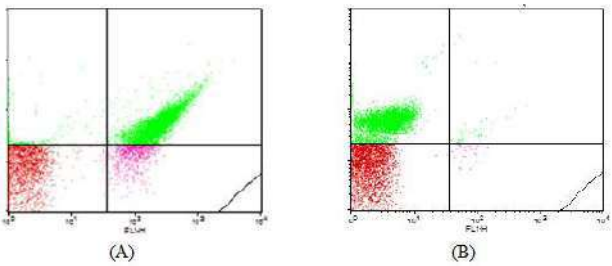

Fig.9. Negative (A) and positive (B) control of Listeria innocua

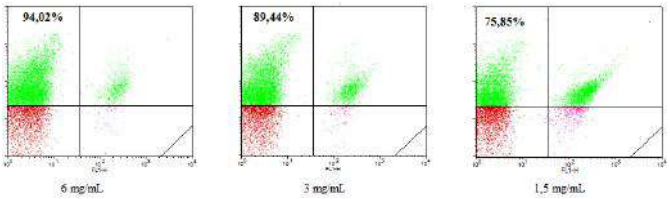

Fig.10. Percentage of dead cell in Listeria innocua bacterial culture

According to Figure 10, when the drug concentration is 6 mg/mL, it is understood that 94,02% of the living *Listeria innocua* cells die and this ratio is 75,85% while the concentration is 1.5 mg/mL.

Verbascum pinetorum

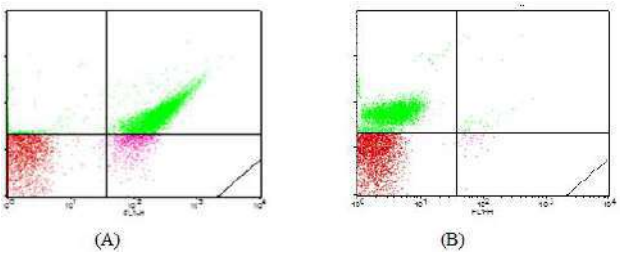

Fig.11: Negative (A) and positive (B) control of Listeria innocua

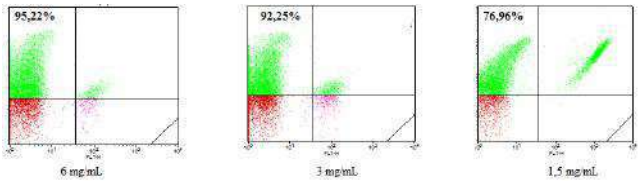

Fig.12: Percentage of dead cell in Listeria innocua bacterial culture.

According to Figure 12, when the drug concentration is 6 mg/mL, it is understood that 95,22% of the living *Listeria innocua* cells die and this ratio is 76,96% while the concentration is 1.5 mg/mL.

Table: Percentage of dead Listeria innocua bacteria cells for Verbascum species.

	6 mg/mL	3 mg/mL	1,5 mg/mL
V.tripolitanum	95,01	88,69	78,93
V.sinuatum L	86,13	75,91	54,02
V.caesareum	93,86	89,47	82,38
V.antiochium	93,15	89,33	70,97
V.gaillardotti	94,02	89,44	75.85
V.pinetorum	95,22	92,25	76,96

Escherichia coli tests

Verbascum tripolitanum

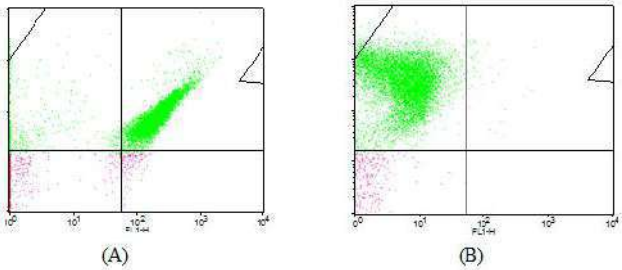

Fig.13: Negative (A) and positive (B) control of Escherichia coli.

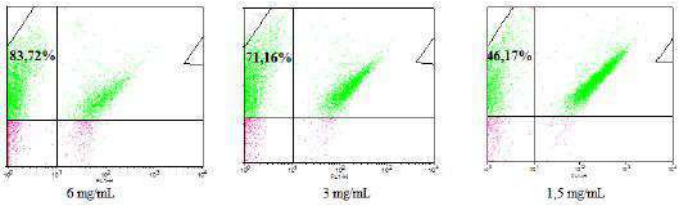

Fig.14: Percentage of dead cell in Escherichia coli bacterial culture.

According to Figure 14, when the drug concentration is 6 mg/mL, it is understood that 83,72% of the living *Escherichia coli* cells die and this ratio is 46,17% while the concentration is 1.5 mg/mL

Verbascum sinuatum L

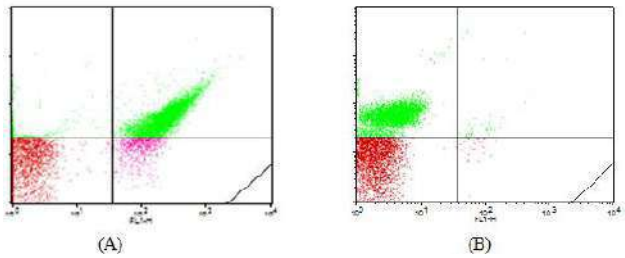

Fig.15: Negative (A) and positive (B) control of Escherichia coli

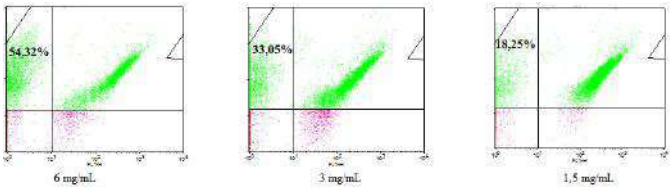

Fig.16: Percentage of dead cell in Escherichia coli bacterial culture

According to Figure 16, when the drug concentration is 6 mg/mL, it is understood that 54,32% of the living *Escherichia coli* cells die and this ratio is 18,25% while the concentration is 1.5 mg/mL.

Verbascum caesareum

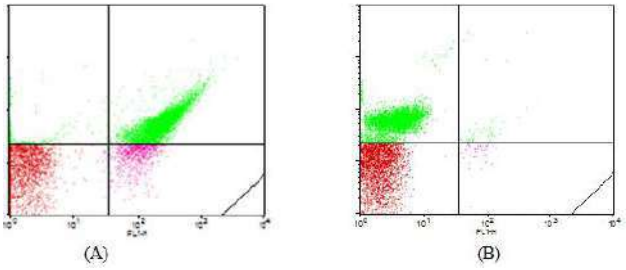

Fig.17. Negative (A) and positive (B) control of Escherichia coli

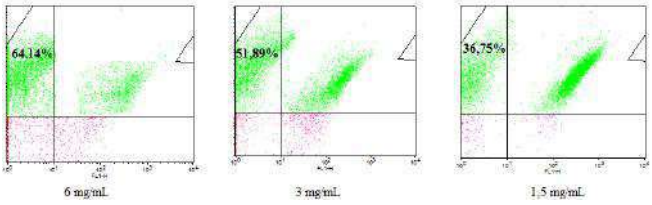

Fig.18: Percentage of dead cell in Escherichia coli bacterial culture

According to Figure 14, when the drug concentration is 6 mg/mL, it is understood that 64,14% of the living *Escherichia coli* cells die and this ratio is 36,75% while the concentration is 1.5 mg/mL.

Verbascum antiochium

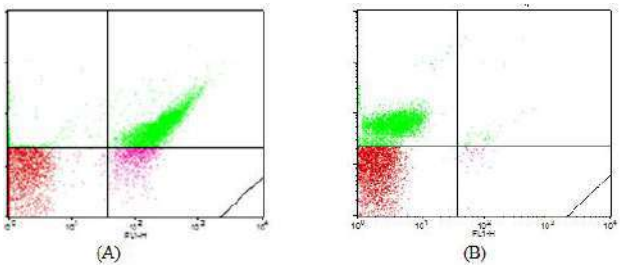

Fig.19: Negative (A) and positive (B) control of Escherichia coli

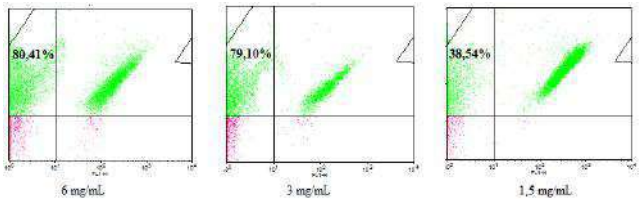

Fig.20. Percentage of dead cell in Escherichia coli bacterial culture.

According to Figure 20, when the drug concentration is 6 mg/mL, it is understood that 80,41% of the living *Escherichia coli* cells die and this ratio is 38,54% while the concentration is 1.5 mg/mL.

Verbascum gaillardotti

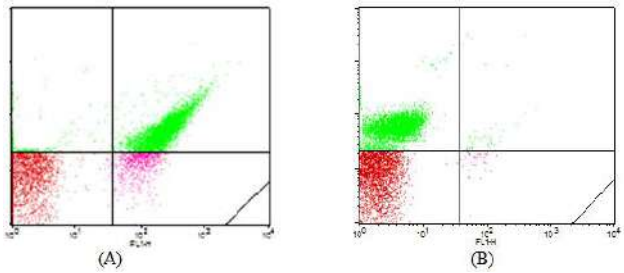

Fig.21: Negative (A) and positive (B) control of Escherichia coli

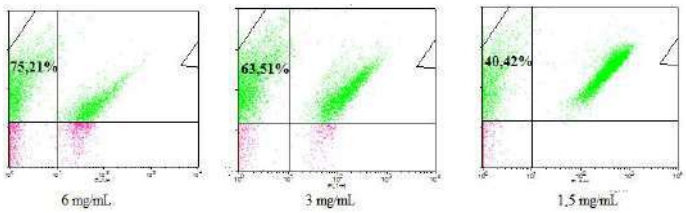

Fig.22: Percentage of dead cell in Escherichia coli bacterial culture

According to Figure 22, when the drug concentration is 6 mg/mL, it is understood that 75,21% of the living *Escherichia coli* cells die and this ratio is 40,42% while the concentration is 1.5 mg/mL.

Verbascum pinetorum

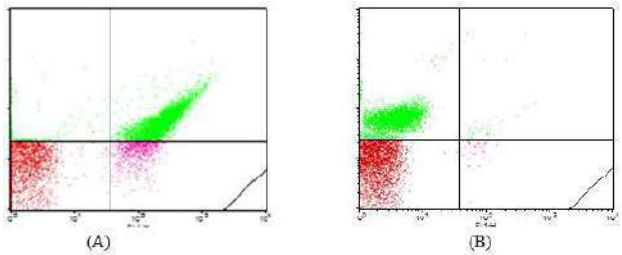

Fig.23: Negative (A) and positive (B) control of Escherichia coli

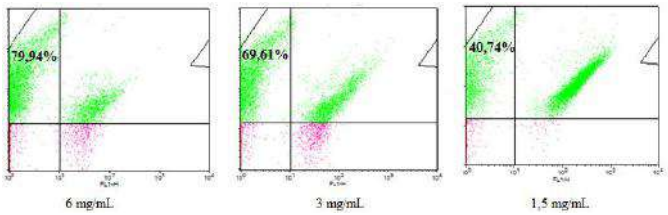

Fig.24: Percentage of dead cell in Escherichia coli bacterial culture

According to Figure 24, when the drug concentration is 6 mg/mL, it is understood that 79,94% of the living *Escherichia coli* cells die and this ratio is 40,74% while the concentration is 1.5 mg/mL.

Table 2: Percentage of dead Escherichia coli bacteria cells for Verbascum species

	6 g/mL	3 mg/mL	1,5 mg/mL
V. ripolitanum	83,72	71,16	46,17
V. sinuatum L	54,32	33,05	18,25
V.caesareum	64.14	51,89	36,75
V.antiochium	80,41	79,10	38.54
V.gaillardotti	75,21	63,51	40,42
V.pinetorum	79,94	69,61	40,74

Verbascum species studied in this study showed antibacterial effects for both *Listeria innocua* and *Escherichia coli*. However, when looking at the results given in Table 1 and Table 2, its resistance against *Listeria innocua* is higher than that of *Escherichia coli*. While *Verbascum pinetorum (*95,22%) showed the antibacterial effect against *Listeria innocua* at the highest concentration, it showed the lowest effect against *Verbascum sinuatum L. (*86,13%). While *Verbascum tripolitanum* (83,72%) showed the antibacterial effect against *Escherichia coli* at the highest concentration, it showed the lowest effect against *Verbascum sinuatum L*. (54,32%). *Verbascum sinuatum* showed the least resistant effect on both bacterial species.

IV. CONCLUSION

It can be said that the *Verbascum* species extract may disrupt the membrane barrier and allow exogeneous solutes such as PI and SYTO 13 to permeabilize into the bacterial cells. Secondary metabolites in the *Verbascum* species may have the ability to increase the permeability of bacterial membranes and allow antibiotics to access the bacterial targets. These results show that the flow cytometry method is more advantageous than traditional methods, such as disk diffusion and mikro dilution, for quickly generating a large amount of data. This technique can serve as a powerful tool in optimally combining different preservative factors in order to design an effective antimicrobial system for selected foods. As a result, This studyshows that methanolic extracts of *Verbascum* species is a potential source of natural antioxidants and antimicrobial agent and can form the basis for pharmacological studies.

ACKNOWLEDGMENTS

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Effect of *Ulva lactuca* L. Seaweed Biostimulant on Seed germination, Growth, and some Biochemical properties of *Vigna radiata* L.

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Abstract— *The effect of green marine biostimulant Ulva lactuca evaluated as Biofertilizer to improve growth of Vigna radiata L. Seeds waspresoaked with different concentration seaweed extract of Ulva lactuca such as 0.2%, 0.5%, 0.7%, 1.0%, 1.5%, 2.0% and control (without treatment) in different time period such as 0h, 1h, 2h, 3h, 4h, 5h, 6h, 12h and 24h. In Petri dish, after 5 days plant growth parameter like % seed germination, root length, shoot length, seedling length, seed vigour index, seed stamina index, No. of lateral roots, wet weight, dry weight, % moisture contentwere observed. After 40, 50 and 60 days some growth parameter like No. of branches per plant, No. of leaves per plant, total height of the plant, No. of flower per plant, No. of pod per plant, length of the pod, No. of seed per pod and leaf area were observed in field trial. In this study, after 50 days Bio-chemical parameter of chlorophyll-a, chlorophyll-b and total chlorophyll, carotenoid, protein, total soluble sugar and reducing sugar measured in plant material. Better result of plant growth observed in higher concentration such as 1.5% & 2.0% of seaweed extract of U. lactuca in Vigna radiata L. and bio-chemical parameter observed in lower concentration such as 0.2% and 0.7%of seaweed extract, respectively. Some biochemical parameter like protein,reducing sugar and carotenoid were decrease the amount of concentration compared to control.*

Keywords— ***Bio-chemical parameter, Growth parameters, Ulva lactuca, Vigna radiata.***

I. INTRODUCTION

India has a huge coastline of more than 7000km which possesses a large diversity of marine seaweeds. India, the Gujarat state covers a total coastline of 1600km, which is the longest among all the states of the country, and it covers almost 21.9% of the Indian coastline of India. Port Okha coast, which is known for its luxuriant growth of a diverse assemblage of seaweeds on Saurashtra coast, is found to have abundant quantities of seaweeds being drifted and washed ashore every year. Port Okha is located at 22º28' N latitude and 69º05' E longitude and is situated within the opening of Gulf of Kachchh, has a flat rocky intertidal belt, with many tide pools, gullies and crevices. Marine algae found attached to the bottom on solid substrates such as rocks, pebbles, shells, dead corals and plants. Seaweed has great importance, rich in vitamins, minerals, proteins, carbohydrates, amino acids, lipids etc. Due to the richness of bioactive constituents, it revealed many applications such as antioxidant, anticancer, antimalarial, pharmacological and cosmeceutical, activities. Approximately, 271 genera and 1153 species of marine algae, including forms and varieties have been enumerated till date from the Indian waters. Seaweed extracts are soluble, non-toxic and non-polluting extracts contribute to the uptake of more nutrients from the soil. Seaweeds extract as organic biostimulant is fast becoming accepted practice in agriculture and horticulture due to its beneficial effects (Battacharyya et al., 2015; du Jardin 2015; Khan et al., 2009). In market, seaweed extracts available for several years as fertilizer additives and beneficial result from their use have been reported (Booth, 1969). The biostimulant present in seaweed extract increase the vegetative growth (Di Filippo-Herrera et al., 2018; Briceno-Dominguez et

al., 2014; Basher et al., 2012; Demir et al., 2006), the leaf chlorophyll content (Vijayakumar et al., 2018; Castellanos-Barriga et al., 2017; Kalaivanan et al., 2012; Matysiak et al., 2011), the stomata density (Spinelli et al., 2010), photosynthetic rate and the fruit production of the plant (Spinelli et al., 2010; Sivasankari et al., 2006). Also the seaweed extracts increased levels of plant defense enzymes (Hernandez-Herrera et al., 2014; Raghavendra et al., 2007), and reduction of harmful seed microflora (Moller & Smith, 1999) and faster emergence and seedling vigour in several species including wheat (Kumar & sahoo, 2011), maize (Farooq et al., 2008), pepper (Sivritepe & Sivritepe 2008), faba bean (El-Sheekh& El-Saled, 2000), barley (Burchett et al., 1998), lettuce (Moller & Smith, 1998), green gram (Selvam, Balamurugan, Thinakaran & Sivakumar, 2013) and table beet (Wilczek& Ng, 1982). Successful application of pre-germination techniques in vegetable crop are remarkable since they increase seed germination, are easy to apply and non-risky as well as being environmental friendly. Seaweed extracts are reported to improve seed germination. Traditional priming procedures include hydropriming (seeds primed with water), osmopriming (soaking seeds in osmotic solutions like polyethylene glycol), halopriming (imbibing seeds in salt solution), thermopriming (management of seed with low or high temperatures), solid matrix priming (the agent solid matrices and water are mixed completely and then seeds are added) and biopriming (hydration by biological compounds). Each treatment has benefits and complications and may have variable effects depending upon the type of test, selection of crop, stage of plant development, method of application concentration/dose and duration of treatments (Ashraf &Foolad, 2005). One of the most important stages of plant life cycle is seed germination. Accelerating the seed germination is one of the important mechanisms on increasing the survival rate of plants. In addition, increasing seed germination rate subsequently increases the yield. Several studies have described the priming of seeds to enhance the germination rate and equal opportunity of growth thereby reducing the emergence time of many horticultural and agricultural crops (Basra et al., 2002; Lee & Kim, 1999; Brocklehurst & Dearman, 1983). Several researchers have used different types of soaking treatments of various crops seeds to increase the rate and uniformity of emergence and better establishment of seedlings (Basra et al., 2005; Ashraf et al., 2003; Bose & Mishra, 1999).The biopriming technique can also be carried out with macroalgae at low concentrations (parts per million) (Sharma et al., 2014). Muthuraman and Ranganathan (2004) selected six species of marine macro algae viz., Caulerpa scalpelliformis, Cladophora vagabunda, Enteromorpha compressa, Halimedamacroloba, Ulva faciata and Chaetomorphaantennina to investigate protein, amino acids, total sugars and lipid contents. Venkatesalu et al (2004).

II. MATERIAL & METHOD

2.1 Collection of Seaweed:

The green seaweed sample*Ulva lactuca* Linnaeus belonging to Chlorophyceae family were collected during 2020-2021 at Okha coast is situated at $22^0$28'7.3272 N & $69^0$4'11.3664 E in the "Gulf of Kutch" on the north-western most part of Saurashtra in Gujarat, India. After collection of seaweeds, its washed immediately with seawater to remove other debris, other epiphytes and sand particles. Then it was transferred in laboratory and washed thoroughly with simple water up to 3 to 4 times for removal of extra salts on the surface.

2.2 Preparation of Seaweed Liquid Fertilizer (SLF)

The seaweed of *Ulva lactuca* was shade dried followed by oven drying at 60^o C for 3-5 hours according to the species. The dried sample was grounded with blender to get fine powder and it was stored for future use. These fine powder 10g was weighed on electronic balance, 100ml distilled water was added. The mixture was incubated for One day (24h). Thereafter, the extract was filtered through What-man No. 1 filter paper (Bhosle et al. 1975). The collected filtrate was stored in refrigerator (0-20^{o}C). The obtained filtrate was considered as 100%. Six different concentrations of solutions such as 0.2%, 0.5%, 0.7%, 1.0%, 1.5% and 2.0% were prepared using this 100% extract and were used for future study.

2.3 Collection of Vegetable Seeds:

The *Vigna radiata* L. seed collected from Vasundhara Agro Agency, Bhavnagar, Gujarat during 2020-21.

2.4 Seed Soaking:

The seeds surface was sterilized with 0.1% $HgCl_2$ up to 1-2 minutes and washed help of distilled water. Seeds was presoaked with different concentration seaweed extract of *Ulva lactuca* such as 0.2%, 0.5%, 0.7%, 1.0%, 1.5%, 2.0% and control (without treatment) in different time period such as 0h, 1h, 2h, 3h, 4h, 5h, 6h, 12h and 24h at room temperature for better germination and early growth. After 5 days all the vegetative parameter was observed.

2.5 Seed Germination

Germination was observed daily over a period of 5 day in *Vigna radiata*L, with the methods of the Association of Official Seed Analysis (AOSA 2005). Three groups of 10

seeds were tested for germination per treatment (AOSA 2005). Tested *Vigna radiata* L. seeds were placed on a filter paper in sterilized 90-mm Petri dishes and then treated with 5 ml- 1 distilled water (Control) and different concentrations (0.2, 0.5, 0.7, 1.0, 1.5 and 2.0%) of Seaweed biostimulant. The plates were incubated at 25±1ºC and a photoperiod of 16h light/8h dark under laboratory. After 5 days measurement of growth parameter like germination percentage, root length, shoot length, seedling length, seed viguor index, seed stamina index, % Moisture content, No. of lateral roots.

2.6 Preparation field for further analysis:

All healthy and specific time selected *Vigna radiata* L. seed primed in each concentration of seaweed extract and sawing in enough space in each field. Water is sprinkle after sawing seed for maintaining the moisture content. Seaweed extract applied by soil drench method with different concentration with selected time duration. After 30 days, 40 days and 50 days measurement of growth parameter like No. of branches per plant, No. of leaves per plant, No. of flower per plant, No. of pod per plant, Total height of the plant (cm), No. of seed per pod, pod length (cm), Leaf area (cm^2).

2.7 Physicochemical Analysis of Seaweed Extract:

All Physicochemical parameters were analyzed by Handheld Multiparameter meters – Aquasol Digital (Testing Equipment) Model No. AM-AL-01

2.8 Biochemical Analysis:

After 50 days biochemical parameter of chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoidwere observed. Protein was determined by Lowry method, Total Soluble Sugar was determined by Anthrone meyhod, reducing sugar was determined by Nelson-Somogyi's method.

III. RESULTS

3.1 Germination and growth parameters of Vigna radiata L. seedling

Vigna radiata L. showed 80% germination in the control and 88.88% in the 1.0% and 2.0% seaweed biostimulant treated seeds. A minimum of 81.11% germination was observed at 0.2% seaweed biostimulant when compared to all other concentrations of treatment (Table 1 to 7). Germination occurred in all treatments in the 2nd day. The effect of seaweed extract showed significantly highest seed germination than control (Table 1 to 7). The maximum seedling length of 18.30cm was recorded when the plants applied with 1.5% of Seaweed biostimulants in 12h time duration (Table 5), and minimum seedling length was recorded in 0.2% (13.2cm) of Seaweed biostimulants in 2h time period (Table 1). A maximum total plant height of 28 cm was recorded applied with 2.0% (4h) of seaweed biostimulants and minimum in 1.5% (12h) (18cm). Also, the plants applied with 2.0% (4h) seaweed biostimulants increased the No. of branches, No. of leaves, length of the pod and leaf area. A maximum seed vigour index of 1830 in 1.5% seaweed biostimulants.

3.2 Physicochemical properties of seaweed extract

The values of P^H in *Ulva lactuca* 2.0% (Table 11) extract was slightly neutral and slightly basic in other concentration of seaweed extract. The value of Salt concentration (ppm) and EC (µs) continue increases in all concentration of seaweed extract. The value of ORP and TDS were fluctuating in all concentration of seaweed extract.

Table 1: Effect of Ulva lactuca Seaweed biostimulant (0.2%) on Vigna radiata L.

Time Period	Day- 5	Germination % (GP)	Root length (radicle length) (cm)	Shoot length(cm)	Seedling length (cm)	Seed Stamina Index (SSI)	Seed Vigour Index (SVI)	Freshweight(g)	Dry weight(g)	% Moisture content	No. of Lateral roots
0h	8	80	5.8±0.1	6.2±0.1	12.0±0.1	9.60±0.012	960±1.23	0.33±0.02	0.14±0.01	57.57	9
1h	9	90	7.0±0.2	4.2±0.1	11.2±0.2	10.08±0.12	1008±12.2	0.26±0.01	0.08±0.01	69.23	25

2h	10	100	6.6±0.2	6.6±0.1	13.2±0.1	13.20±0.23	1320±23.20	0.38±0.01	0.20±0.01	47.36	10
3h	5	50	6.2±0.1	9.0±0.1	15.2±0.1	7.60±0.013	760±1.35	0.37±0.02	0.22±0.02	40.54	20
4h	9	90	4.9±0.1	6.4±0.2	11.3±0.2	10.17±0.23	1017±23.12	0.38±0.01	0.21±0.01	44.73	11
5h	9	90	7.3±0.2	6.0±0.3	13.3±0.2	11.97±0.24	1197±24.61	0.36±0.01	0.20±0.01	44.44	20
6h	9	90	4.4±0.1	8.0±0.1	12.4±0.1	11.16±0.24	1116±24.11	0.35±0.02	0.18±0.02	48.57	16
12h	6	60	5.0±0.1	6.0±0.2	11.0±0.2	6.60±0.012	660±1.23	0.08±0.01	0.02±0.01	75.00	12
24h	8	80	3.2±0.2	10.0±0.1	13.2±0.1	10.56±0.22	1056±22.10	0.43±0.01	0.18±0.01	58.13	18

(Result=Mean±std)

Table 2: Effect of Ulva lactuca Seaweed biostimulant (0.5%) on Vigna radiata L.

Time Period	**Day- 5**	**Germination % (GP)**	**Root length(radicle length) (cm)**	**Shoot length(cm)**	**Seedling length (cm)**	**Seed Stamina Index (SSI)**	**Seed Vigour Index (SVI)**	**Fresh weight (g)**	**Dry weight(g)**	**% Moisture content**	**No. of Lateral roots**
0h	9	90	3.4±0.1	7.2±0.1	10.6±0.1	9.54±0.012	954±1.23	0.22±0.01	0.08±0.01	63.63	6
1h	9	90	7.6±0.1	4.2±0.2	11.8±0.1	10.62±0.12	1062±12.21	0.36±0.02	0.10±0.02	72.22	21
2h	10	100	9.0±0.2	5.0±0.2	14.0±0.2	14.00±0.23	1400±23.24	0.39±0.03	0.12±0.01	69.23	15
3h	8	80	6.4±0.1	2.9±0.1	9.3±0.1	7.44±0.023	744±2.31	0.18±0.04	0.06±0.01	66.66	14
4h	9	90	5.2±0.1	4.0±0.1	9.2±0.1	8.28±0.031	828±3.11	0.29±0.01	0.10±0.01	65.51	20
5h	8	80	2.2±0.2	2.0±0.1	4.2±0.2	3.36±0.011	336±1.10	0.29±0.03	0.09±0.006	68.96	13
6h	8	80	6.2±0.1	5.4±0.2	11.6±0.1	9.28±0.010	928±1.09	0.25±0.02	0.08±0.001	68.00	20
12h	7	70	4.2±0.2	13.0±0.1	17.2±0.2	12.04±0.25	1204±25.12	0.51±0.03	0.23±0.02	54.90	18
24h	8	80	5.9±0.1	7.2±0.1	13.1±0.1	10.48	1048	0.33	0.11	66.66	15

(Result=Mean±std)

Table 3: Effect of Ulva lactuca Seaweed biostimulant (0.7%) on Vigna radiata L.

Time Period	Day- 5	Germination % (GP)	Root length(radicle length) (cm)	Shoot length(cm)	Seedling length (cm)	Seed Stamina Index (SSI)	Seed Vigour Index (SVI)	Wet weight(g)	Dry weight(g)	% Moisture content	No. of Lateral roots
0h	8	80	6.6±0.1	7.2±0.1	13.8±0.1	11.04±0.12	1104±12.23	0.33±0.01	0.12±0.01	63.63	15
1h	7	70	5.2±0.1	4.2±0.1	9.4±0.1	6.58±0.021	658±2.14	0.24±0.02	0.09±0.01	62.50	15
2h	8	80	7.8±0.2	10.6±0.2	18.4±0.2	14.72±0.21	1472±21.14	0.66±0.01	0.33±0.02	50.00	16
3h	8	80	7.8±0.1	5.4±0.2	13.2±0.1	10.56±0.22	1056±22.31	0.32±0.01	0.15±0.01	53.12	6
4h	8	80	6.5±0.1	4.0±0.2	10.5±0.1	8.40±0.012	840±1.23	0.31±0.01	0.12±0.02	61.29	14
5h	9	90	6.5±0.1	6.0±0.1	12.5±0.2	11.25±0.23	1125±23.11	0.31±0.01	0.14±0.01	54.83	18
6h	9	90	6.0±0.2	10.0±0.1	16.0±0.2	14.40±0.11	1440±11.56	0.34±0.01	0.13±0.01	61.76	17
12h	9	90	6.2±0.2	6.0±0.1	12.2±0.1	10.98±0.32	1098±32.08	0.30±0.02	0.11±0.01	63.33	15
24h	9	90	7.0±0.1	6.0±0.2	13.0±0.1	11.70±0.24	1170±24.10	0.22±0.02	0.09±0.01	59.09	22

(Result=Mean±std)

Table 4: Effect of Ulva lactuca Seaweed biostimulant (1.0%) on Vigna radiata L.

Time Period	Day- 5	Germination % (GP)	Root length(radicle length) (cm)	Shoot length(cm)	Seedling length (cm)	Seed Stamina Index (SSI)	Seed Vigour Index (SVI)	Wet weight(g)	Dry weight(g)	% Moisture content	No. of Lateral roots
0h	10	100	5.4±0.1	4.4±0.1	9.8±0.1	0.98±0.012	980±1.23	0.12±0.01	0.06±0.01	50.00	12
1h	9	90	4.0±0.1	7.4±0.1	11.4±0.1	10.26±0.25	1026±25.10	0.24±0.02	0.09±0.01	62.50	15
2h	9	90	6.6±0.2	10.5±0.1	17.1±0.2	15.39±0.36	1539±36.21	0.42±0.01	0.21±0.01	50.00	16
3h	8	80	6.2±0.1	11.0±0.2	17.2±0.1	13.76±0.25	1376±25.10	0.45±0.01	0.22±0.01	51.11	13
4h	9	90	6.6±0.1	9.1±0.2	15.7±0.1	14.13±0.26	1413±26.12	0.35±0.01	0.16±0.01	54.28	10
5h	9	90	5.8±0.2	7.6±0.1	13.4±01	12.06±0.22	1206±22.21	0.20±0.02	0.08±0.02	60.00	16

6h	8	80	5.3±0.1	5.2±0.1	10.5±0.2	8.40±0.011	840±1.12	0.27±0.02	0.12±0.01	55.55	15
12h	9	90	8.0±0.2	9.2±0.1	17.2±0.1	15.48±0.28	1548±28.21	0.48±0.02	0.24±0.01	50.00	13
24h	9	90	4.0±0.1	4.6±0.1	8.6±0.1	7.74±0.010	774±1.09	0.17±0.01	0.08±0.01	52.94	9

(Result=Mean±std)

Table 5: Effect of Ulva lactuca Seaweed biostimulant (1.5%) on Vigna radiata L.

Time Period	**Day- 5**	**Germination % (GP)**	**Root length(radicle length) (cm)**	**Shoot length(cm)**	**Seedling length (cm)**	**Seed Stamina Index (SSI)**	**Seed Vigour Index (SVI)**	**Wet weight(g)**	**Dry weight(g)**	**% Moisture content**	**No. of Lateral roots**
0h	10	100	4.6±0.1	3.0±0.1	7.6±0.1	7.60±0.012	760±1.23	0.13±0.02	0.03±0.01	76.92	13
1h	9	90	4.2±0.1	1.4±0.1	5.6±0.1	5.04±0.032	504±3.21	0.42±0.01	0.21±0.01	50.00	8
2h	9	90	4.0±0.1	3.8±0.1	7.8±0.1	7.02±0.024	702±2.41	0.09±0.01	0.03±0.01	66.66	9
3h	10	100	3.9±0.2	9.0±0.2	12.9±0.2	12.90±0.15	1290±15.23	0.29±0.02	0.12±0.01	58.62	8
4h	9	90	6.2±0.1	6.4±0.2	12.6±0.1	11.34±0.23	1134±23.11	0.27±0.02	0.14±0.01	48.14	9
5h	8	80	3.9±0.2	8.2±0.1	12.1±0.2	9.68±0.031	968±3.10	0.26±0.01	0.09±0.01	65.38	16
6h	6	60	7.5±0.1	11.0±0.1	18.5±0.1	11.10±0.25	1110±25.09	0.28±0.01	0.10±0.01	64.28	19
12h	10	100	7.5±0.1	10.8±0.1	18.3±0.2	18.30±0.12	1830±12.21	0.38±0.02	0.16±0.01	57.89	19
24h	8	80	6.3±0.1	8.0±0.1	14.3±0.1	11.44±0.20	1144±20.08	0.22±0.01	0.10±0.01	54.54	15

(Result=Mean±std)

Table 6: Effect of Ulva lactuca Seaweed biostimulants (2.0%) on Vigna radiata L.

Time Period	**Day-5**	**Germination % (GP)**	**Root length(radicle length) (cm)**	**Shoot length(cm)**	**Seedling length (cm)**	**Seed Stamina Index (SSI)**	**Seed Vigour Index (SVI)**	**Wet weight (g)**	**Dry weight(g)**	**% Moisture content**	**No. of Lateral roots**
0h	7	70	5.7±0.1	6.8±0.1	12.5±0.1	8.75±0.023	875±2.31	0.27±0.01	0.1±0.01	62.96	7
1h	8	80	4.2±0.1	2.8±0.1	7.0±0.1	5.60±0.052	560±5.21	0.1±0.01	0.04±00.2	60.0	10

2h	10	100	5.8±0.2	8.2±0.2	14.0±0.1	14.00±0.32	1400±32.10	0.3±0.02	0.01±0.1	96.66	17
3h	9	90	4.1±0.2	5.2±0.1	9.3±0.2	8.37±0.041	837±4.11	0.27±0.01	0.1±0.01	62.96	8
4h	9	90	5.4±0.1	6.6±0.1	12.0±0.1	10.80±0.25	1080±25.56	0.24±0.02	0.1±0.01	58.33	18
5h	6	60	6.2±0.1	4.0±0.2	10.2±0.1	6.12±0.061	612±6.10	0.09±0.02	0.006±0.001	93.33	15
6h	8	80	5.6±0.2	2.4±0.2	8.0±0.2	6.40±0.071	640±7.12	0.2±0.01	0.08±0.01	60.0	11
12h	9	90	4.6±0.1	5.0±0.2	9.6±0.2	8.64±0.091	864±9.12	0.17±0.01	0.09±0.01	47.05	9
24h	6	60	5.2±0.1	2.4±0.1	7.6±0.1	4.56±0.051	456±5.14	0.16±0.01	0.06±0.01	62.5	7

(Result=Mean±std)

Table 7: Control plant on Vigna radiata L.

Time Period	**Day- 5**	**Germination % (GP)**	**Root length(radicle length) (cm)**	**Shoot length(cm)**	**Seedling length (cm)**	**Seed Stamina Index (SSI)**	**Seed Vigour Index (SVI)**	**Wet weight(g)**	**Dry weight(g)**	**% Moisture content**	**No. of Lateral roots**
0h	**6**	**60**	**1.6±0.1**	**6.6±0.1**	**8.2±0.1**	**4.92±0.021**	**492±2.11**	**0.36±0.01**	**0.16±0.01**	**55.55**	**10**
1h	**9**	**90**	**5.4±0.1**	**6.0±0.1**	**11.4±0.1**	**10.26±0.23**	**1026±23.11**	**0.18±0.01**	**0.09±0.02**	**50.00**	**16**
2h	**9**	**90**	**6.1±0.1**	**6.8±0.1**	**12.9±0.1**	**11.61±0.21**	**1161±21.10**	**0.20±0.01**	**0.10±0.01**	**50.00**	**12**
3h	**9**	**90**	**6.8±0.2**	**6.1±0.2**	**12.9±0.2**	**11.61±0.20**	**1161±20.15**	**0.28±0.02**	**0.12±0.02**	**57.14**	**21**
4h	**10**	**100**	**6.4±0.1**	**8.0±0.2**	**14.4±0.1**	**14.40±0.18**	**1440±18.41**	**0.38±0.02**	**0.18±0.01**	**52.63**	**16**
5h	**10**	**100**	**5.4±0.2**	**8.2±0.1**	**13.6±0.1**	**13.60±0.24**	**1360±24.36**	**0.26±0.01**	**0.13±0.02**	**50.00**	**12**
6h	**9**	**90**	**8.3±0.1**	**9.8±0.2**	**18.1±0.2**	**16.29±0.30**	**1629±30.11**	**0.49±0.02**	**0.23±0.01**	**53.06**	**16**
12h	**10**	**100**	**6.6±0.1**	**4.8±0.2**	**11.4±0.2**	**11.40±0.20**	**1140±20.14**	**0.28±0.01**	**0.13±0.01**	**53.57**	**17**
24h	**8**	**80**	**5.2±0.1**	**8.6±0.1**	**13.8±0.1**	**11.04±0.10**	**1104±10.25**	**0.25±0.01**	**0.11±0.01**	**56.00**	**17**

(Result=Mean±std)

Table 8: Treatment of Ulva lactuca on Vigna radiata L. (After 40 days)

Concentration	No. of branches per plant	No. of leaves per plant	Height of the plant (cm)	No. of flower per plant	No. of pod per plant	No. of seed per pod	Length of the pod (cm)	Leaf area (cm^2)
0.2% (2h)	4	14	21	3	----	----	----	12.0
0.5% (2h)	4	14	22	2	----	----	----	10.5
0.7% (6h)	5	10	20	1	1	----	2	10.0
1.0% (12h)	4	10	23	1	1	----	2	9.0
1.5% (12h)	4	11	20	----	1	----	4	8.5
2.0% (4h)	7	16	24	----	1	----	2	13.0
Control (2h)	5	14	15	----	----	----	----	12.5

(Result=Mean)

Table 9: Treatment of Ulva lactuca on Vigna radiata L. (After 50 days)

Concentration	No. of branches per plant	No. of leaves per plant	Height of the plant (cm)	No. of flower per plant	No. of pod per plant	No. of seed per pod	Length of the pod (cm)	Leaf area (cm^2)
0.2%	4	14	22	----	2	6	7	12.5
0.5%	5	14	23	----	2	4	5.5	11.5
0.7%	6	14	27	----	2	8	8	10.0
1.0%	5	11	23	----	2	2	5.6	9.0
1.5%	4	11	20	----	1	3	6	8.5
2.0%	7	16	26	----	2	5	7.6	13.5
Control	5	17	17	----	3	4	7	12.5

(Result=Mean)

Table 10: Treatment of Ulva lactuca on Vigna radiata L. (After 60 days)

Concentration	No. of branches per plant	No. of leaves per plant	Height of the plant (cm)	No. of flower per plant	No. of pod per plant	No. of seed per pod	Length of the pod (cm)	Leaf area (cm^2)
0.2%	6	14	23	4	1	----	2	12.5
0.5%	6	14	24	2	----	----	----	11.5
0.7%	6	12	24	1	4	----	1.2	10.0
1.0%	5	11	24	2	----	----	----	9.0
1.5%	5	11	18	1	----	----	----	8.5
2.0%	7	17	28	3	1	----	0.8	13.5
Control	7	15	17	2	----	----	----	12.5

(Result=Mean)

Table 11: Physicochemical analysis of Ulva lactuca L. extract at different concentration

Name	Salt Conc. (ppm)	P^H	ORP (mV)	COND (µs)	TDS (ppm)	Temp (°C)
Ulva lactuca (0.2%)	125	8.96	-97.6	260	168	26.6
Ulva lactuca (0.5%)	167	7.70	-20.6	346	227	26.3
Ulva lactuca (0.7%)	150	7.79	-27.6	302	198	26.2
Ulva lactuc (1.0%)	187	7.68	-19.8	383	252	26.3
Ulva lactuca (1.5%)	216	7.94	-34.4	442	289	26.8
Ulva lactuca (2.0%)	230	7.62	-20.7	474	312	27.1
Control	----	7.0	----	0	----	----

3.3 Biochemical properties of *Vigna radiata* L. treated with seaweed extract *Ulva lactuca*

Maximum concentration of 0.2371mg/g dry weight of chlorophyll a, 0.5491mg/gm dry weight of chlorophyll b and 0.5451mg/gm dry weight of total chlorophyll was recorded at 0.5% of *U. lactuca* seaweed extract treatment (Figure 1). Their decreases more than 43%, 54.87% and 53.47% respectively, when compared to control. Carotenoid also continue decreases amount of all seaweed extract concentration (Figure 2). The maximum concentration of 3.8475mg/gm dry weight of protein was recorded at 1.5% seaweed extract in *U. lactuca* (Figure 3). A maximum concentration of 7.9677 µg/ml dry weight of total soluble sugar was recorded at 0.2% *U. lactuca* seaweed extract treatment (Figure 4). In total soluble sugar amount of concentration was high compared to control. Maximum concentration of 11.4245 µg/ml dry weight of reducing sugar was recorded at 0.7% of *U. lactuca* seaweed extract treatment (Figure 5). Their decrease 62.72% compared to control.

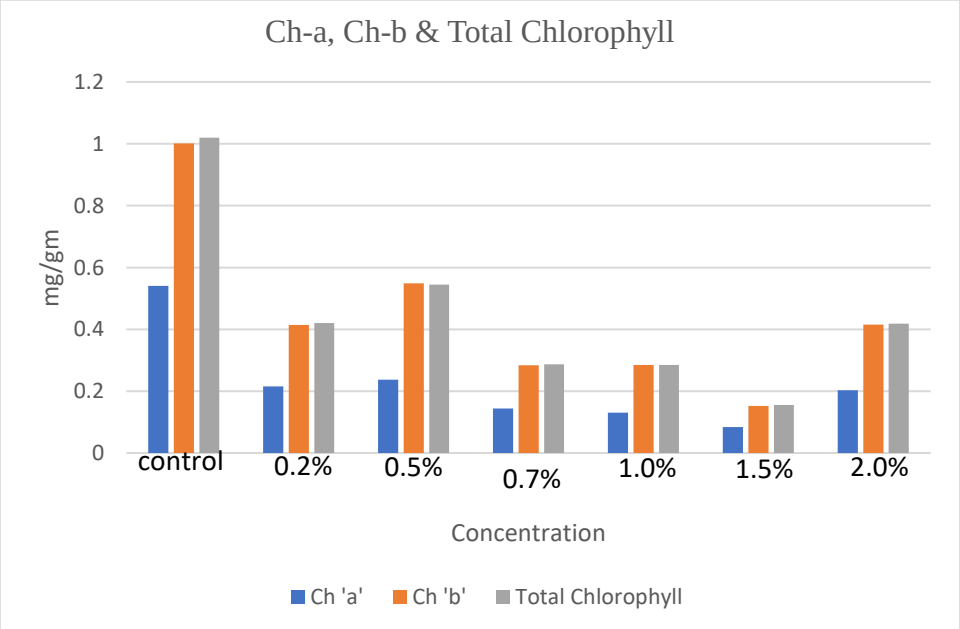

Fig.1: Effect of Ulva lactuca Seaweed biostimulant on the Chlorophyll-a, chlorophyll-b & Total chlorophyll content of Vigna radiata L.

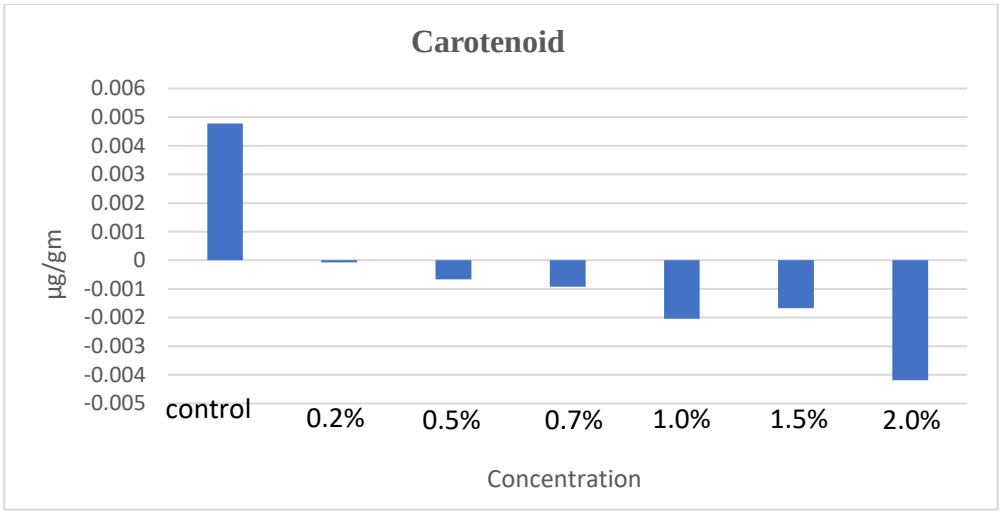

Fig.2: Effect of Ulva lactuca Seaweed biostimulant on the Carotenoid content of Vigna radiata L.

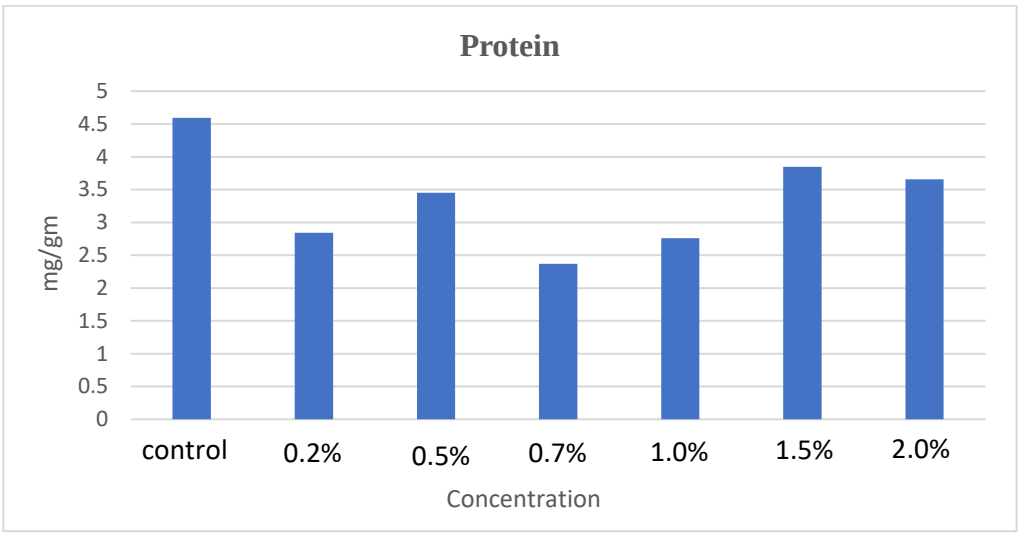

Fig.3: Effect of Ulva lactuca Seaweed biostimulant on the Protein content of Vigna radiata L.

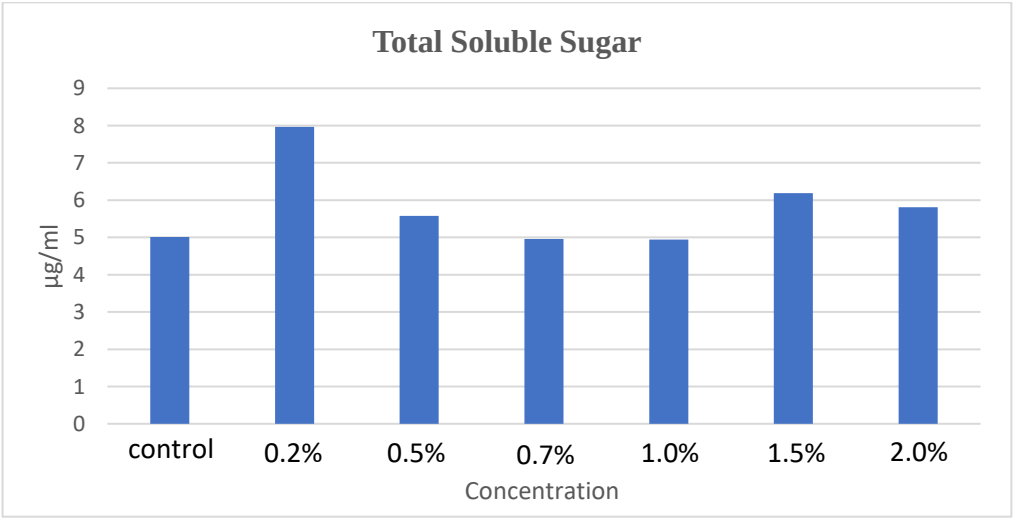

Fig.4: Effect of Ulva lactuca Seaweed biostimulant on the Total Soluble Sugar content of Vigna radiata L.

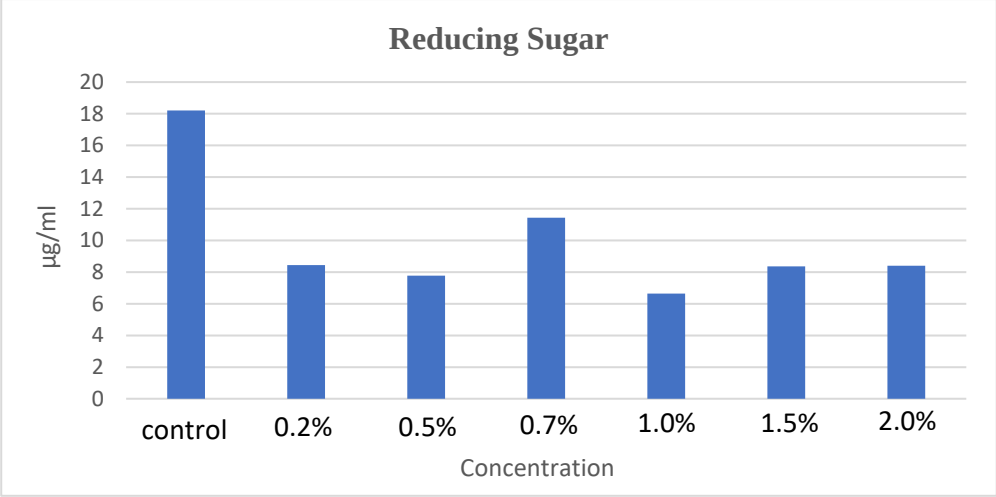

Fig.5: Effect of Ulva lactuca Seaweed biostimulant on the Reducing Sugar content of Vigna radiata L.

IV. DISCUSSION

The *Vigna radiata* L. seeds soaked with higher concentrations of the seaweed biostimulant showed higher rates of germination, while the lower concentrations of the extracts inhibited the germination. The value of seaweed as fertilizer is not from the nitrogen, phosphorous, potash and organic matter but from trace element and metabolites similar to plants growth regulators (Booth, 1969). Seaweed liquid fertilizer was found superior than chemical fertilizer because of the presence of high level of organic matter (Aitken and Senn, 1965). The increased germination percentage at high concentrations may be due to the presence of some growth promoting substances such as IAA and IBA, Gibberellins (A&B), cytokinins, micronutrients (Fe, Cu, Zn, Co, Mo, Mn, Ni), vitamins and amino acids (Challen and Hemingway, 1965). The increased seed germination percentage at high concentrations may be due to the presence of growth promoting substances (Jennings and Yulloch, 1965). The present study highlights the efficiency of Seaweed biostimulants obtained from the green seaweed, Ulva lactuca. Similar results were recorded in Cajanus cajan red gram (Kumar, Mohan, Murugeswari and Muthusamy, 1993), Oryza sativa (Kumar, 2009) and Vigna mungo (Ganapathy, Balamurugan, thinakaran and Sivakumar, 2013). Reported that the presence of plant growth regulators, trace elements, vitamins and macronutrients in the seaweed liquid fertilizer enhance the growth of root length and shoot length of *Vigna mungo* (Challen and Hemingway, 1965). The present investigation is in agreement with the earlier studies (Xavier and Jesudas, 2007). The lower concentrations showed a decreasing trend. Some similar results were recorded in Pedina which induced maximum seedling growth at lower concentrations in *C. cajan* and *Vigna radiata*. Reported similar findings with *Hypneamusciformis*, *Spatoglossum asperum*, *Stoechospermum marginatum* and Sargassum on the growth of crops such as green chillies, turnips and pineapple (Dhargalkar and Untawale, 1983). Additionally, it is known that higher concentrations of (1.0%) the *Ulva lactuca* liquid extract can inhibit the germination of mung bean (Castellanos-Barriga, 2017). In this study, total chlorophyll contents highest in 0.5% concentration in *U. lactuca* seaweed biostimulants and lowest in 1.5% seaweed biostimulants in *U. lactuca*. Protein, total soluble sugar and reducing sugar was highest in 1.5%, 0.2% and 0.7% concentrations respectively, and lowest in 0.7% and1.0% respectively. Vigna catajung when treated with 10% Caulerpa racemose extract contained highest protein and amino acid contents (Anantharaj and Venkatesalu, 2001). The amount of protein content was found highest the shoot system under the treatment of Enteromorpha and Jania extract (El-Sheekh and El-Saied, 1999).

V. CONCLUSION

The results obtained in this experiment showed this presoaking experiment seed with seaweed extract for improve and increase seed germination and plant growth of selected plant of *Vigna radiata* L. the study showed that there is significant effect of treatments with seaweed extract from Ulva lactuca on bio-chemical parameter of chlorophyll a, b & total, carotenoid, protein, total soluble sugar and reducing sugar. The seed priming is a new and unique method for improvement of healthy and fast seed germination. Seaweed biostimulants as a Bio-primer material and this Bio-priming method is a organic, very easy, very cheap, easily available and eco-friendly. Based on this experiment, green algae of Ulva lactuca on giving a result of in a plant of Vigna radiata L. higher growth are

observed in 1.5% and 2.0% concentration, Biochemical parameter are observed in lower concentration for better result are observed. The possibility of the existence of minerals and polysaccharides in algal extracts auxin-like activity is high and could be responsible for the effect on growth parameters.

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Development Potential of Beef Cattle under the Coconut Trees in East Bolangitang District Regency of North Bolaang Mongondow

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Abstract— *Farmers in East Bolangitang District were trying to increase beef cattle population as a source of their income, but the problem was the lack of feed caused by cattle being cultivated on agricultural land. The purpose of this study was to determine the potential for land development under coconut trees for forage. The research method used was a survey method, with the determination of the location was by purposive sampling, namely three villages that have the largest cattle population. The data collected was primary and secondary data with the type of data was cross section and time series. Respondents were determined by purposive sampling, namely 40 farmers who use land under coconut. Analysis of the data used descriptive analysis. Coconut area was 0.2-2 Ha or the average was 0.94 Ha. 35 percent ownership status by farmers and 65 percent farmers as tenants. Beef cattle ownership was 148 or 2-6 cattle per farmer. Feed consumption per head per day consisted of 6.21 kg of grass, 10.40 kg of corn waste and 6.26 kg of rice waste. Forage production for 0.94 Ha can be utilized by 8.83 ST. In conclusion, coconut land in the study area has the potential to be used as a forage development. Need for socialization for farmers to introduce quality forage.*

Keywords— ***beef cattle, land, coconut, potential.***

I. INTRODUCTION

The phenomenon of achieving self-sufficiency in beef that was difficult to achieve was influenced by various factors, including the insufficient population of beef cattle. Meat production from beef cattle in traditionally developed areas was highly expected to support the national demand for meat.This includes local cattle raised by small farmers in rural areas (Rusdiana, 2019). However, the production of meat needed was closely related to the availability of feed both in quantity and quality. The availability of forage both quantity and quality depends on the land potential of each region.

Several research results show that beef cattle farmers face various obstacles related to feed. The existence of land conversion causes the narrowness of the land to be used for feed development. Land conversion is a problem faced by various beef cattle farming development areas (Mulyani et al. 2016; Elly et al. 2018 b; Elly et al. 2019 a).

Cattle farmers together with the government were trying to solve the problem of forage in the development of beef cattle. Beef cattle must be encouraged considering the need for beef every year has increased. Beef cattle as ruminants had the largest contribution as meat producers. This contribution greatly supports the fulfillment of food needs, especially animal protein. In addition, beef cattle had a role in income distribution and had significant market potential (Rusdiana and Talib. 2019).

Beef cattle breeders in East Bolangitang District were trying to increase the population of beef cattle as their source of income. Increasing the productivity of beef cattle farming as an effort to increase economic growth in rural areas was aimed at increasing added value and farmers' income (Romjali, 2018). On the other hand, the

development of beef cattle was sought to supply the shortage of beef availability regionally. This means that beef cattle in the regions had the opportunity to support the demand for beef whose demand continues to increase from year to year.The need for national beef consumption had not been met even beef tends to be in deficit until 2018 (Sodiq et al. 2018). The beef cattle business is developed so that the population achieved is balanced with the consumption needs of food of animal origin (Rusdiana and Praharani, 2018).

The problem was that the productivity of beef cattle in this area was low due to many obstacles. Lack of feed because cattle were grazed on agricultural land was one of the obstacles faced by farmers.Whereas in this area the land under coconut trees had not been used optimally.Other farmers develop food crops under coconut trees and the waste was used as feed. However, the quality of food crop waste was considered low, especially dry waste. The problem was how far the land under coconut trees can be used for forage development. Based on the background and thoughts above, a study has been carried out regarding the use of land under coconut trees. The purpose of this study was to determine the potential for land development under coconut trees through the introduction of forage fodder.

II. RESEARCH METHODS

The research method used was a survey method to farmers through interviews using a list of questions. The location of the research was determined by purposive sampling, namely three villages (Bohabak, Binjeta and Nunukan villages). The sample village was determined based on the consideration that the village had the largest population of cattle. The data collected was primary and secondary data with the type of data was cross section data. Respondents were determined by purposive sampling, namely 40 farmers who used the land under coconut trees. Data analysis used descriptive analysis and carrying capacity.

III. RESULTS AND DISCUSSION

Farmers develop beef cattle as a sideline. This condition was supported by various studies related to beef cattle farming in rural areas. The main occupation of the respondents was as a farmer (100 percent). This condition was supported by the potential of research areas in agricultural development, such as the availability of dry land and rice fields. The optimally managed regional potential will support the successful development of the livestock subsector (Yulia et al. 2015).

Most of the farmers in the research location develop food crop farming, both in open land and under coconut trees. The area of coconut land used by farmers was 0.2-2 Ha or an average of 0.94 Ha. The results showed that the coconut land ownership status was 35 percent owned by farmers and 65 percent of farmers were cultivators. The land under coconut trees was used for grazing cattle. The number of cattle ownership was 148 heads or 2-6 heads per farmer. Various efforts were made to increase the scale of business, including the government in this case facilitating the increase of business scale (Ministry of Agriculture. 2017). Cattle in the study area, during the day grazed and transferred from one coconut land to another. Farmers had not built cages so that cattle were left on agricultural land. The respondent's cattle consume grass that grows wild under coconut trees. Corn and rice waste were also used as cattle feed in the study area. Consumption of cattle feed at the study site can be seen in Table 1.

Table 1. Beef Cattle Feed Consumption in the Research Area

No.	Consumptionof Feed	Quantity (Kg/Cattle/Day)	Percentage (%)
1.	Grass	6.21	27.16
2.	Waste of Corn	10.40	45.47
3.	Waste of Rice	6.26	27.37
	T o t a l	22.87	100.00

The data in Table 1 shows that the amount of corn waste was the largest consumption of beef cattle (45.47 percent). Then followed by the amount of rice waste by 27.37 percent and grass 27.16 percent. Agricultural waste of food crops in the area can be relied on as cattle feed. Beef cattle farming was generally integrated with food crops (Susanti et al. 2014). Waste management strategies were needed to support the development of beef cattle.

The problem was that the quality of food crop waste was low, especially since the waste was dry. The indications need to introduce technology to improve the

quality of the food crop waste. This is because the feed given to livestock must contain good nutritional value (Saputra et al. 2016). Land under coconut trees and other unused land can be used for the development of forage for livestock. This condition shows that land for forage development in the research area was not an obstacle. However, the importance of land cannot be ignored even though its role is small (Nur et al. 2018). The grass that had been developed by several breeders in North Bolaang Mongondow Regency was dwarf grass. The results showed that forage production for 0.94 Ha could be utilized by 8.83 AU. Coconut land area of 1 Ha, if the content was calculated to be 0.8 Ha, it requires 16,000 dwarf grass seeds. Grass was planted with 1 m x 0.5 m, and the grass production obtained was 4 kg per m2. Grass under coconut trees can be cut as much as 9 times per year, resulting in 288 tons per year per Ha. The amount produced is 288 tons, which is equivalent to 22.5 AU/year (Salendu and Elly. 2012). The types of dwarf grass that can be developed in the research area can be seen in Figure 1.

Fig.1: Dwarf Grass Grown under Coconut Trees

Figure 1 shows that the land under coconuts can be used by farmers to support feed needs in the study area. The introduction of this grass had been carried out in other areas and had been well received by the farming community. The grass had been given to cattle in pens (Elly et al. 2018 a; Elly et al. 2019 b).

IV. CONCLUSION AND SUGGESTION

Based on the results of the study, it can be concluded that coconut land in the research area had the potential to be used as forage development for beef cattle. There is a need for socialization for breeders for the introduction of quality forage.

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Impact of agricultural supervision on the performance of cotton producers in the north of Côte d'Ivoire

Impact de l'encadrement agricole sur les performances des producteurs du coton au nord de la Côte d'Ivoire

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Abstract— *The cotton sector in Côte d'Ivoire has long been under state control, through state structures and companies. But, faced with numerous macroeconomic imbalances, the government had to engage in sector reforms in the early 1990s. Despite its reforms, the supervision of producers is encountering difficulties. The objective of this article is to know the structuring of agricultural supervision by identifying the role, activities, actors as well as the constraints faced by both agricultural advisers and producers of Korhogo. This is a joint study carried out in the villages of Bafimé, Dassoungbo and Dassounblé as well as at the General Directorate of the Ivorian Cotton Company (COIC). 68 individuals made up of cotton producers, Agricultural Advisers and officers of the Directorate were interviewed. It emerges that the slowness of the structuring of the supervision leads to dysfunctions in the supervision process of the Ivorian cotton company. Local knowledge is a limiting factor in the application of the practices advocated for Agricultural Advisors. Finally, the lack of human and material resources explains the ineffectiveness of the support actions of cotton producers.*

Keywords— ***Agriculture, Cotton sector, agricultural supervision, Agricultural Advisers, producers.***

Résumé— *La filière coton en Côte d'Ivoire a longtemps été sous le contrôle de l'État, à travers des structures et sociétés d'État. Mais, face aux nombreux déséquilibres macroéconomiques, legouvernement a dû s'engager dans des réformes sectorielles au début des années 90. Malgré, ces réformes, l'encadrement des producteurs rencontre des difficultés. L'objectif de cet article est deconnaitre la structuration de l'encadrement agricole en identifiant le rôle, les activités, les acteurs ainsi que les contraintes auxquelles font face les différents acteurs. Il s'agit d'une étude mixte réalisée dans les villages de Bafimé, de Dassoungbo et de Dassounbléainsi qu'à la Direction Générale de la Compagnie Ivoirienne de Coton (COIC).68 individus composés de producteurs de coton, de Conseillers Agricoles et d'agents de la Direction ont été enquêtés. Il en ressort d'une part que la structuration de l'encadrement entraine des dysfonctionnements dans le processus d'encadrement de la Compagnie Ivoirienne de Coton.D'autre part, l'insuffisance de ressources humaines et matérielles explique l'inefficacité des actions d'encadrement des producteurs de coton sur le terrain. Enfin, les savoirs locaux des paysans constituent des facteurs limitant dans l'application des pratiques prônées pour les Conseillers Agricoles.*

Mots-clés— ***Agriculture, Filière coton, encadrement agricole, Conseillers Agricoles, producteurs.***

I. INTRODUCTION

Le coton a toujours joué un rôle important dans l'économie agricole de la Côte d'Ivoire. Il représente entre 5 et 10% des exportations du pays et génère annuellement de l'ordre de 120 Milliards FCFA de chiffre d'affaires dont 70 à 80% en devises (Dugué, 2001). Avec l'anacarde, il constitue le « poumon » de l'économie des zones septentrionales qui couvrent les deux tiers du territoire. C'est l'une des principales sources de revenus des paysans de ces régions. En effet, environ 3,5 millions de personnes vivent directement ou indirectement de la filière coton qui a participé de façon significative à la réalisation d'infrastructures communautaires tels que l'ouverture et l'entretien des pistes rurales, les écoles, les centres de santé etc.

Cette filière a longtemps été sous le contrôle de l'État à travers la Compagnie pour le Développement des fibres Textiles en Côte d'Ivoire (CIDT). Cependant, la mauvaise conjoncture économique des années 1990 a contraint le gouvernement à engager des réformes dans ce secteur.En effet, la chute des cours mondiaux de la fin des années 1980 et le contexte de crise économique générale ont conduit le Gouvernement à décider la restructuration et l'assainissement de la filière coton dans le cadre d'un programme fondé sur l'amélioration des performances de la CIDT et la responsabilisation des opérateurs. En 1996, le gouvernement ivoirien, en accord avec ses partenaires au développement, s'est engagé dans un processus de privatisation et de libéralisation de la filière. Mi-1998, une partie des actifs de la CIDT a été attribuée à deux groupes privés à savoir La Compagnie Cotonnière de Côte d'Ivoire (LCCI) et Ivoire-Coton. Un cadre libéralisé de fonctionnement de la filière s'est mis en place à la campagne 1999-2000 qui a été couronné par la création de l'Autorité de Régulation du Coton et de l'Anacarde (ARECA) en septembre 2002. La crise sociopolitique qu'a traversée la Côte d'Ivoire depuis septembre 2002 et la conjoncture internationale particulièrement défavorable ont remis en cause de nombreux acquis. Cela a conduit les décideurs à s'interroger sur la pertinence du modèle choisi pour la gestion de la filière et sur les orientations et les priorités à donner aux programmes de développement.

La restructuration a consisté également au transfert de compétences aux organisations des producteurs, à la libéralisation des sous-filières intrants et égrenage et à la mise en place d'une plate-forme des structures d'encadrement agricole. Le transfert de ces fonctions aux organisations des producteurs n'a pas été suffisamment accompagné afin de doter celles-ci de capacités nécessaires (Dugué, 2001). La mauvaise gestion des prestations et des ristournes de coton accompagnée de la gouvernance approximative de certaines organisations paysannes a fortement affecté la cohésion des producteurs et affaibli leurinfluence dans de la filière. Dans les faits, les nouvelles organisations paysannes agricoles avaient du mal à fournir des services d'encadrement technique et managérial de qualité à leurs membres ou à influencer la qualité des services offerts par des tiers. Or, ces services sont importants car ils permettent aux producteurs d'améliorer leur performance grâce à l'augmentation des rendements, à l'amélioration de la qualité du coton et à l'utilisation rationnelle des intrants (Kotchikpa, 2016). Aussi, les hausses de production observéesétaient le résultat d'uneaugmentation des surfaces emblavées.Cela n'a pas véritablement évolué avec le temps. A la campagne 2003-2004, la situation de la filière coton s'est considérablement détériorée. En 2007-2008, la production a été de 119 716 tonnes de coton graine alors qu'en période vertueuse, elle avoisinait les 350.000 tonnes (Koffi, 2013). La reprise attendue en 2009-2010 n'a pas été au rendez-vous en raison des problèmes structurels liés à l'annonce tardif du prix d'achat du coton graine, au placement tardif des intrants etc. (CIRAD, 2009). Cet effondrement de la production est la conséquence de plusieurs facteurs notamment la crise sociopolitique de 2002, l'effondrement des prix sur le marché international dû à un excédent de l'offre créé en partie par les subventions accordées par certains pays développés à leurs producteurs. En plus de ces facteurs exogènes on peut ajouterla baisse des rendements qui sont passés de 1400 kg/ha en moyenne jusqu'au début des années 2000, à moins de 800 kg/ha actuellement. C'est la conséquence de la maîtrise approximative des itinéraires techniques par les producteurs, des retards dans la mise en place des intrants et aussi une maîtrise insuffisante du processus de réforme lié à la libéralisation du secteur(CIRAD, 2009). En effet, contrairement aux attentes, le dispositif organisationnel et fonctionnel de la filière a perdu de son efficacité. Il ne sécurise ni les producteurs ni les égreneurs. En ce qui concerne les producteurs, les approvisionnements en intrants, l'enlèvement de la productionet le paiement des revenus sont incertain. Quant aux égreneurs,ils connaissent des difficultés tant au niveau del'approvisionnement en coton graine, le recouvrement du crédit intrant qu'au niveau de la fourniture d'intrants et de l'octroi de crédit des banques commerciales (op cit).Les récents diagnostics ont relevé des difficultés à tous les niveaux de la filière coton. Les acteurs privés ou publics se trouvent dans un contexte de libéralisation marqué par la baisse des revenus des producteurs et des sociétés cotonnières. Le diagnostic note également l'affaiblissement continu des organisations de producteurs ainsi que la baisse de la qualité de coton (Chiapo, 2001).

En Côte d'Ivoire, le tissu industriel du secteur coton est composé de 14 unités d'égrenage appartenant à 06

sociétés que sont la Nouvelle CIDT, Ivoire Coton, COIC, SECO, SICOSA.2.0 et Global Cotton. Pour ce qui est de la compagnie ivoirienne de coton dont la zone fait objet de cette étude,le taux de coton graine de deuxième choix est passé de 7,2% pour la campagne 2013/2014 à 49% la campagne suivante 2014/2015. De plus, le taux de premier choix est passé de 92,8 à 51% durant la même période (Kossonou, 2016). Les efforts sont fournis par les structures d'encadrement agricole plus particulièrement celles qui s'occupent de la culture de coton. Mais, sans une administration efficace, une amélioration de la qualité ne peut avoir lieu.

Un telconstat pose le problème de l'efficacité interne des structures d'encadrement agricole. C'est pour cela qu'il parait important de connaitre le système d'encadrement de la COIC ainsi que ses contraintes.L'objectif de cette étude est de connaitre la structuration de l'encadrement agricole en identifiant le rôle, les activités,les acteurs ainsi que les contraintes auxquelles font face aussi bien les conseillers agricoles que les producteurs de la Compagnie Ivoirienne de Coton de Korhogo

II. MÉTHODOLOGIE

La présente étude porte sur l'encadrement agricole dans le processus de la production du coton : cas de la zone de Korhogo 1 de la compagnie ivoirienne de coton au nord de la Côte d'Ivoire. Elle s'appuie sur une approche à la fois qualitative et quantitative. Elle a été réalisée dans la zone cotonnière de la Compagnie Ivoirienne de Coton de Korhogo.Les villages de**Bafimé, Dassounblé et Dassoungbo** dans la région du Poroont été les zones d'enquête. La collecte de donnéess'est faite à travers une recherche documentaire et des enquêtes de terrain à travers un questionnaire, des guides d'entretien et une grille d'observation. Elle s'est faite auprèsde la Direction Générale de COIC, des conseillers agricoles etdes producteurs de cotonencadrés par ladite entreprise.La collecte des données s'est faite auprès des producteursde coton grâce à un tirage aléatoire.Pourles responsables et agents d'encadrement de la COIC, c'est la méthode de choix raisonné qui a été utilisée. Au final, c'est un ensemble de soixante-huit (68) acteurs qui ont été interrogés. Il s'agit de 47 producteurs, 18 agents Conseillers Agricoles et 03 responsables de la Direction de COIC. L'analyse de contenu des données d'entretien et d'observation s'est effectuée manuellement.Le traitement des questionnaires s'est fait grâce à Excel et au logiciel PSPP

III. RÉSULTATS

3 Présentationet production de coton de la Compagnie Ivoirienne de Coton Korhogo

3.1 Présentation de la Compagnie Ivoirienne de Coton Korhogo

La Compagnie Ivoirienne de Coton (COIC) est une société anonyme basée à Korhogo.Elle a pour rôle l'encadrement des producteurs de coton et l'égrenage du coton graine. Elle est née suite à la crise qu'a connue La Compagnie Cotonnière Ivoirienne (LCCI). En effet, en 2002, la LCCI a rencontré des difficultés financières et n'apu régler ses créances. Pour ce faire, au cours de la campagne 2005-2006, la faitière YEBE WOGNON a été créée afinde venir en aide aux producteurs délaissés par la LCCI.En 2008, cette structure a été liquidéeet ses actifs ont été acquis par YEBE WOGNON.Deuxusines d'égrenages de Korhogo 1 et de Korhogo 2 sur les quatre usines que possédait la LCCI ont été rachetées. Cette faitière va donner naissance en janvier 2009 à une société anonyme spécialisée dans le domaine de l'agriculture principalement celui du coton. Elle est dénommée Compagnie Ivoirienne de Coton Société Anonyme (COIC SA). Cette dernière a créé deux usines supplémentaires portant sa capacité à quatre usines. Son aire de compétence est divisée en deux grandes parties subdivisées en zones. Ainsi, Korhogo Nord est composée de Niellé- Kassére-Ouangolodougou- Ferkessédougou- Sinematiali. Korhogo Sud comprend Napié- Dikodougou- Marandalla- Bielou. Toutes ces zones se trouvent dans le bassin cotonnier ivoirien au nord du pays et sont gérées par des chefs de zone. Les producteurs de ces zonessont regroupés en Organisations Professionnelles Agricoles (OPA) qui signentdesconventions avec la structure d'encadrement qui leur fourni les intrants. En contrepartie, elles luilivrent leur production de coton.

Les activités de la COIC-SA se structurent autour des activités agricoles, des activités industrielles et des activités commerciales. Les premières concernent la culture du coton graine. En début de campagne agricole, les Conseillers Agricoles(CA) recensent les intentions des producteurs. Ils assurent leur suivi technique depuis la production jusqu'à la commercialisation. Outre la culture de coton, la COIC appuie la culture vivrière grâce à la fourniture d'intrants et de conseils. Les cultures concernées sont entre autres le riz, le maïs, le sorgho, le mil et l'igname. Les activités industriellessont axées sur l'égrenage du coton graine. Les produits issus de cette transformation sont la fibre et la graine du coton. Une partie de la quantité de graine est utilisée comme semence pour la campagne à venir. Enfin, l'activité commerciale consiste en la commercialisation de la fibre et de la graine de coton. Une partie de ces produits est vendue localement. Le reste est destiné àl'exportation.

3.2 Production de coton-graine de COIC

La qualité du coton graine a un impact aussi bien sur les revenus des producteurs que des égreneurs et des filateurs. En cas de défauts de qualité du coton graine, les prix aux producteurs sont réduits carcela entraine des pertes lors du conditionnement, de la commercialisation et de la transformation. C'est pour cela qu'il est de l'intérêt des entreprises cotonnières que la production soit de bonne qualité. Le tableau ci-dessous consigne la production cotonnière cumulée de la période 2013 à 2018 par zone et selon la qualité.

Tableau 1 : La production cumulée de COIC en tonnes par zone et selon la qualité sur la période 2013-2018

Zone de production	Production (en tonne)	Qualité	
		1er choix	2éme choix
Korhogo nord	65.628	61.194	4.434
Korhogo sud	52.025	47.488	4.537
Total	**117.653**	**108.682**	**8.971**

Source : Données de terrain, Mars 2019

La Compagnie Ivoirienne de Coton a fait une production totale de 117 653 tonnes de 2013 à 2018 pour les deux zones confondues. Soit 65.628 tonnes pour Korhogo Nord et 52025 tonnes pour Korhogo Sud. En ce qui concerne la qualité, Korhogo Nord a enregistré 61.194 tonnes de coton graine de premier choix et 4434 tonnes pour le deuxième choix. Korhogo Sud a estimé à 47488 tonnesle coton de premier choix et à 4537 tonnes le deuxième choix. Le coton graine premier choix est toujours supérieur au deuxième choix. Le prix du coton graine était fixé à 250 f CFA pour le premier choix et deuxième choix à 225 F CFA de 2013 à 2017.Lors de la campagne 2017-2018, le prix de coton graine premier choix est passé à 265 F CFA.Celui de deuxième choixa connu une progression de 15 F CFA soit 240 F CFAsur la même période.

4 Profils sociodémographiques des enquêtés

4.1 Situation matrimoniale des Conseillers Agricoles

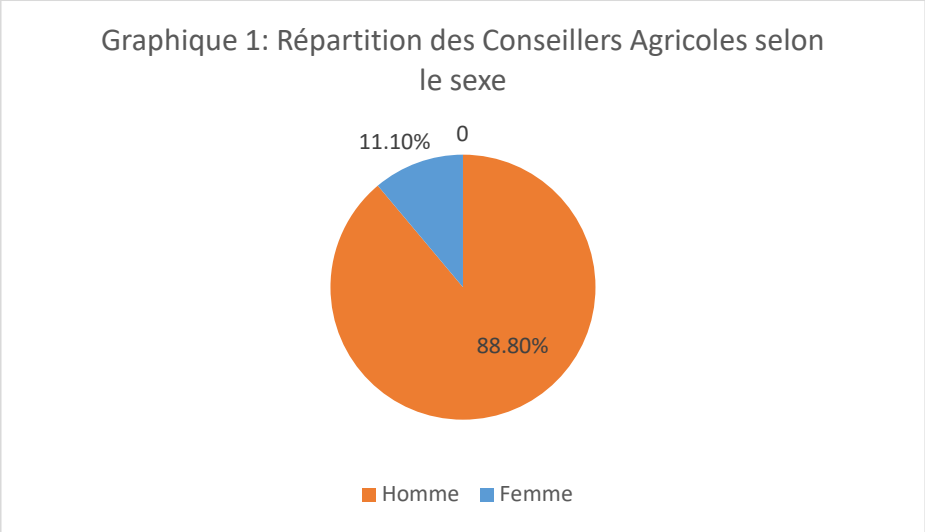
Tableau 2 : Répartition des Conseillers Agricoles selon la situation matrimoniale

Situation Matrimoniale	Effectifs	Fréquences(%)
Célibataire	11	61,1%
Marié(e)	02	11,1%
Union libre	05	27,7%
Divorcé(e)	00	00
Veuf (ve)	00	00
Total	18	100%

Source : Données de terrain, Mars 2019

Sur les 18 agents Conseillers Agricoles enquêtés, 11sont célibataires (61,1%), 02 sont mariés (es) (11,1%) et 05 sont en unions libres (27,7%). Au regard de ces chiffres, on constate que les agents d'encadrement sont majoritairement célibataires. Le métier d'encadrement étant un métier dynamique, les célibataires sont plus adaptés sur le terrain. Car, souvent, il faut faire des mois sur le terrain ce qui n'est pas facile pour les hommes et femmes mariés(es).

4.2 Répartition des Conseillers Agricoles selon le sexe



Source : Données de terrain, Mars 2019

Cediagramme fait ressortir que la majorité des Conseillers Agricolesde la Compagnie Ivoirienne de Coton sont à 88,8% des hommes.Le reste est constitué de personnelde sexe féminin. Cette proportion de 88,8% sous-entend que le domaine d'encadrement dans la compagnie de coton est majoritairement dominé par les hommes.Cela s'explique parle peu d'intérêt des femmes pour cet emploi. En effet, les conditions de l'exercice de ce métier sont très contraignantes. Il fait appel à beaucoup de privation. Les Conseillers agricoles sont mobiles et donc obligés de partir loin de leur famille pour des périodes plus ou moins longues. Au regard de l'état des pistes rurales, leurs déplacements sur le terrain se font sur des motos. Les propos ci-dessous d'un responsable expliquent la faible proportion des femmes dans le maillon de l'encadrement àla COIC.

« Nous n'employons pas assez de femmes dans le domaine d'encadrement car nous pensons que cette activité est dotée de plus d'engins déplacement sur le terrain et aussi il faut avoir la maitrise des engins et enfin, nous pensons ce métier est plus destiné aux hommes qu'aux femmes ».

4.3 Situation matrimoniale des producteurs de coton-graine

Tableau 3 : Répartition des producteurs de coton selon la situation matrimoniale

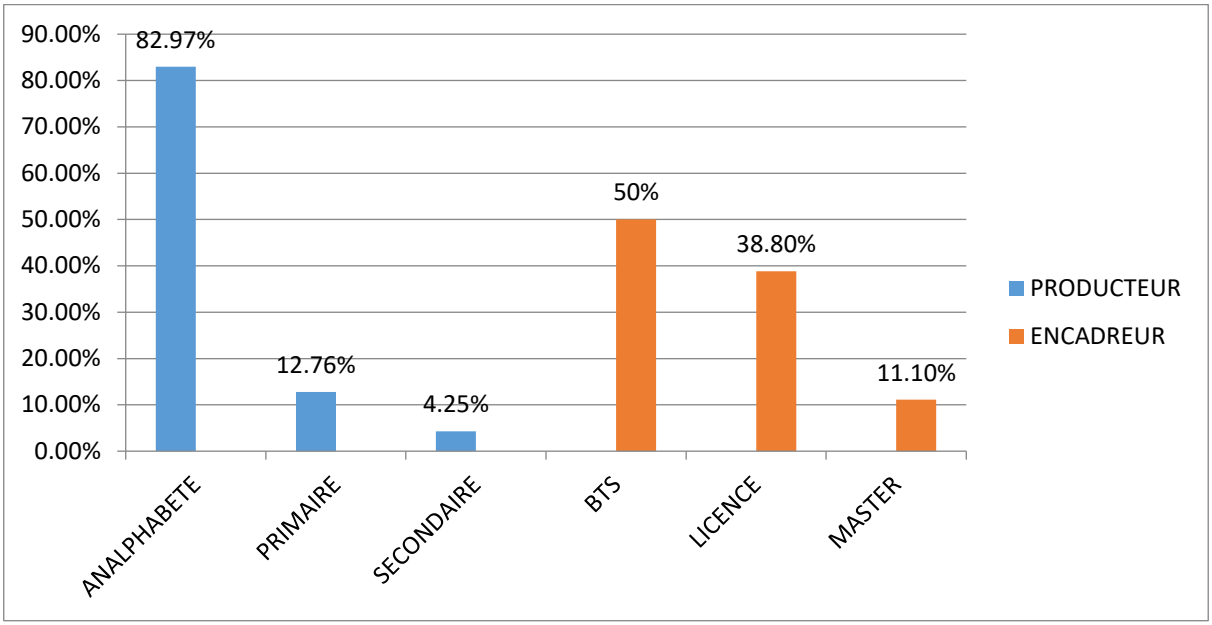
Situation Matrimoniale	Effectifs	Fréquences (%)
Célibataire	01	02,12%
Marié(e)	37	78,73%
Union libre	09	19,15%
Divorcé(e)	00	00
Veuf (ve)	00	00
Total	47	100%

Source : Données de terrain, Mars 2019

Au regarddu tableau ci-dessus, sur les 47 producteurs de coton enquêtés, 78,72% sont mariés.Les célibataires sont de 02,12% et ceux en union libre s'élève à 19,14%. La culture du coton est dominée par les hommes mariés. Il faut noter que la main d'œuvre comprend les hommes, les femmes et les enfants issues généralement de la même famille que le chef d'exploitation. Les tâches sont partagées par le chef de famille en fonctions des différentes étapes de l'itinéraire cultural du coton. Pour ce faire, le mariage s'avère être important dans la mesure où les femmes alimentent la main d'œuvre autant que les enfants.Au-delà de cette réalité le mariage revêt une signification sociale très importantepour les peuples des localités d'enquête. Il confère un rôle de responsabilité un statut social plus valorisant dans de la communauté.

4.4 Niveau d'instruction des Conseillers Agricoles et des producteurs

Graphique 2 :	Répartition des encadreurs et des producteurs selon le niveau d'instruction



Source : Données de terrain, Mars 2019

Le graphique montre que les producteurs sont majoritairement an alphabètes 82,97 %. On dénombre 12,76 % de niveau primaire et 04,25 % de niveau secondaire. Ce taux élevé d'analphabétisme constitue un handicapdans l'encadrement des producteurs. Cela freine l'assimilationdes pratiques culturales enseignées par les Conseillers Agricole. Ce qui rend l'apprentissage difficile et lent. Les propos de ce Conseiller Agricole le relève si bien.

« Le fait que nos producteurs n'ont pas un niveau élevé de connaissance et qu'ils ont leurs savoirs locaux et leurs expériences vécues ils ont du mal à mettre en pratique nos conseils donnés »

Cette situation décrit les difficultés que les CA rencontrent dans l'exercice de leur travail d'encadrement des paysans. En effet, les formations se font en français. Certains conseillers utilisent souvent le malinké lorsqu'ils le parlent. Ainsi, la langue constitue une barrière à l'assimilation des enseignements faits à l'endroit des paysans.

En ce qui concerne les CA, le niveau minimum requis pour exercer ce métier est le BTS ou BAC+2 en agriculture. Au regard des données du graphique on constate que 38,80% de ces derniers ont un niveau de Licence en agronomie contre 50% pour le niveau de BTS et 11,10%pour le niveau de Master. Dans l'ensemble, les agents de la COIC en charge de l'encadrement agricole des producteurs de coton de la zone d'étude ont un niveau d'étude supérieur à celui qui est requis. Selon un responsables COIC :

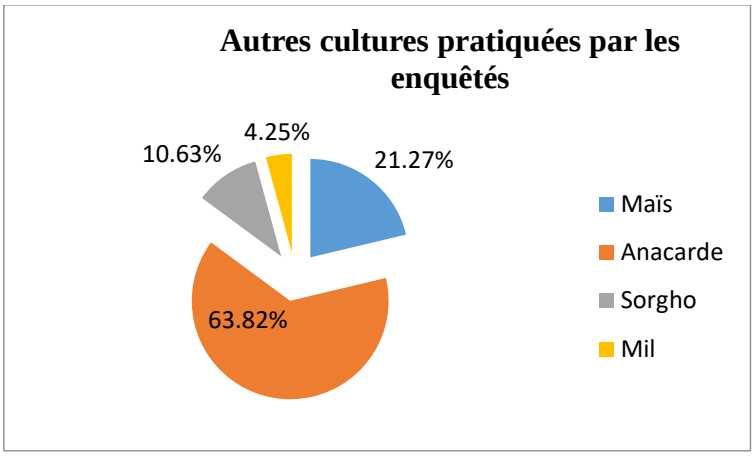
« Pour être CA, il faut avoir un niveau de BAC +2. Lorsque le poste est occupé par un agent qui a un diplôme supérieur, il a le même traitement salarial que les autres.Seule une formation complémentaire ou un concours professionnel peut permettrede changer de catégorie et d'améliorer la situation salariale. »

Le graphique ainsi que les propos du responsable de COIC montrent que beaucoup d'agents occupent ces postes alors qu'ils ont des qualifications supérieures à ce qui est demandé. Cela peut constituer une faiblesse dans l'encadrement des producteurs. En effet, bien qu'informés du traitement lorsdu recrutement, ces derniers peuvent ressentir des frustrations sur le terrain. Cela peut les pousser souvent à mal faire son travail d'encadrement.

Par ailleurs, le niveau d'étude élevé des CA au lieu de constituer un atout devient un handicap dans l'encadrement. L'une des difficultés est qu'ils emploient un langage technique soutenu qui empêchent les producteurs qui sont en majorité des analphabètes, à assimiler leurs messages. Ce qui entrave souvent le bon fonctionnement de l'encadrement.

5 Autres cultures pratiquées par les producteurs de coton

Graphique 3 : Les différentes cultures pratiquées par les producteurs en dehors du coton



Source : Données de terrain, Mars 2019

En dehors du coton, les paysans de la zone COIC pratiquent d'autres cultures agricoles.L'anacarde vient en tête avec 63,82%. Le maïs occupe le deuxième rang avec 21,27% des enquêtés qui en sèment. Ensuite, 10,63% cultivent le sorgho.Et enfin, 04,25% des enquêtés s'adonnent à la culture du mil. La culture de l'anacarde devance de loin les cultures vivrières. Elle se positionne comme la deuxième spéculation agricole dans cette zone après celle du coton. Les revenus de cette culture permettent de compléter ceux issus de la commercialisation du coton. L'anacarde est cultivé pour mettre à l'abri les paysans contre les aléas pouvant entrainer la chute de la production ou des cours du coton. Les revenus tirés de la culture de l'anacarde permettent de renforcer les gains des producteurs de coton.

Quant aux cultures vivrières, leurs productions sont encadrées par la COICI. Celles-ci, sont la plupart du temps associées à celle du coton. Elles sont destinées essentiellement à l'autoconsommation du ménage. Mais, le surplus peut être vendu sur les marchés locaux.

6 Système et organisation des activités d'encadrement agricole des producteurs de coton

6.1 Le système d'encadrement agricole des producteurs de coton de la COIC

L'encadrement agricole consiste à apporter aux producteurs de coton des innovations et des appuis techniques culturaux dans le but d'améliorer et de renforcer la productivité de leurs exploitations. Il se fait autour d'une interrelation collaborative entre le paysan et le Conseiller Agricole. Pour la réalisation de leurs taches, ces dernierssont formés.Ils sont également dotés des moyens techniques et matériels nécessaires pour lamission qui leur est confiée. Les paysans reçoivent l'appui des organisations paysannes qui sont elles aussi soutenues par les encadreurs. Les deux sous-systèmes interagissent par des activités collaboratives.Au cours des rencontres,des informations et des connaissances techniques sont échangées.

La conception des technologies se fait en partenariat avec des organismes notammentl'Agence Nationale d'Appui au Développement Rural (ANADER), l'Interprofession du coton (Intercoton), le Centre National de Recherche Agronomique (CNRA). La COIC élabore les stratégies et les politiques de conseil agricole. Elle assure la formation en cascade des différents acteurs de la chaîne de production au niveau du sous-système encadrant. Elle fait le suivi-évaluation de la mise en œuvre des activités. En effet, lorsqu'une technologie est prête pour être diffusée, la COIC se l'approprie et en assure sa vulgarisation en formant les responsables concernés. Elle effectue plusieurs missions de formation et de supervision sur le terrain en vue de suivre la mise en œuvre des activités par les agents d'encadrement et organise au besoin des recyclages. Les solutions apportées aux préoccupations des agents d'encadrement et des paysans tiennent compte du diagnostic. Des ateliers de revue de technologie sont organisés pour corriger les insuffisances. Le travail d'encadrement se fait surtout dans les unités de vulgarisation qui regroupent un ou plusieurs villages dans lesquels les agents mettent en place des associations. La mise en place d'une action requiert la participation des coopératives de producteurs qui sont souvent consultées.

En général, dans le processus d'encadrement agricole en Côte d'Ivoire,ce sont les organisations paysannes (OP)qui représentent et qui défendent les intérêts des producteurs.En ce qui concerne le coton, c'est l'Inter-coton qui joue ce rôle desrégions de production. Les OP participent aux formations de masse. Elles constituent des groupes de contact. Elles organisent les paysans en groupement de production, enregistrent leurs besoins en intrants, évaluent le tonnage des récoltes et facilitent la commercialisation. Elles ont en charge le suivi, la progression de la vente des produits et le paiement des revenus aux producteurs. Les OP font également des plaidoyers auprès du gouvernement pour l'allègement des conditions de production notamment l'augmentation des crédits et le prix d'achat du coton graine, la diminution du coût des intrants, etc.

En somme, le système d'encadrement est un agrégat d'institutions dans chaque sous-système qui agissent les unes sur les autres dans une relation de coopération et de partage d'informations pour atteindre les objectifs communs de production.

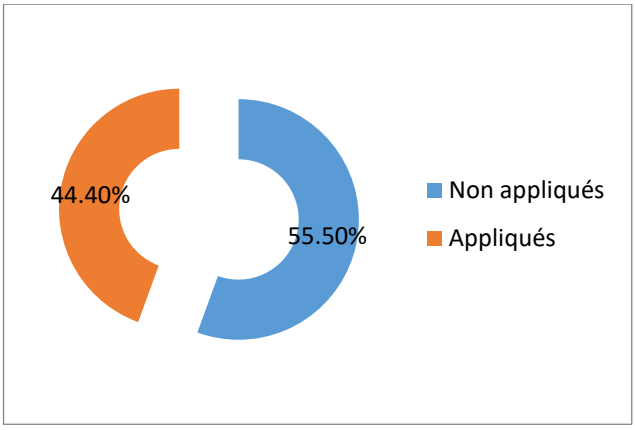
6.2 Organisation des activités d'encadrement

Les activités d'encadrement sont organisées autour de la formation, du financement des activités etc. Ellesconcernent les techniques de semis à plat qui consistent à semer directement sans labour. Elles prennent également en comptela distribution des intrants et la démonstration de leur utilisation.En clair, l'encadrement consisteà apporter les intrants aux producteurs tout en mettant en œuvre leur utilisation. Il apporte d'innovation pour améliorer les techniques de production. Il consiste à faire des démonstrations des techniques de traitement phytosanitaire, de fertilisation des sols par la rotation et l'assolement. La formation de masse, l'aide au transport de la production de coton font partie également des activités d'encadrement.

Les champs de démonstration servent de cadre pour la mise en pratique des activités de conseils agricoles. Ils réunissent des groupes de paysans pour une formation de masse. Il peut s'agir également d'une intervention directe auprès des grands producteurs de coton. La planification des interventions se fait en fonction du cahier de charge grâce à un emploi de temps fixe.Elle peut se faire parfois sur sollicitation des agriculteurs. Le contrôle et le suivi des activités du paysan se font à toutes les étapes de la production. Cependant, les missions de contrôle et d'inspection dans les champs et la mise en œuvre des innovations sous le contrôle Conseiller Agricole sont plutôt règlementées à travers des visites inopinées dans les exploitations.

- **Opinion des Conseillers Agricoles sur l'assimilation des conseils par les producteurs**

Graphique 4 : Niveau d'application des conseils agricoles

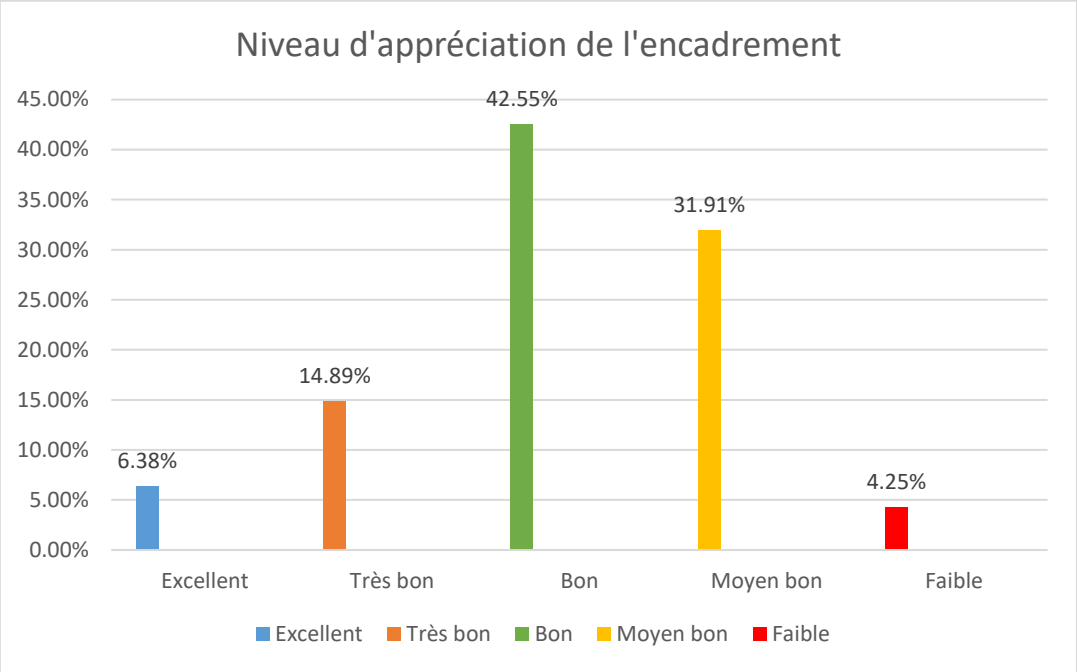


Source : Données de terrain, Mars 2019

Ce graphique indique que 55,5% des conseillers agricoles ne sont pas satisfaits de l'application des instructions par les producteurs. Ils estiment que les paysans n'appliquent pas bien les conseils. A contrario, les exploitants estiment que les techniques qu'on leur enseigne sont contraignantes. Pour faciliter leur assimilation, les producteurs demandent beaucoup plus d'investissement et beaucoup plus de temps.Ce qui leur permettrait selon eux de respecter rigoureusement les dosages des intrants, le calendrier d'épandage des produits phytosanitaires, l'écartement des lignes, le sarclage régulier du champ.

Dans le même temps, 44,4% des agents d'encadrement agricole reconnaissent que les producteurs qui appliquent les consignes de l'encadrement ont rompu avec leurs anciennes pratiques. Il s'agitgénéralement des gros producteurs qui ont beaucoup de moyens financiers et matériels. Ceux-ci ont recours parfois à la mécanisation et ils emploient une main d'œuvre agricole rémunérée durant toute la campagne en fonction des contrats. L'adoption et l''application des conseils agricoles peuvent garantir le succès de l'encadrement lorsque tous les moyens sont réunis.

- **Opinion des producteurssur l'encadrement agricole**



Source : Données de terrain, Mars 2019

On observe que 6,38% des paysans trouvent que d'encadrement agricole est excellent. 14,89% le trouvent très bon contre 42,55% qui le jugent bon. Dans l'autre sens, 31,91% de producteurs estiment que l'encadrement qui leur est proposé est moyennement bon et 4,25% trouvent faible. Au total, on peut dire qu'environ 36,16 de producteurs de coton ne sont pas très satisfaits du mode d'encadrement. Ces différents niveaux d'appréciation sont explicables grâce aux différentes méthodes mise en place pour l'encadrement. Pour eux, les missions d'inspection, les méthodes de démonstrations de l'utilisation des intrantsentre autres ne sont pas suffisantes pour leur permettre de comprendre et d'assimiler ce qu'on leur demande de faire. En outre, ils auraient souhaité avoir plus fréquemment les visites du Conseillers agricole afin de lui exposer leurs difficultés.

7 Contraintes de l'encadrement des producteurs de coton à Korhogo

Les obstacles sont de divers ordres. Au niveau des producteurs, ils relèvent des menaces verbales et des injures lors des rencontres de suivi-évaluation de la part des conseillers agricoles. Ils notent aussi le retard de paiement des revenus de coton. Les paysans entrent en possession de leurs fonds parfois six mois après l'enlèvement de la production. Lors de l'enquête, qui a coïncidé avec le début de la nouvelle campagne, le paiement des revenus de la campagne précédente étaient encore en cours. Cela constitue une faiblesse du système d'encadrement.

Les retards dans la mise en place des intrants, de la commercialisation de la production et du paiement des fonds aux producteurs sont selon les conseillers agricoles la faiblesse du processus d'encadrement. Ces derniers notent aussi des contraintes inhérentes à leur métier. Il s'agit notamment de la vétusté du matériel roulant, le manque d'équipements spécifiques pour les démonstrations, le difficile accès aux sites à cause de l'impraticabilité des pistes surtout en saison de pluies. Ils relèvent également la question du traitement salariale qui n'est pas des plus incitatifs. Mais, ils expliquent le désintérêt des producteurs à l'appui-conseil par le manque de professionnalisme des paysans. Pour eux, les producteurs ont du mal à se départir des pratiques anciennes de culture. Ils sont dans la routine des informations et des conseils. Selon les propos de l'un de nos enquêtés :

« Les paysans ne viennent pas tous aux formations de masse, car ils croient tout savoir ».

Les producteurs sont pour la plupart des anciens dans la culture du coton. Certains ont connu plusieurs encadreurs agricoles. Au fils des années, ils ont acquis des connaissances techniques et savoirs empiriques. Ils trouvent parfois que les nouvelles innovations sont soit inutiles soit difficiles à appliquer en comparaison avec leurs précédentes connaissances. Cette situation impactent l'application des nouvelles techniques et le niveau de productivité des exploitations.

En plus des entravent exposées plus haut, il y a les lourdeurs de la bureaucratiequi constituent également des contraintes dans l'efficacité des actions sur le terrain. Les décisions sont prises essentiellement par le supérieur hiérarchique après consultation des parties et structures impliquées dans la gestion et production du coton. Celles-ci affectent toute la chaîne de production. La lenteur avec laquelle elles parviennent aux producteurs impacte négativement leur efficacité.

Enfin, les actions des organismes non gouvernementaux et de certaines structures privées intervenant dans l'encadrement créent un déséquilibre dans les messages véhiculés. Ces interventions ne se font pas dans une synergie avec la COIC. Les organismes ne collaborent toujours pas avec les organismes publics.Les multiples acteurs avec des messages parfois divergents et non coordonnés font perdre de l'efficacité aux producteurs de coton de la zone. Tous ces facteurs mis ensemble rendent lourde la machine de l'encadrement. Ils entravent ainsi la production et le bon fonctionnement de la Compagnie Ivoirienne de Coton.

IV. DISCUSSION

L'étude a pour objectif de décrire la structuration de l'encadrement des agriculteurs en identifiant le rôle, les activités et les implications des différents acteurs.Plusieurs résultats ont été obtenus. En effet, il en ressort que le système d'encadrement des agriculteurs est constitué d'un ensemble de dispositifs mis en place par le gouvernement. Les personnels de ces structures sont essentiels dans l'accompagnement, la vulgarisation, le suivi et l'évaluation des activités et la commercialisation des produits du milieu rural. Enfin, la structurationde l'encadrement agricole se décompose en sous-systèmes qui partent de la rechercheà l'application des résultats de la recherche à travers la vulgarisation.

Des travaux ont été effectués sur le système d'encadrement des paysans. Ce maillon du processus de production agricole est même constitué d'un réseau d'acteurs qui travaillent en synergie afin de faciliter l'innovation dans le domaine de l'agriculture (Totin, 2004). Dans cette étude, il s'agit de l'ensemble des personnes etdes institutions qui apportent les intrants nécessaires à la production du coton et qui veillent à la récolte, au transport et à la commercialisation. La pluralité de structures d'encadrement génère des conflits. C'est l'une des causes de la lourdeur du système. Il découle de tout ce qui précède que la structuration de l'encadrement, quand bien même elle converge vers les objectifs de production accrue n'offre pas au bout de compte une fluidité dans la prise des décisions et la coordination des interventions de chacun des services publics auprès des populations. Cela corrobore avec la lenteur des décisions et la lourdeur des interventions révélées par cette recherche.

Par ailleurs, plusieurs situations créent et complexifient les besoins de survie et de sécurité des producteurs de coton. D'abord, il faut noter que les besoins de sécurité alimentaire ne sont pas totalement comblés. La désertification et le changement climatique entraînent la rareté des pluies et partant de mauvaises récoltes. Ils ne sont donc pas à l'abri de la disette. À cela, s'ajoutent les problèmes de sécurisation et de fertilité des terres. Dans cette atmosphère d'insécurité, les cultures d'exportation comme le coton dont le prix est fixé non pas par eux, mais plutôt par l'Etat sur le plan national constitue un élément important de démotivation.Dans cette situation, les logiques paysannes prennent le pas sur celle des pouvoirs publics. Parlant de l'adoption de la culture du soja dans le Nord de la Côte d'Ivoire, Kam (2013) a noté que les logiques des paysans sont de « satisfaire aux besoins sociaux fondés sur l'empirisme et le pragmatisme ». Les besoins alimentaires à travers les cultures vivrières, les activités sociales sont priorisés par rapports à l'augmentation des revenus. Ainsi, alors que les pouvoirs publics visent une meilleure productivité, une augmentation des recettes de l'état et la satisfaction des besoins alimentaires du grand nombre, les paysans misent sur l'unité de production familiale (idem). Cette opposition des visions expliquent les échecs des politiques d'encadrement agricole.

L'étude a montré que les paysans ne sont pas satisfaits des comportements et du traitement des Conseillers Agricoles. Cela met en évidence des failles dans tout le système d'encadrement. Pour ce faire, parlant des contraintes de l'encadrement agricole en Côte d'ivoire, JP Colin et B. Losch (1990) dans leur étude ont relevé le caractère directif et hiérarchique du système. Ce modèle de gestion installe les paysans dans une dépendance vis-à-vis de la « tutelle » dont à travers le contrôle de l'accès au

matériel végétal sélectionné, aux intrants,à la formation technique, aux débouchés de la production. Pour eux, cela entraine la fragilité de tout le système de production. Les résultats corroborent avec ceux de Ouattara (2021). En effet, ce mode dirigiste et rigide de l'encadrement des producteurs a été un facteur déterminant dans l'abandon de la culture du coton au profit de l'adoption de l'anacarde dans le département de Katiola

Les résultats de la présente étude rejoignent les travaux de Folefack (2010). Ce dernier a noté qu'au Cameroun, plusieurs contraintes entravent le développement de la filière coton. Il a relevé notamment le paiement tardif du coton, le retard d'évacuation de la production, le mauvais état des pistes rurales, l'inefficacité du classement du coton graine dans les villages, la faiblesse des prix d'achat. Cette situation est identique à celle que vivent les producteurs de coton de Korhogo

V. CONCLUSION

L'étude sur l'encadrement agricole dans le processus de la production du coton : cas de la zone de Korhogo 1 de la compagnie ivoirienne de coton au nord de la Côte d'Ivoirea relevé les entravent dans l'encadrement paysan. Il s'agit de l'insuffisance de ressources humaines et matérielles qui inhibent l'inefficacité des actions d'encadrement des producteurs de coton sur le terrain. On note également l'analphabétisme et les savoirs locaux des paysans qui constituent des facteurs limitant dans l'application des conseils agricoles. Pour se faire, pour le rendre plus pertinent, l'encadrement doit valoriser aussibien l'agriculture que les paysans. Il doitmettre l'agriculteur au centre du processus en tenant compte de ses connaissances empiriques.

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Reconstruction of the phylogeny of Anopheles sp. Based on the Cytochrome Oxidase Sub Unit 1 (CO1) gene in the Minahasa Peninsula, North Sulawesi

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Abstract— *Indonesia is a country with the highest malaria cases in the world. North Sulawesi is known as one of the malaria endemic areas in Indonesia. Malaria can only be transmitted through the bite of the Anopheles sp. Thus, the high case of malaria infection in an area is linear with the high population of Anopheles sp. The identification method to the species level that has high accuracy is by molecular identification using the cytochrome oxidase sub unit 1 (CO1) gene. Based on the CO1 gene, the mitochondrial DNA of Anopheles sp from Tombatu was 92% similar to Anopheles maculatus [KT382822.1] from China. Anopheles sp from Ratahan based on the CO1 gene has a similarity level of 80% with Anopheles barbirostris [KM610029.1] from China. Anopheles sp from Pineleng has a 77% similarity with Anopheles aquasalis [AF417697.1] from Brazil. The CO1 gene sequences of Anopheles sp from Southeast Minahasa (Tombatu and Ratahan), and Anopheles sp from Minahasa (Pineleng) had a nitrogen base size difference of more than 6%. Thus, the variation of the Anopheles sp CO1 gene is relatively high compared to similar sequences that have been recorded on the NCBI gene bank site.*

Keywords— ***Reconstruction of Phylogeny, Anopheles sp. Cytochrome Oxidase Sub Unit 1 (CO1) gene, Minahasa***

I. INTRODUCTION

Malaria is still a major health consideration, especially in tropical countries. Malaria is the world's most dangerous parasitic infection, causing more than a million death and 500 million cases annualy (Penet et al.,2007; Ravichandran et.al. 2007). Malaria may decrease the productivity of individuals, families and the whole due morbidity and mortality (Ravichandran et.al. 2007, Namdeo, et.al., 2006). Malaria remains a leading cause of morbidity and mortality worldwide with an estimated 500 million cases and 2.5 million deaths annually (Stauffer & Kamat 2003). Malaria is a reemerging disease, which is a disease that is re-infected en masse (Arsin, 2012).

Indonesia is a country with the highest malaria cases in the world. North Sulawesi is known as one of the malaria endemic areas in Indonesia. Some malaria endemic areas in North Sulawesi are Minahasa, Southeast Minahasa and North Minahasa. Malaria is an infectious disease caused by a protozoan parasite of the genus Plasmodium, which is transmitted through the bite of the Anopheles mosquito. Malaria can only be transmitted through the bite of the Anopheles sp. Thus, the high case of malaria infection in an area is linear with the high population of Anopheles sp. In Indonesia, vector confirmation has been carried out from 1919 to 2009, and during that period 25 species were found to be positive for the malaria parasite. As a tropical rain forest area, the Minahasa area is separated by forests and mountains. Based on a survey from the Ministry of Health of the Republic of Indonesia in 2009, in North Sulawesi, three main species of Plasmodium vector were

found in humans, namely Anopheles subpictus, Anopheles vagus and Anopheles annullaris (Ministry of Health RI, 2009). Identification is based on morphological characteristics.

However, identification was carried out based on morphological characteristics. Identification of mosquitoes by morphological analysis method has many limitations. The observed specimens often have undergone morphological changes due to immersion with alcohol or formalin from the sampling location. This greatly affects the stage of species identification in the laboratory.

The identification method to the species level that has high accuracy is by molecular identification. Molecular identification using the cytochrome oxidase sub unit 1 (CO1) gene has been widely carried out. CO1 is one of the genes in mitochondrial DNA that has been designated as a molecular barcode. The cytochrome C oxidase sub unit I (COI) gene has special characteristics that are suitable as a tool in evolutionary studies, namely (1) as a final catalyzer in the respiratory chain in mitochondria, so COI is widely studied at the biochemical level, and shows that the structure and size of the COI gene conserved in all aerobic organisms (Rivera et al. 2009). (2) The amino acid sequence correlates with the function of each part of the COI, thus showing the characteristics of the species that possess it (Rivera et al. 2009 Roe & Sperling 2007). (3) A sequence of 658 basepair (bp) at the 5' end was proposed as an animal barcode (Hebert et al. 2003 a,b). These barcodes have been successfully demonstrated to be able to differentiate between species in Lepidoptera (Hebert et al. 2003a; Hajibabaei et al. 2005), beetles (Funk et al. 1995), several insect pests (Toda & Murai 2007) moth Hamona mermerodes (Hulrc et al. 2007). al. 2007), mosquitoes (Cywinska et al. 2006).

Identification of insects from North Sulawesi using the CO1 gene has been successfully carried out on Aedes sp (Kaunang et.al. 2013; Timah et.al. 2016), Apis dorsata Binghami (Mokosuli et.al. 2013), subterranean termites (Ngangi et.al. 2014), Demselfly (Rantung et.al. 2015) and Marine Gerridae (Warouw et.al. 2015). Molecular identification using the CO1 gene as the basis for reconstructing the phylogeny of Aedes sp. in North Sulawesi as a malaria endemic area. Reconstruction of phylogeny will break the distribution of Anopheles sp. in Minahasa. The results of the phylogeny reconstruction will be very useful for the prevention of malaria vector mosquitoes.

II. MATERIALS AND MEHODS

Sample

Adult mosquito collected used modified method Cheng et. al. (2010). Collection on the fields area randomly. Insects that have been collected will insert in a bottle sample that has been labeled with place and time of data sampling. The bottle was filled with 70% alcohol for identification and preservation.

DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted larva and adults mosquito using Qiagen DNA Blood and Tissue, according to the manufacturer's protocol. PCR was performed in a total volume of 25 µL containing 1 × reaction buffer, 3 mM MgCl2, 0.24 mM dNTPs, 1.4 µM of each primer LCO1490 : 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 : 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et. al., 1994), 1U Go Taq Flexi DNA polymerase (Promega Corp.) and 2.5 µL of DNA (a 100 time dilution of the original DNA). The PCR program was as follows: 94 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, 48 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega Corp). Purified PCR products were analyzed by electrophoresis in 1% agarose gel. The molecular size of the amplified products was estimated using 1 kbp DNA ladder (Biometra). PCR products were sequenced using AB1 PRISM Dye Terminator Cycle Sequencing Ready Reaction System, version 1.1. (Applied Biosystems) in FIRST BASE Singapura

Sequences Analyses and Phylogeny trees reconstructed

Obtained sequences were aligned using MEGA 6.0 and Geneous 6.0 software. Sequences were subjected to Basic Local Alignment Search Tool (BLAST) in order to perform sequence similarity searches (www.ncbi.nih.gov.com). Nucleotide frequencies were calculated using MEGA 6.0 software (Tamura et. al. 2013). The genetic distances (number of nucleotide substitutions per site) among sequences were calculated using the Maximum Composite Likelihood model in Geneous 6.0 software. Phylogenetic trees were reconstructed using two different reconstruction methods: (1) neighbor joining (NJ) and (2) maximum parsimony (MP). The NJ tree was reconstructed using the Maximum Composite Likelihood method. Phylogenetic analyses were conducted in MEGA 6.0 software. Bootstrap support values were obtained by 1,000 replications using both methods (Tamura et. al. 2013).

III. RESULTS AND DISCUSSION

Total DNA Extraction and Purification

Tissue in adult insects is found in the exoskeleton. Thus, to obtain good DNA purity and concentration, the selection of the right organ will determine the success of DNA extraction. In this study, a series of trials were carried out using mosquito organs to obtain total dsDNA with standard purity and concentration. The total DNA in this study was nuclear DNA and mitochondrial DNA isolated from mosquito organ cells which were extracted using the DNA blood and tissue kit. The organs used are the head, thorax, legs and abdomen. From the extraction carried out, it was found that the use of the thoracic organ resulted in the best concentration and purity (table 1). Proteinase K is a key enzyme in DNA extraction. Proteinase-K functions to damage membrane proteins and other proteins in the cytoplasm and nucleoplasm as well as other cell compartments to isolate DNA. The results showed that proteinase K immersion time affected the total DNA purity but had no effect on the total DNA concentration obtained (Table 1).

Table 1. Purity and Concentration of total Mosquito DNA

No	Organ	Sample weight (mg)	Modified Immersion Proteinase-K (hours)	Purity (A260/A280)	Concentration µg/ml
1	Head	35	12	1,10	25,80
		35	24	1,23	25,84
2	Thorax	35	12	1,72	37,50
		35	24	1,87	36,24
3	Limbs	35	12	1,35	32,10
		35	24	1,45	33,40
4	Abdomen	35	12	1,42	34,50
		35	24	1,52	36,20

Amplification and Visualization of Gene CO1

The results of the CO1 gene amplification were shown by electrophoresis electrogram. Based on the formed band, it shows a high concentration of amplicon in both samples AR1, KL1 and SG1 (Figure 1)

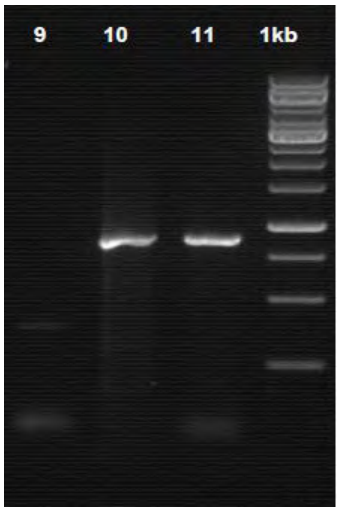

Fig.1 Visualization of the PCR product of Anopheles sp CO1 gene amplicons through 0.8% agarose gel electrophoresis. Anopheles sp from Pineleng/PSA (no. 9), Anopheles sp from Ratahan/RTA (no. 10) and Anopheles sp from Tombatu/TLA (no. 11).

The results of partial sequencing of Gen CO1 in the form of an ABI file, interpreted using Geneous 6.0 software. The sequence lengths of RTA (Ratahan), TLA (Tombatu) and PSA (Pineleng) were 870 bp, 774 bp and 862 bp, respectively. Based on the sequencing chromatogram, it showed that the CO1 gene sequence formed was good (Figure 2).

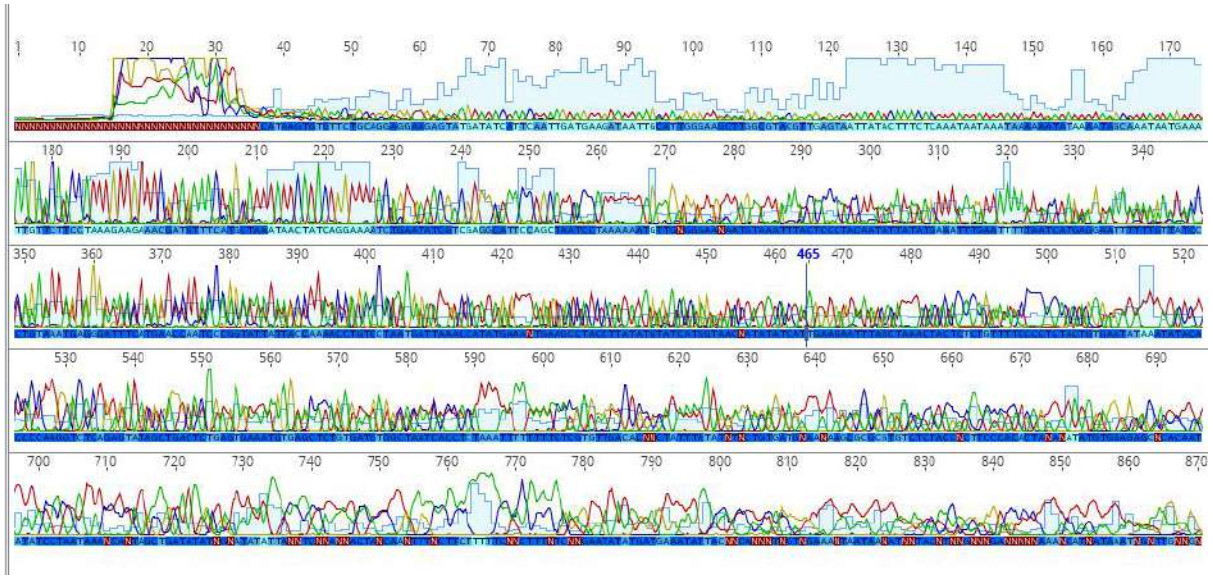

Fig.2a. The nitrogen base sequence of the CO1 RA gene was read with the Geneous Program 6.0

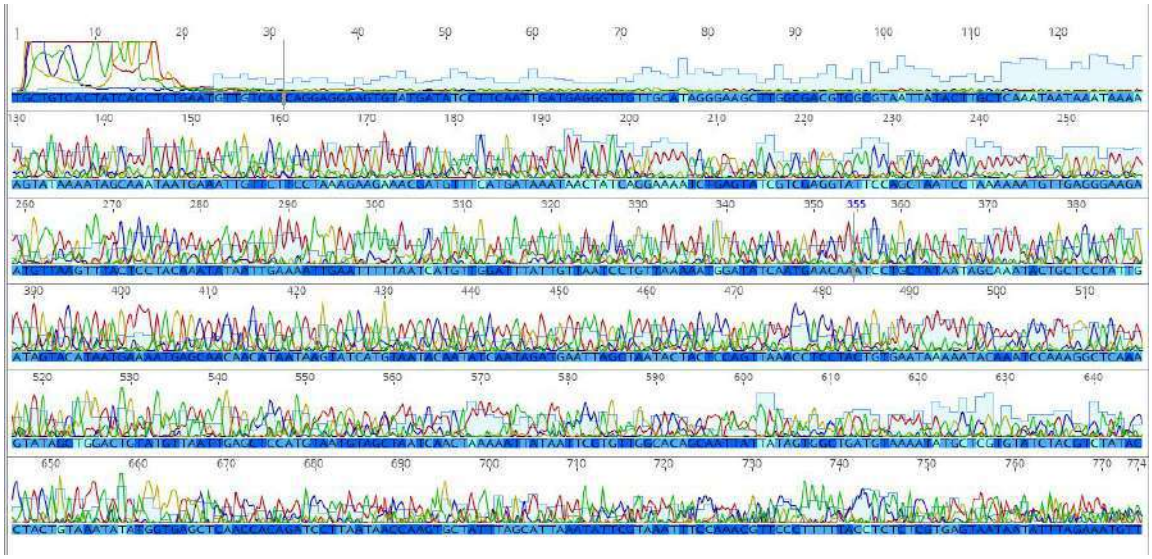

Fig.2b. The nitrogen base sequence of the CO1 gene HAS been read with the Geneous Program 6.0

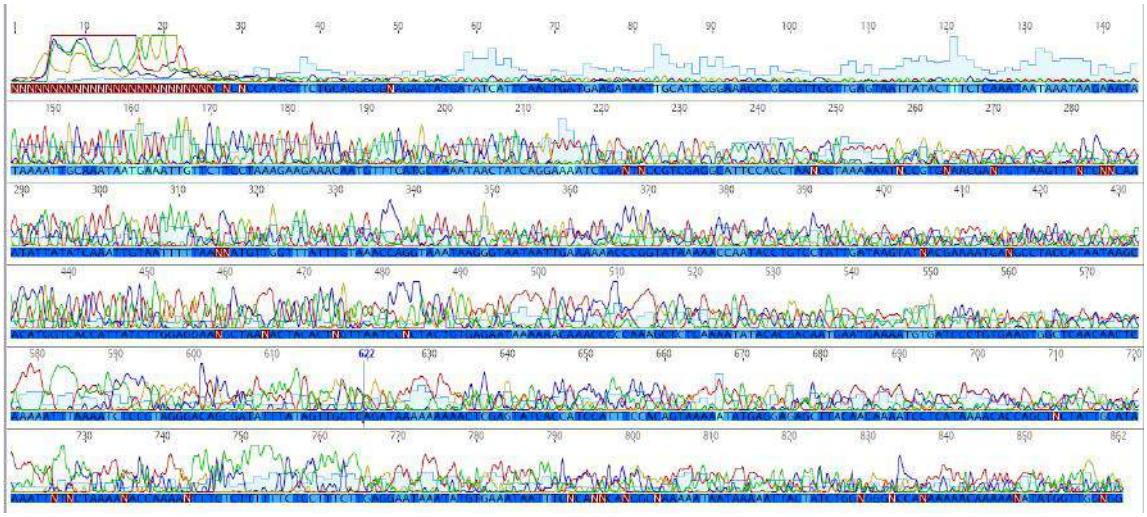

Fig.2c. The nitrogen base sequence of the PSA CO1 gene was read with the Geneous Program 6.0

Table 2. Percentage similarity of TLA CO1 gene sequences compared with the top ten Sequences recorded in the NCBI gene bank (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

No	Description	E value	Identic (%)	Accession
1	Anopheles maculatus voucher AMAC20150811V4 mitochondrion, complete genome	0,00	92	KT382822.1
2	Anopheles albitarsis cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product	0,00	89	AF417696.1
3	Anopheles deaneorum isolate D6 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	0,00	89	DQ076230.1
4	Anopheles deaneorum mitochondrion, complete genome	0,00	88	HQ335347.1
5	Anopheles marajoara cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product	0,00	88	AF417699.1
6	Anopheles albitarsis isolate A3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	0,00	88	DQ076206.1
7	Anopheles aquasalis isolate GUA109012 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	0,00	88	KC354821.1
8	Anopheles oswaldoi haplotype H11 cytochrome oxidase subunit I gene, partial cds; mitochondrial	0,00	88	DQ784837.1
9	Anopheles oswaldoi haplotype H10 cytochrome oxidase subunit I gene, partial cds; mitochondrial	0,00	88	DQ784836.1
10	Anopheles marajoara isolate C8 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	0,00	88	DQ076223.1

Table 3. Percentage similarity of CO1 RTA gene sequences compared with the top ten Sequences recorded in the NCBI gene bank (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

No	Description	E value	Identic (%)	Accession
1	Anopheles aquasalis isolate aqua28 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product	4e137	85	AF548901.1
2	Anopheles aquasalis isolate aqua10 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product	4e137	84	AF548894.1
3	Anopheles barbirostris subgroup clade III isolate SMMULZ3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	5e136	83	KM610037.1
4	Anopheles barbirostris subgroup clade III isolate SMMUPR3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	5e136	83	KM610022.1
5	Anopheles oswaldoi haplotype H09 cytochrome oxidase subunit I gene, partial cds; mitochondrial	5e136	83	DQ784835.1
6	Anopheles oswaldoi haplotype H08 cytochrome oxidase subunit I gene, partial cds; mitochondrial	5e136	83	DQ784834.1
7	Anopheles barbirostris subgroup clade III isolate SMMUPR10 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	4e142	80	KM610029.1
8	Anopheles barbirostris subgroup clade III isolate th1.10 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	6e140	80	EU797223.1
9	Anopheles barbirostris subgroup clade III isolate kh3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1e136	79	EU797224.1
10	Anopheles barbirostris subgroup clade III	1e136	79	EU797218.1

	isolate th1.9 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial			

Table 4. Percentage similarity of PSA CO1 gene sequences compared with the top ten sequences recorded in the NCBI gene bank (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

No	Description	E value	Identic (%)	Accession
1	Anopheles aquasalis isolate aqua10 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product	2e100	78	AF548894.1
2	Anopheles aquasalis isolate aqua21 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product	7e100	78	AF548900.1
3	Anopheles aquasalis cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product	1e121	77	AF417697.1
4	Anopheles oswaldoi haplotype H05 cytochrome oxidase subunit I gene, partial cds; mitochondrial	2e119	77	DQ784831.1
5	Anopheles oswaldoi haplotype H01 cytochrome oxidase subunit I gene, partial cds; mitochondrial	2e119	77	DQ784827.1
6	Anopheles oswaldoi haplotype H04 cytochrome oxidase subunit I gene, partial cds; mitochondrial	1e117	77	DQ784830.1
7	Anopheles oswaldoi haplotype H02 cytochrome oxidase subunit I gene, partial cds; mitochondrial	1e117	77	DQ784828.1
8	Anopheles oswaldoi haplotype H03 cytochrome oxidase subunit I gene, partial cds; mitochondrial	5e116	77	DQ784829.1
9	Anopheles marajoara isolate C1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1e111	76	DQ076216.1
10	Anopheles punctipennis cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product	9e104	76	AF417720.1

The results of BLAST NCBI analysis of the CO1 gene sequences of Anopheles sp from Tombatu showed a 92% similarity with Anopheles maculatus [KT382822.1] from China. Anopheles sp from Ratahan based on the CO1 gene has a similarity level of 80% with Anopheles barbirostris [KM610029.1] from China. Anopheles sp from Pineleng has a 77% similarity with Anopheles aquasalis [AF417697.1] from Brazil. (Table 4, Table 5 and Table 6). Alignment results of Anopheles sp from Tombatu showed 6 different nitrogen base sites with sequences similar to Anopheles maculatus [KT382822.1]. Meanwhile, Anopheles sp from Ratahan showed 39 different nitrogen base sites with similar sequences to Anopheles barbirostris [KM610029.1]. Anopheles sp from Pineleng showed 38 different nitrogen base sites with sequences similar to Anopheles aquasalis [AF417697.1] (Table 4, Table 5 and Table 6). The position of the difference in nitrogen bases indicates a mutation that occurs in Anopheles sp from Minahasa.

Table 5. Alignment of Anopheles from Pineleng with Sequences Similar to NCBI Anopheles aquasalis. (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Anopheles aquasalis cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial gene for mitochondrial product
Sequence ID: **AF417697.1** Length: 899 Number of Matches: 1
Range 1: 132 to 884

Score	Expect	Identities	Gaps	Strand	Frame
448 bits(242)	1e-121()	595/773(77%)	38/773(4%)	Plus/Minus	

Features:

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Query  35   TGTTCTGCAGGCGGNAGAGTATGATATCATTCAACTGATGAAGATAATTGCATTGGGAAA  94
Sbjct  884  TGTTCTGCAGGAGGAAGAGTATGATATCATTCAATAGATGAAGATAATTGCATTGGGAAT  825
Query  95   CCTGGCGTTCGTTGAGTAATTATACTTTCTCaaataataaataagaaatataaaattgca  154
Sbjct  824  GCTGGCGTTCGTTGAGTAATTATACTTTCTCAAATAATAAATAAAAAGTATAAAATAGCA  765
Query  155  aataatgaaatTGTTCTTCCTAAAGAAGAAACAATGTTTCATGCTAAATAACTATCAGGA  214
Sbjct  764  AATAATGAAATTGTTCTTCCTAAAGAAGAAACAATATTTCAAGTTAAATAACTATCAGGA  705
Query  215  AAATCTGANTNCCGTCGAGGCATTCCAGCTAANCCTAAAAAATNCCGTGNAACGANTGTT  274
Sbjct  704  AAATCAGAGTATCGTCGAGGTATTCCTGCTAATCCTAAAAAATGTTGAGGGAAAAATGTT  645
Query  275  AAGTTTN-TCNN-CAAATATTATATCAAATTGTAATTTTTAANNATGTTGG-TTTATTTG  331
Sbjct  644  AAATTTACTCCAACAAATATTATAGAAAATTG-AATTTTTAATCAAGTAGGGTTTATTGT  586
Query  332  TAAACCAGGTAAATAAGGGTAATAATTGAAAAAACCCGG-TATAAAAACCAATACCTG-T  389
Sbjct  585  TAATCCTGTTAAAAGAGGGTA-TCAATGAATAAATCCTGCTATAATAGCAAATAC-TGCT  528
Query  390  CCTATTGATAAGT-ATNACGAAAATGANGC--CTACCATAATAAGCA-CATG--GTCACC  443
Sbjct  527  CCTATTGATAATACATAATGGAAATGA-GCTACTAC-ATAATATGTGTCATGTAGT-ACA  471
Query  444  AT-TATCTTGGAGGA-ANGCTAANACTACACTNGTTAATCCNC-TACTGTGAGAATAAAA  500
Sbjct  470  ATGTCAATTGAAGAATTAGCTAAAACTACCCCAGTTAATCCACCTACAGT-A-AATAAAA  413
Query  501  A-ACAAACCCCCAAAGCTCTCAAAATATA-CACGACAATGAATGAAAA-TGTGATCCCTG  557
Sbjct  412  ATACAAATCC-AAATGCTC--AAAGTATAGCTGGGCTAT-A-TGTTAATTGTGTTCCATG  358
Query  558  TGAAGTGGCTCAACAACTCAAAAATTTAAAATCTCC-GTAGGGACAGCGATATTTATAGT  616
Sbjct  357  CAAAGTGGCTAATCAACT-AAAAATCTTAA-T-TCCTGTAGGAACGGCAATAATTATAGT  301
Query  617  TGGTCAGATaaaaaaaaaCTCGAGTATCACCATCCATTTCCACAGTAAAAATATGAGGAG  676
Sbjct  300  AGCTGAAGTAAAATAAG-CTCGAGTATCTACGTCTATTCCAACAGTAAATATATGATGAG  242
Query  677  AGCTTACAACAAAATCCTCATAAAACACCAGCTNCTATTGCATAAAATTNTNCTAAAANA  736
Sbjct  241  CTCAAACAATAAA-TCCTAATAATCCAATTGCTAGTATAGCATAAATTATTCCTAAATTT  183
Query  737  CCAAAANTTTCTCTTTTTTCTCCTTTCTTGAGGAATAAATATGTGAAATAATT  789
Sbjct  182  CCAAAAGTTTC-CTTTTTACCTCTTTCTTGAGTAATAA-TGTGTGAAATTATT  132
```

Table 6. Alignment of Anopheles from Tombatu with Similar Sequences on NCBI Anopheles aquasalis. (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Anopheles maculatus voucher AMAC20150811V4 mitochondrion, complete genome

Sequence ID: **KT382822.1** Length: 14850 Number of Matches: 1

Range 1: 2177 to 2919

Score	Expect	Identities	Gaps	Strand	Frame
1044 bits(565)	0.0()	686/745(92%)	6/745(0%)	Plus/Minus	

Features:

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Sbjct  2919  GCAGGAGGAAGGGTATGATATCATTCAATTGATGAAGATAGTTGTATAGGAAAACTTGGT  2860

Query  91    G-ACGTCGCGTAATTATACTTGCTCAAATAATAAATAAAAAGTATAAAATAGCAAATAAT  149
Sbjct  2859  GTTCGTTGTGTAATTATACTTTCTCAAATAATAAATAAAAAGTATAAAATAGCAAATAAT  2800

Query  150   GAAATTGTTCTTCCTAAAGAAGAAACGATGTTTCATGATAAATAACTATCAGGAAAATCT  209
Sbjct  2799  GAAATTGTTCTTCCTAAAGAAGAAACGATGTTTCATGATAAATAACTATCAGGAAAATCT  2740

Query  210   GAGTATCGTCGAGGTATTCCAGCTAATCCTAAAAAATGTTGAGGGAAGAATGTTAAGTTT  269
Sbjct  2739  GAATATCGTCGAGGTATTCCTGCTAATCCTAAAAAATGTTGAGGGAAGAACGTTAAATTT  2680

Query  270   ACTCCTACAAATATAATTGAAAATTGAATTTTTAATCATGTTGGATTTATTGTTAATCCT  329
Sbjct  2679  ACTCCAACAAATATAATTGAAAATTGAATTTTTAATCATGTTGGATTTATTGTTAATCCT  2620

Query  330   GTTAAAAATGGATATCAATGAACAAATCCTGCTATAATAGCAAATACTGCTCCTATTGAT  389
Sbjct  2619  GTTAATAATGGATATCAATGAACAAATCCTGCTATAATAGCAAATACTGCTCCTATTGAT  2560

Query  390   AGTACATAATGAAAATGAGCAACAACATAATAAGTATCATGTAATACAATATCAATAGAT  449
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Query  450   GAATTAGCTAATACTACTCCAGTTAAACCTCCTACTGTGAATAAAAATACAAATCCAAAG  509
Sbjct  2499  GAGTTAGCTAATACTACTCCAGTTAATCCTCCTACTGTAAATAAAAAAACAAATCCAAAG  2440

Query  510   GCTCAAAGTATAGCTGGACTGTATGTTAATTGAGCTCCATGTAATGTAGCTAATCAACTA  569
Sbjct  2439  GCTCAAAGTATAGCTGGACTGTACGTTAATTGAGTTCCGTGTAATGTAGCTAGTCAACTA  2380

Query  570   AAAATTATAATTCCTGTTGGCACAGCAATTATTATAGTGGCTGATGTAAAATATGCTCGT  629
Sbjct  2379  AAAATTTTAATTCCTGTAGGTACAGCAATAATTATAGTAGCTGATGTAAAATATGCTCGT  2320

Query  630   GTATCTACGTCTATACCTACTGTAAATATATGGTGAGCTCAACCAC-AGATCCTTAATAA  688
Sbjct  2319  GTATCTACGTCTATTCCTACTGTAAATATATGATGAGCTCAAACAATAAATCCT-AATAA  2261

Query  689   -CCAAGTGCTATT-TAGCATTAAATATTCGTAAATTTCCAAACGTTCCCTTTTTACCTCT  746
Sbjct  2260  TCCAATAGCTAGTATAGCATAAATTATTCCTAAATTTCCAAATGTTTCCTTTTTACCTCT  2201

Query  747   CTCGTGAGTAATAATATTTAGAAAT  771
Sbjct  2200  TTCTTGAGTAATAATATG-AGAAAT  2177
```

Table 7.Alignment of Anopheles of Ratahan with Similar Sequences in NCBI Anopheles aquasalis. (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Anopheles barbirostris subgroup clade III isolate th1.10 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
Sequence ID: **EU797223.1** Length: 756 Number of Matches: 1
Range 1: 62 to 756

Score	Expect	Identities	Gaps	Strand	Frame
508 bits(275)	6e-140()	570/715(80%)	39/715(5%)	Plus/Minus	

Features:

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Query  5    AGTGTGTTCTGCAGGAGGAAGAGTATGATATCATTCAATTGATGAAGATAATTGCATTGG  64
Sbjct  756  AGTATGTTCAGCAGGTGGAAGAGTATGATATCATTCAATTGATGAAGATAATTGTATTGG  697
Query  65   GAA-GCTTGGCGTACGTTGAGTAATTATACTTTCTCaaataataaataaaaaatataaaa  123
Sbjct  696  GAAAGC-AGGTGTACGTTGAGTAATTATACTTTCTCAAATAATAAATAAAAAATATAAAA  638
Query  124  tagcaaataatgaaatTGTTCTTCCTAAAGAAGAAACGATGTTTCATGCTAAATAACTAT  183
Sbjct  637  TTGCAAATAATGAAATTGTACTACCTAAAGAAGAAACAATATTTCAAGCTAAATAACTAT  578
Query  184  CAGGAAAAATCTGAATATCGTCGAGGCATTCCAGCTAATCCTAAAAAAATGTTGNGGGAANA  243
Sbjct  577  CAGGAAAAATCAGAATATCGTCGAGGTATTCCAGCTAATCCTAAAAAAATGTTGTGGAAAGA  518
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Sbjct  517  AAGTTAAATTTACTCC-TACAAATATTATAGCAAATTGTAACTTT-AATCAAGAAGGATT  460
Query  302  TTTTGTTATCCCTGT-AAATGAGGGATTTC-ATGAACCAATCCCGGTATTATTACCAAA-  358
Sbjct  459  TATAGTTAATCCTGTTAAAAGAGG-ATATCAATGAACAAATCCTGCTATAAT-AGCAAAT  402
Query  359  ACCTGTCCTAATGATTAAACCATA-TGAAANTGAAGC--CTACCTTATATGTATCATGGT  415
Sbjct  401  ACAGCTCCTATTGATAAAAC-ATAATGGAAATGA-GCAACTACATAATATGTATCATG-T  345
```

This genetic variation is supported by the results of morphometric analysis which indeed show differences in several morphometric characters, including the shape of the strip on the pronotum and the color of the antennae tip on Rhynchoporus sp. cream-colored sago palm and Rhynchoporus sp. in black sugar palm (Korua et.al. 2015). Polymophism can occur in a population if more than one morphological variation is found at the same location and time (Ford, 1965, Abad et. al. 2014). If random mating occurs and each individual has the potential to mate, then morphological changes can take place in a population (Abad et. al. 2014).

Phylogenetic Analysis and Construction

The substitution matrix between Anopheles sp from Tombatu, Ratahan and Pineleng was compared with 22 BLAST sequences at the NCBI site built using the Maximum Likelihood Model on the MEGA 6.0 program. The form of transitional substitution is indicated by the numbers in bold in table 6. While the transversional substitution is written in italics in table 6. Nucleotide frequency A = 33.70%, T/U = 36.75%, C = 14.11% and G = 15.44 %. The maximum value of the Log Likelihood from the calculation results is 9678,156.

Table 8. Estimation of the Maximum Likelihood Model Substitution Matrix in the MEGA 6.0 . Program

	A	T/U	C	G
A	-	*8.24*	*3.16*	**3.71**
T/U	*7.55*	-	**12.03**	*3.46*
C	*7.55*	**31.33**	-	*3.46*
G	**8.09**	*8.24*	*3.16*	-

Phylogenetic construction

The phylogeny construction was carried out using two models, namely Neighbor Joining and UPGMA. These two models are used because they have similarities, namely the evolutionary approach and to see the position of the Anopheles sp species from Minahasa. The Neighbor Joining model phylogeny construction was built with 22 sequences similar to the NCBI BLAST results. Three monophyletic clades were formed, where Anopheles sp from Tombatu and Ratahan were in the same clade while Anopheles from Pineleng was in its own clade. The phylogenetic tree construction using the UPGMA model also placed Anopheles sp from Pineleng in its own node, while Anopheles sp from Tombatu and Ratahan formed the same node but still in one monophyletic clade. In the UPGMA model, only 2 monophyletic clades were formed.

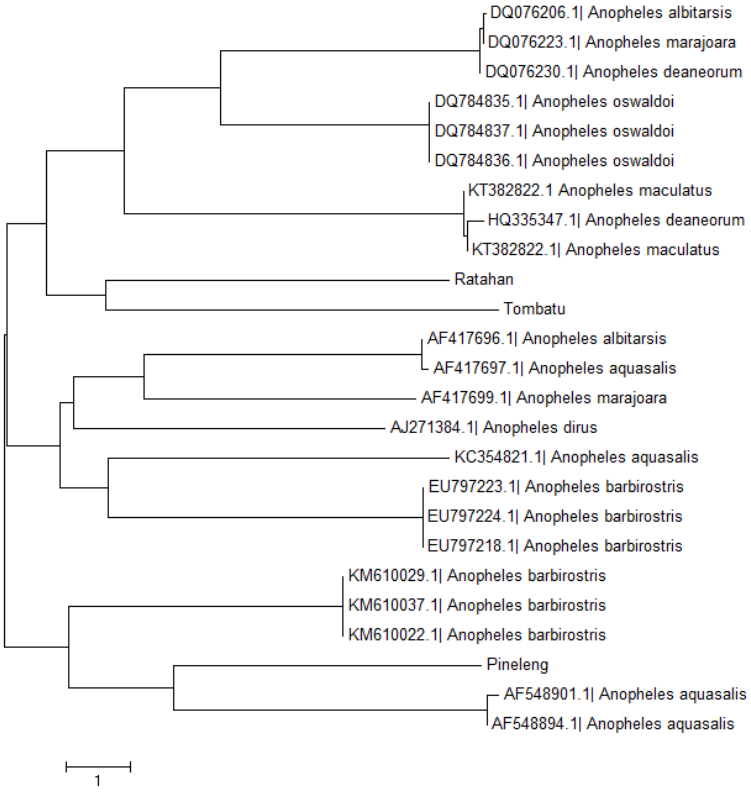

Fig.3: Anopheles sp phylogeny tree from Tombatu, Ratahan and Pineleng compared to 22 BLAST sequences at the NCBI site, built using the Neighbor Joining Model, bootstrap 1000 x.

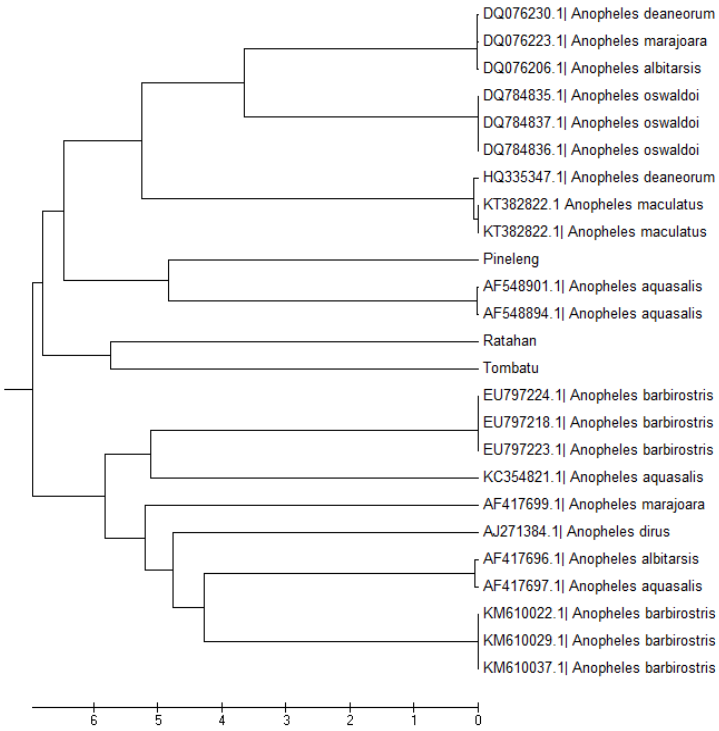

Fig.4: Anopheles sp phylogenetic tree from Tombatu, Ratahan and Pineleng compared to 22 BLAST sequences at the NCBI site, constructed using the UPGMA Model, bootstrap 1000 x.

IV. CONCLUSION

1. Based on the CO1 gene, the mitochondrial DNA of Anopheles sp from Tombatu is 92% similar to Anopheles maculatus [KT382822.1] from China. Anopheles sp from Ratahan based on the CO1 gene has a similarity level of 80% with Anopheles barbirostris [KM610029.1] from China. Anopheles sp from Pineleng has a 77% similarity with Anopheles aquasalis [AF417697.1] from Brazil.

2. The CO1 gene sequences of Anopheles sp from Southeast Minahasa (Tombatu and Ratahan), and Anopheles sp from Minahasa (Pineleng) have nitrogen base size differences of more than 6%. Thus, the variation of the Anopheles sp CO1 gene is relatively high compared to similar sequences that have been recorded on the NCBI gene bank site.

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Green Logistics and Alternative Methods: A Review

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Abstract**—** *When logistics activities are evaluated, it creates awareness on consumers. The understanding of sustainability, which has developed and grown in recent years, has brought important developments in logistics applications. These developments have found a place in the understanding of the green logistics approach. With this understanding, it has found a place in the methods and practices of logistics. With this understanding, it has developed the organizational structures of logistics. In this understanding, the aim is not to harm the environment. On the other hand, rationalizing high energy costs is considered within this scope. Sustainability policies, which have become more important in the recent period, also show their effect in logistics applications. This also aims to give companies a competitive advantage. In this context, sustainability studies on social, ecological and economic issues have highlighted the "green logistics" approach. In addition to the developing retail sector, companies in this sector are trying to implement green logistics practices within the scope of trend logistics activities in order to stay in the market. In this study, the environmental impact of the measures taken by the companies in the field of green logistics practices and different sales strategies were examined. In this context, the effects of energy on the usage areas are evaluated and green logistics applications are discussed. In addition, a common framework was created by examining the success rates of green logistics applications. In this context, the scope of green logistics and its basic applications, followed by alternative green logistics applications are listed.*

Keywords**— Sustainability, Logistics, Green Logistics.**

I. INTRODUCTION

Towards the end of the 80s, the concept of "green", which opened a new era in the transportation sector, allowed to create new searches with the problems occurring in transportation. In the late 80s and early 90s, the concept of green became a new competitive slogan. The World Commission on Environment and Development Report provided support on green issues in the political and economic field (Banister et al. 1993). At the beginning of the 90s, studies were started on how the concept of green and the logistics sector are blended together.

Along with green logistics, it envisaged measuring the environmental effects of different distribution methods for goods produced and distributed in a sustainable way. At the same time, it aimed to reduce the use of energy in logistics operations. Again, the goal of green logistics is to protect biodiversity. However, sustainable activities and reducing carbon emissions are also included in the scope of environmentally friendly logistics systems. Another goal of green logistics, such as reducing environmental pollution, has been an application area that allows the method of operation of a logistics system to be viewed from a different perspective.

With the effect of increasing environmental awareness in the world, the importance and necessity of green logistics has increased with the legal infrastructure prepared by the green agreement in Europe. As a result of the activities of not only logistics, but also all sectors, there is a carbon emission released to the nature. The investments made within the scope of incentive investments of around 1 trillion Euros that the European Union will make for the studies in this field show the importance of the transformations in this field. Compliance and transformation in this field is much more important because when companies operating in this field are not compatible with this field, tax burdens such as carbon tax

at the border will increase, competition conditions will become more difficult, and commercial reputations will be damaged due to the increasing awareness in this field.

Turkey is developing many environmentally friendly practices within the scope of green logistics by many companies. While it is possible to reach earlier studies on the concept of green logistics in foreign countries, compared to Turkey, Turkey has only recently become acquainted with this concept.

Our aim in the study is to examine and research today's green logistics studies and to investigate to what extent companies include green logistics practices in their activities. At the same time, in this context, it offers suggestions about how the legal legislation should be. The method of the study consists of a literature review. After the general outlines of the subject were formed, the literature review was reduced to a summary by researching world examples.

Importance of Green Logistics and Effects on Businesses to Green Logistics

Sustainability, legal obligations, environmental concerns and the social responsibility understanding that emerged in the logistics sector, together with the recently increasing environmental sensitivity to the issue, pushes companies to participate in green logistics activities. The importance of green logistics, economic factors, has become an important label that guarantees customer satisfaction in the market. The importance of green logistics is increasing day by day due to factors such as green image and environment-oriented government programs (Acar and Köseoğlu, 2014: 352).

Logistics activities should be managed in an environmentally friendly manner. The use of more environmentally friendly raw materials in packaging and the search for alternative transportation methods are typical examples of this. Companies take various measures to reduce the harmful effects of traffic on the environment. Judging from these measures, they focus on noise pollution and traffic congestion, while considering reducing fuel costs to the most economical level. When buying a vehicle, a green company should prefer unleaded gasoline-powered vehicles and apply for long-distance rail transport. On the road, where road transport is essential, transport methods in accordance with the green understanding should be selected. The fuel used should be consumed in a way that does not pollute the environment. Environmentally friendly motor vehicles should be preferred (Aslan, 2007: 40).

II. GREEN LOGISTICS PRACTICES

There are many applications related to green logistics in the world and in our country, if we will consider them mainly (Gültaş and Yücel, 2015:75, www.tobb.org.tr);

- Use of alternative fuels
- Encouragement and dissemination of the use of environmentally friendly motor vehicles
- Promotion of public transportation and support of infrastructures
- Promotion of cycling
- Obligation to use noise and sound-preventing equipment in vehicles
- Use of environmentally friendly transportation and distribution systems
- Reducing overall packaging and materials used
- Use of materials with shorter recycling in packaging
- Use of sustainably produced pure products
- Increasing and planning environmentally friendly recycling activities
- Social awareness and educational practices
- Promotion of reverse logistics practices.

Advantages of green logistics

- By adding originality to the service production system and operating procedures, it provides learning how to deal with environmental impacts and current or future regulations and these changes.
- Increases the quality and efficiency of service and product delivery.
- Needs green economy enables innovative decisions to be made. These restrictions push companies further in their efforts to reduce operating costs.
- When you develop products that meet environmental requirements, a difference is made in the services and products offered.
- Being environmentalist strengthens the company's marketing and advertising policy. It gives strength especially in the sales area.
- It raises the awareness of sensitive consumers in the fields of sustainability and social responsibility.

GREEN APPROACH IN SUPPLY CHAIN MANAGEMENT

Green supply chain can be defined as a chain of processes in which environmentally friendly inputs are used in production and these inputs are naturally transformed into products that can be easily recycled and do not cause pollution after process changes (Temur, Ayvaz and Bolat,

2015: 1). Green supply chain refers to a system created by integrating environmental objectives into all branches of the supply chain. Procurement consists of a combination of green production (materials management), green distribution, green marketing and reverse logistics studies and the advantages of green technology.

Supply chain management of companies can be listed as follows (Temur, Ayvaz and Bolat, 2015: 2);

Cost reduction profit maximization

Ensuring an effective risk management

Preventing a possible increase in waste costs

Inexpensive fulfillment of environmental legal obligations now and in the future,

Increasing product and service quality

Meeting market expectations is the management of customer relations.

Green logistics in road transport

As road transport, trucks, trucks, etc., which only make intercity and international transport operations in mind. vehicles should not come. In addition, with increasing prosperity and wealth, road transport is used very seriously to provide personal transportation. When we look at today, 40% of the world's oil demand is demanded by road transport. This situation is increasing rapidly from year to year. Naturally, at the end of the process, the share of road transport in carbon emissions increases even more. According to TUIK 2020 data, while CO2 emissions in road freight and passenger transport in Turkey were 30 million tons in 1990, this figure increased threefold in 2018 and reached 90 million tons. Therefore, worldwide road freight and passenger transport has a serious impact on CO2 emissions. In the coming days, it will be extremely necessary for those who want to gain a competitive advantage in this field and who want to continue to exist in the sector, to minimize the effects of CO2 emissions and to update themselves with alternative methods for this. For this, firstly, instead of petroleum-derived fuels, they should use alternative fuels that emit less CO2 emissions to the nature. At the beginning of these is the most widely used "Electricity" energy. However, considering the increasing electricity demand in the world, this is not enough on its own. In addition to electricity, "Biodiesel energies obtained from vegetable oils" and "Bioethanol energies obtained from sugar-based plants", which are also obtained through plants, also constitute an alternative method. Likewise, newly developed and and hydrogen, "Hydrogen energy obtained as a result of combining oxygen and hydrogen" is an alternative method.In addition to these, alternative methods that are more used, "LPG, liquefied petroleum gas and CNG, liquefied natural gas" Among these methods, the method that releases the most CO2 to the nature is the use of energy obtained from LPG or CNG. However, it is expressed as more environmentally friendly when compared to petroleum.1 liter of gasoline, 2500 grams of CO2 While releasing its emissions to nature, this amount is 1600 grams in LPG (Synak, Ghana, Rievaj and Mokrickova: 2019).

Alternative Methods for Green Logistics

1) Electric Bus

It is one of the environmentally friendly transportation methods created for urban public transportation. Electric buses, which are more comfortable and easier to use for cities where the sun is abundant, work with zero CO2 emissions with the solar energy panels placed on them. It is thought that its use will become widespread in other cities, along with its use in cities such as Izmir and Malatya in Turkey. According to the data obtained from the 2-month usage results of the ESHOT Electric Bus Project, which is 40% domestic and used in İzmir, it provides an energy saving of up to 75% (www.emo.org.tr). Likewise, electric buses produced by Bozankaya, which are 100% domestic, are used in Malatya.

2) Trambus

It is one of the alternative methods developed for urban public transportation, which started to be implemented in Malatya in 2015 in Turkey. It is a transportation vehicle with a high passenger capacity due to the fact that it does not have a rail system body, which takes its power from the power line (cataner system) hanging along the road, provides ease of use by charging with the battery system in places where there is no power line (www.bozankaya.com.tr). Since it does not need a rail system infrastructure, the infrastructure cost is low. They are electric buses with freedom of route due to the use of the highway. Trambuses, which are economical because they do not require a rail system and work with zero emissions, are a transportation system that is expected to become widespread even though it is still new.

3) Electric Tram

Developed in 1881 by the German electrical engineer and industrialist Werner von Siemens (1816-1892), the electric tram was put into service on the Berlin-Lichterfelde trial line (https://core.ac.uk). Later, in 1914, it was first used on short lines in the Ottoman Empire, in the city of Istanbul. The system has started to be used on the Şişli-Karaköy line in Istanbul. Electric tram technology, which was very new at that time, could not become widespread in the Ottoman Empire. However, today in Turkey, with the rapid increase in the population of metropolitan cities, its use has

increased. Today, it has become a very preferred transportation system. Recently, electric trams have started to take on green colors and zero-emission electric trams have also started to be produced.

4) Silent night distribution

The European Union, which is the starting point of the Green Agreement, acts more effectively and effectively for green logistics than many other developed countries. For this, it uses special transport systems, transport hours, special transport places and environmentally friendly systems. One of the best examples of this is a pilot study in Barcelona using a special transport clock called the "silent night distribution system". Within the scope of the application, the trucks that will be required for transportation made the distribution at night. In this application, trucks with a capacity of 40 tons were used. These trucks were equipped with a noise-canceling system and were allowed to drive twice, from 11 am to 5 am. At 11 o'clock at night, long-lived goods were transferred, and at 5 o'clock in the morning, short-lived goods that need to be transported within the framework of the cold air chain were transferred (Güvercin, 2018:475). Thus, as the city traffic is not more concentrated during the daytime, a further reduction in the amount of emissions has been observed.

5) Bicycle transport

Logistics is not just a long-distance transportation system. At the same time, the concept of logistics is used within short distances. Many vehicles and engines are used in this. Although it is not very common today, there is actually "Bike Transport". It is more common in countries with green consensus and environmentally friendly laws. "Getir" brand, which is one of the most preferred brands for urban logistics in Turkey, offers its services with motorcycle couriers. However, in countries with environmentally friendly laws, such as Germany, this service is by bicycle and electric bicycle. In the transportation sector, if we go back to the history of an old transportation system, which is not really new, it will be necessary to go as far as India. Lunch has been delivered since 1890, with the oldest bicycle transport service in Mumbai, India. Today, in this area, more than 175,000 light goods are transported in Mumbai every day by 5,000 carriers (Maes and Vanelslander, 2012: 415).

Image:1 An official delivering food in Mumbai

Although it is more common in Europe, the use and transportation of bicycles is also encouraged in Turkey. For this, necessary bicycle paths, bicycle lanes and necessary infrastructures are prepared. Cycling is much more advantageous in urban transportation and transportation, as there is no parking problem and it has the advantage of being used in areas with heavy traffic. In the future, with the increase in e-commerce, small deliveries will increase even more, and with the increasing traffic density, it will be inevitable that the advantages of bicycle transportation will be reflected in logistics applications. It is thought that this situation will increase much faster once the logistics companies are noticed. Because cycling not only reduces carbon emissions, but also brings other advantages such as time savings and cost reduction. Again, in a review, it was investigated how much of a freight transport could be converted from road to bicycle transport in European cities. According to the findings obtained from the research, it was concluded that 7 km distances are acceptable distances for bicycle transportation. Within the scope of the research, 7 km distance is assumed as the transformation potential. This distance was accepted as the acceptable distance and calculations were made over this distance. According to the report in question, the crowd created by freight vehicles in traffic was also examined. According to the findings, freight transport vehicles constitute 8-15% of vehicle mobility for French traffic. This is 15% for Switzerland. According to the information obtained from the report, it has been observed that freight transport vehicles constitute 20% of Berlin traffic. (http://www.cyclelogistics.eu).

6) Logistic Villages

With the increase in the necessity and importance of green logistics, logistics has created logistics villages as a new concept and method. Although it is used in definitions such as freight village, logistics area, logistics center, logistics focus, logistics park, logistics base, logistics distribution park in Turkey (Tanyaş, 2010), these areas heavily overlap with the concept of logistics village. It is said that the idea of the logistics village first emerged in Japan. However, the first application area of the logistics village was in the USA, due to the increasing urban density and rapidly developing industry. Later, it started to be applied in other developed countries such as EU countries and Japan. Along with the widespread logistics villages, logistics activities have also become more efficient. In particular, logistics villages built in places where railway, road and seaway can be used together have become even more efficient. Therefore, the most critical move in the construction of logistics villages has been the choice of location. When choosing a location, the chosen location should be an advantageous location for both the transportation and distribution systems required for logistics. Besides, it should be a place that will not increase the density of city traffic with its distance to the city. Already, the purpose of its establishment is due to the fact that the vehicles in freight transportation increase the density of city traffic. In the USA, logistics villages have been established in order to prevent traffic congestion caused by heavy transport vehicles in the city. In Europe, the areas close to the main transportation networks were preferred for logistics villages by reducing the environmental pollution caused by the development of low-growth industrial areas and truck transportation. In this way, these old industrial areas, which allow intermodal transportation, have been used in a more consolidated way. When all these are considered in terms of Europe, the importance of logistics villages can be understood within the concept of sustainable development (Maes and Vanelslander, 2012: 415).

III. RESULT AND SUGGESTIONS

As a result, the tough competition environment brought by globalization, laws and regulations to protect the environment, customer power, activities in the field of corporate social responsibility bring the green perception to the fore and make it more important. In this context, the management of green logistics practices by logistics companies will bring along strategic decisions. In the context of these strategic decisions, they need to demonstrate environmental awareness in all areas of management and logistics practices. For this purpose, it is essential that they create a stronger entrepreneurial awareness than ever before. Businesses, industries and companies providing logistics services should focus on meaningful applications. At the same time, they should provide more value and benefits to their suppliers in terms of environmental awareness in their production and management processes. In this context, they should develop guidelines and strategies. In the process, they should always be open to development and change in accordance with the green consciousness. If it is desired to leave a world worth living for future generations, it is necessary to act with this awareness in the field of logistics, as in every field. In this context, all players in the logistics sector should define green logistics practices as a mission and target. In their business processes, they should implement green logistics practices as soon as possible.

This situation can be viewed from another angle. Developing countries are in fierce competition with developed countries in the field of technology. In order to compete with developed countries in the field of technology, many infrastructures need to be developed. However, in order to compete in green logistics applications, government incentives and increased awareness are required. Considering from this point of view, in terms of green logistics in Turkey, the necessary breakthrough and legal infrastructure should be made urgently. With the legal implementation of these practices, significant contributions will be made to Turkey economically, along with the contributions to the environment.

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Analysis of Productivity of Purse Seine Catch Fisheries in Tpi Lonrae, Bone Regency of South Sulawesi

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Abstract— *The purpose of this study was to analyze the productivity level of purse seine in Bone Regency, South Sulawesi. The study was conducted from August to November 2020. The method used is Quantitative Descriptive by using questionnaire tools. The method used is the Random Sampling method with the number of 10 fishermen. The data analysis used is the total production of handlers by summing the catches per season in one year. The results showed that the productivity of purse seine fishery products was quite profitable with an average receipt of Rp. 2,690,038,750 per year. Explained that the total revenue in 1 year of fishermen purse seine in TPI Lonrae amounted to Rp. 2,690,038,750 the largest income was in the eastern season of Rp. 1,561,146,340.00 while the smallest income was in the western season of Rp. 323,830,295.00. This difference in income is caused by the difference in sea trip thus resulting in a difference in the number of catches and the amount of operational costs incurred.*

Keywords— ***Operating Expenses, Productivity and Revenue.***

I. INTRODUCTION

Bone County is one of the potential areas in the field of marine and fisheries. Over the past five years the number of fishing gear, especially purse seine fishing gear has increased in 2015 the number of fishing gear by 115, in 2019 increased to 183 units. Fish that are the main destination for fishing from purse seine are fish that pelagic shoaling species" or pelagic fish that cluster (Zulkarnain et al., 2020) Thus, the catch of this seine purse fishing gear affects the production of fishing products (Rosana & Viv Djanat Prasita, 2018).

The production of fishery products achieved through fishing efforts at sea in 2019 production amounted to 34,556 tons, and decreased production when compared to 2020 production of 33,504 tons and 2018 production of 25,073.4 tons (Utami et al., 2020) Of the fishing gear that dominates catches, especially fishing, is purse seine fishing equipment where in 2013 the amount of production is 2,306 tons, While in 2018 there was an increase in the amount of production by 15,137 tons. (Bone county Marine and Fisheries Service, 2015-2019).

Based on the data shows that purse seine fishing gear is still productive and still a prima donna. But on the one hand the condition of the field shows that purse seine fishing gear made tends to vary, this can be seen from the capacity of large ships but fishing gear is small and vice versa the capacity of small boats but fishing gear is long and deep, there are also variations that occur based on the use of ship dimensions and machinery, in addition to the design and construction of purse seine fishing gear is generally assembled by itself and based on the experience of fishermen for generations. (Rumpa A, Najamuddinr, 2017).

Productivity of fish catches using purse seine fishing gear is related to the effect of suitability of fishing gear dimensions, ship capacity and fishing aids. Some studies on the analysis of technical aspects related to the design and construction of purse seine include research on

the speed of sinking purse seine fishing gear (Dwi Ujiti, 2017).

Purse seine is a fishing tool whose productivity and effectiveness are relatively higher when compared to other fishing tools, because in its optimization can catch large amounts of fish, which is as much as 435.79 tons. The development of purse seine fishing methods is influenced by the main target fish resources of purse seine fishing efforts. The existence of fish resources can be known by the level of vertical distribution and horizontal distribution (swimming layer) of pelagic fish, because the type of pelagic fish is a fish that lives in the middle layer (mid layer) to the surface of the water. Pelagic fish can be divided into two groups, namely small and large pelagics (Mirnawati et al., 2019).

II. RESEARCH METHODS

A. Time and Place

This study was conducted during 4 months August-November 2020. As for the location of this research in TPI Lonrae District Tanete Riattang, Bone Regency. The sample of this study is an ABK fisherman who destroys all his work on the ship.

B. Type of Research

This research is conducted by survey methods, namely by conducting observations in the field and interviews directly with respondents, and using questionnaires as data collection tools that have been obtained quantitatively analyzed.

C. Sampling Methods

This study uses the Random Sampling method where respondents or samples are randomly selected by taking the research location as a fishing base, namely TPI Lonrae in Tanete Riattang District, Bone Regency.

D. Data Source

The data sources used in this study are primary and secondary data including:

1. Primary data is primary data conducted with a structured interview using a list of questions (questionnaires) supported by direct observation of the activities of kite fishing fishermen.
2. Secondary data collection is obtained from the Marine and Fisheries Service, Subdistrict Office, Village Office and BPS. The data collected includes geographical conditions and regional administration, population conditions, state of fishery facilities and infrastructure, kite fishing effort data and kite fish production data over the past 10 years (2010-2019).

E. Data Retrieval Techniques

The research data is obtained with the following stages:

1.Field observation to find out the general condition of the research site

2. Questionnaire (questionnaire)

Questionnaire or questionnaire is a technique of collecting data by giving a set of written questions to the recess to be answered. Literature studies are collecting data with documentation studies, reading literature or research results that are considered relevant to the research theme.

F. Data Analysis

Data obtained both primary data and secondary data in quantitative and qualitative analysis.

To answer the first problem how the level of productivity and income of fishermen catch purse seine in Bone Regency, South Sulawesi, then in the following analysis:

a. total production of arrests

To find out the total production of purse seine capture units is calculated by summing the catch per season in one year (eastern season, transition season, and western season).

$$\text{Productivity} = \frac{\Sigma \text{ production of pelagic fish}}{\Sigma \text{ attempted capture of purse seine}}$$

Where:

Σ Production is an attempt to combine input factors at a given level of technology to produce as efficiently as possible.

Σ capture efforts to produce fish production using purse seine fishing gear.

III. RESULTS AND DISCUSSIONS

The results of identification in the field there are 3 types of catches that are dominantly caught in this study. The three types of catches are small pelagic fish. The three small pelagic fish include: Kite (decapterus ruselli), Bentong selar (Selar crumenopthalmus) and Sunglir (Elagatis bipinnnulata). The number of catches per type can be seen in the chart below.

Table 1. Percentage of purse seine productivity during study

Number	Type Of Fish	Month (Kg)				sum (Kg)	Percentage
		August	September	October	November		
1	Layang	5,987	4,345	3,023	3,022	16,377	87%
2	Selar Bentong	1,024	481,25	366,22	300,13	2,171	12%
3	Sunglir	176,25	44,5	21,3	13	254	1%
Sum						18,802	100%

In table 1 above, the number of catches tends to decrease month by month during the study. Sunglir which was still able to be found in the first 3 months of research (August to October), which is from 176, 25; It is then significantly reduced to 20 kg, in the fourth month disappearing as the eastern season enters. Selar bentong which in the 1st month (August) amounted to 1024 Kg, significantly reduced to 481.25 Kg in the 2nd month (May), which was further gradually reduced until the 4th month (November). In contrast to the condition of the kite which although declining significantly, but still consistent with its appearance. The increase in the number of kites at the end of the study amounted to 2,595 Kg in October to 2,645 Kg in November. Consistency is likely to rise slowly as the entry of the western season transition begins (January-March).

The phenomenon of succession of abundance of natural resources from season to season is common, considering the dynamic of the factors forming the seasons. The fishing season by purse seine from Bone County lasts all year round and this reality is not in line with the existence and abundance of some types of catches.

Table 2. Average productivity (Kg/trip)

Month	**Productivity (Kg/Trip)**
August	**898, 375**
September	**608,75**
October	**426,25**
November	**416,875**

In Table 2, the productivity of catches obtained is August amounted to 898,375 Kg, September 608.75 Kg, October 426.25 Kg, and November amounted to 416,875 Kg. Based on the results in table 2 can be seen again where productivity in August-November can be said to decrease because in that month it is known by fishermen in Kab Bone as the famine season.

Fluctuations in fish production above are understood by fishermen as a phenomenon that routinely occurs in the gulf of bone, where January - March by bone regency fishermen is known as the peak fishing season in July - September as the fishing panceklik season. The months that last both seasons are known as the transition season or the regular season. The phenomenon of this fishing season directly affects the efforts of fishing by purse seine fishermen.

IV. CONCLUSION

Based on the results of research that has been conducted in TPI Lonrae District Tanete Riattang Bone Regency, it can be concluded that:

The highest production of purse seine catch unit yields at TPI Lonrae in the eastern season, which led to greater fishermen's revenues in the eastern season in one year.

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Income Contribution of the Fisherman wife Fishing Business Nike (Ophieleotris Aporos) Lake Tondano in increasing Household Income in Kaima Village, Remboken District, Minahasa Regency

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Abstract— *The contribution of the wife of a nike fisherman (Ophioletris aporos) is triggered by increased household needs, while the husband's income as a nike fisherman (O. aporos), seems to be inseparable from the inadequacy. This is also experienced by Nike fishermen in the Lake Tondano area in general and in Kaima Village in particular. Their low income requires the wife to contribute in meeting household needs.The purpose of this research is to find out and learn what are the contributions of the wife of the nike fisherman (O. aporos) in Kaima Village, Remboken District, Minahasa Regency. and knowing how much the wife of the nike (O. aporos) fisherman contributes to improving the welfare and quality of life of the Nike (O. aporos) fisherman's household, so it is necessary to realize effective management as a way of sustaining their life.The method used in this research is purposive sampling, data sources are primary and secondary data, data analysis using qualitative descriptive analysis and qualitative descriptive analysis.The results of this study are the wives of fishermen who catch Nike Lake Tondano in Kaima Village, Remboken District, have a high contribution to the household economy. The contribution given by the wife of a fisherman who catches Nike Lake Tondano in Kaima Village, Remboken District is 50.7%.*

Keywords—***Contribution, Fisherman's Wife, Nike Fish, Kaima Village.***

I. INTRODUCTION

Women especially as wives are citizens who have the same opportunities as men in all fields of fisheries development in addition to their duties and responsibilities as well as their roles to foster healthy, strong future generations in the context of full human development, women's abilities need to be improved in balancing additional tasks. and obligations as a wife or mother imposed on him. This is a national development activity including fisheries management where women as mothers or wives have skills, so they are able to participate in all fields (Suwondo, 1990).

In line with the increasingly complex life and the increasingly heavy burden of the family's economy, the duties and roles of women, especially wives in the family and society, are increasingly needed. The contribution of women today is not only in activities within the family sphere, but many areas of life in society require a woman's touch in handling them, so that every activity carried out is increasingly evident in supporting family life (Murawaro, 2007).

Women as wives of fishermen who catch fish are generally involved in activities to earn a living for their families (Soenarno, 2006). The family or household is one of the smallest social units consisting of father, mother and children (Diknas, 1997). Furthermore, Mantjoro et al. (2003), stated that the family is the smallest economic institution, where one of its functions is as a unit of production and consumption.

The contribution of women fishing in the family refers more to the role of a wife/housewife or daughter in doing productive work that generates income and work that does not directly generate money, but can allow other members of the family to take advantage of opportunities to work. Resusun, 1985).

The role of women in supporting the family economy has been going on since the emergence of the family institution itself. The division of tasks between family members, including women in the context of carrying out household life, is basically an economic activity (Munaroh, 2007).

It is the family's economic limitations that require women as nike fish catchers' wives to work, because women's fish farming activities as wives play a very strategic role, especially in post-harvest and marketing of fishery products. According to Sutadi (2006), the contribution of women as wives of Nike fishermen to household income can reach half of the husband's income. The results of a study by Widaningroem et al., (1999) on the South coast of Yogyakarta showed that although the reproductive contribution made by women, it turned out to reach 80% of the time allocation each day, when they carried out productive activities at home, and the contribution was temporarily abandoned and handed over to the community. their child or mother/ grandmother.

Payangka fish Ophieleotris aporos is the most abundant type of fish that lives in the waters of Lake Tondano (Makmur et al., 2015). In 1980, payangka production accounted for about 35% of all fish production and dominated the catch in Lake Tondano. The young payangka fish referred to are payangka fish, which are popularly called Nike fish, measuring 9.5-27.4 mm and weighing 0.004-> 0.183 mg (Susanto, 2016). The large population of Nike and Payangka fish must be supported by successful reproduction and adequate food in the lake. One of the reproductive factors that support the size of the Nike population is fecundity. According to Bataragoa and Tamananpo (2009), the fecundity of payangka fish in Lake Tondano with a size of 12.5-15.6 cm which is around 30,000-127,000 eggs, according to (Taupetel, 2004) the fecundity of payangka fish with a size of 9.0-16.4 cm ranges from 12,000-46,000, and According to Soeroto (1988) the fecundity of payangka fish with a size of 8.6-20.5cm ranges from 30,000-60,000 eggs.

II. MATERIALS AND MEHODS

This type of research is descriptive research and uses the research base, namely survey. A survey is a research conducted by collecting data, investigating and interpreting data in general as available in the field (Cresswell, 2009).

Data collection is done by observation or direct observation. The population in this study is the wife of the fisherman of the catcher. Nike fish numbered 80 people,

The sample to be taken is 10 - 20% of the total population of Nike (O. aporos) fishing wives in Kaima Village, Remboken District, Minahasa Regency. Sampling is done by purposive sampling or sample purpose. According to Sugiyono (2016) the notion of purposive sampling is a sampling technique with certain considerations.

Data analysis of research results was carried out in two ways, namely quantitative descriptive analysis and qualitative descriptive analysis. The data that has been analyzed is then interpreted as the result of research and discussed or phrased according to field facts, with reference to research journals related to this research (Sugiyono, 2010).

To find out the contribution of women as wives of fishermen fishing Nike to household income can be calculated by the formula (Soekartawai, 1993), namely:

Contribution (%)=(Total income of women)/(income of fishing family) X 100%

Meanwhile, net income for fishing families is calculated from the total household income minus the total household expenditure.

III. RESULTS AND DISCUSSION

1. General Condition of Research Site

Minahasa Regency is located at the northeastern tip of North Sulawesi and Remboken District is one of the sub-districts within it. Kaima Village is included in the Remboken District which is one of the villages in Minahasa Regency. The distance from the center of the sub-district is 15 km and the distance from the center of the capital city of North Sulawesi Province is 42 km.

The lake area is a characteristic of Kaima Village which is the source of livelihood for most of the population. One of the villages located on the shores of Lake Tondano, where the residents have been familiar with fishing activities in the effort to catch fish have been going on for a long time. Residents have carried out fishing activities such as catching nike fish (Ophioleotris aporos), with or without using a boat. In addition, there are also several fishermen who are active in cultivating fish in Lake Tondano using the Karamba Net Cap system.

Types of fish caught vary, namely: tilapia fish, tilapia that grows outside the cage and more and more in the lake, payangka fish, snakehead fish and the research is specifically nike fish (Ophioleotris aporos). The catch and the results of fish cultivation are other than for the family's

daily food, and especially for marketing, in increasing the family's income to support daily needs.

2. Productive Contribution of Fishermen's Wives

The wife plays a productive role as a manifestation of how much she contributes as a Fisher Mother/Wife, according to Astuti and Alghaasyiyah (2014), a basic role that a wife has due to work to increase the needs for her household. This is a big contribution because it is undergoing a transition period in increasing family income. Productive contribution is an award that is valued in money or goods that can generate income in the form of services and materials related to economic activities.

The results of the research on the activities of Fishermen's Wives who do work in helping their household lives include fish sellers in the market (retail traders), collectors traders (petibo), Staff/kiosk traders, selling cakes and food, housemaids, etc. The productive contribution of the wife of a fisherman who catches Nike Lake Tondano in Kaima Village, as a fish seller in the market (retail trader) is closely related to the state of Nike fishing, because Kaima Village is one of the villages on the coast of Lake Tondano as a supplier of Nike fish (Ophileotris aporos) biggest.

Kaima Village Fishermen who go down to Lake Tondano to catch Nike Fish and other types of fish, do not use additional labor. Every time fishermen land their caught fish, their wives from several fishermen take part in landing the fish. Because the fisherman's wife was so alert to help her husband in removing the fish from the boat, then the nike fish were put in a bucket and carried ashore.

The habit of the Fisherman's Wives is that every fish that is landed is immediately brought into the building which is an auction building or selling fish. The fish are then weighed and separated according to type and placed on concrete tables and that's when the selling process begins. For Nike Lake Tondano Fish, they are no longer weighed like other types of fish, because the fish are directly sold based on the existing buckets. The selling price of Nike Fish during the fish season ranges from Rp. 100,000 to Rp. 200,000 while Nike's fish sales when it is not fish season, the price increases from Rp. 300,000 to even Rp. 700,000. During the study in April the price of fish when it was not fish season was Rp. 450,000.

The process of selling Nike fish was fast because the fish were sold by the fishermen's wife as retailers and there were also collectors, so they did not last long at the Kaima Village auction place. By the Fisherman's Wife Nike fish is sold to the Remboken market which is only 1 Km from Kaima Village. So since 06.00 the fish have moved, except for other types of fish, sometimes sales are from 06.00 - 3.00 WITA. Fishermen's wives as traders, buy fish from fishermen and then take them outside Remboken markets such as: Tomohon Market, Kawangkoan market, Langowan market, even Manado Market. Nike Fish Sales sometimes there are consumers who come from other villages, namely Talikuran Village, because the distance is close to the blades, they are reached by motorbikes or pick up cars, these consumers come to buy fish at a cheaper price per bucket, which is only Rp. 100,000 per bucket. The weight of 1 bucket of Nike Fish is 10 Kg, so for 1 Kg of Nike fish it costs Rp. 10,000, this price is cheaper than the selling price of Nike fish in Remboken Market.

2.1. Nike Fish Production (Ophileotris aporos) Lake Tondano

Lake Tondano Nike (O. aporos) fish, one of which comes from Kaima Village, is very popular as a fish that tastes good and has high nutritional content. No wonder that big restaurants in Manado City provide food that comes from Nike Fish. The catch of fishermen in Kaima Village, is not always the same, usually changes in a month, meaning that the catch depends on the availability of fish, especially if the people of Kaima Village call it the month of death, then the catch is minimum. Dead moon is synonymous with no fishing season according to Lake Tondano coastal fishermen. For a clearer view of Nike's fish catch, see Table 1 below.

Table 1. Production of Nike Lake Tondano fish in Kaima Village

No	Production (Kg)	Fish Season		Not Fish Season	
		Total	Percerntage (%)	Total	Percentage (%)
1.	10 - 100	0	0	10	100
2.	101 - 200	0	0	0	0
3.	201 - 300	7	70	0	0
4.	301 - 400	2	20	0	0
5.	> 400	1	10	0	0
	Total	10	100	10	100

Source: Research data, April 2021

The research data in Table 1 shows that the results of catching Nike Lake Tondano Fish are very different, during the fishing season 70% of fishermen can produce 300 kg of fish per month, the remaining 20% of their production varies from 390 - 400 kg even 10% of fishermen can resulted in more than 400 Kg/month of fishing, this is the highest number of all observed respondents. The results of the interview with the fisherman were because the time spent was higher than other fishermen, for the production of Nike Lake Tondano

Fish carried out by the fishermen of Kaima Village, during the non-fishing season (Dead month), it turned out that 100% of the fishermen who caught fish could only produce 45Kg up to 75 kg of fish. In this situation, fishermen cannot do anything because when the moon is dead, this Nike fish will not appear on the surface, because by its nature this fish likes light. To catch this fish, fishermen must prepare tools that are designed in such a way that they can produce light. Materials and tools prepared to assist fishing by means of electric lamp lighting made by the fishermen themselves (Figure 1).

Fig.1: Tools and Materials used to catch Nike Fish

The price of Nike Fish sold by the Fisherman himself and the Fisherman's Wife, because most of the pricing and sales are carried out by the Fisherman's Wife. Observations turned out that there were also some fishermen who directly sold them to retailers who were already at the sales location, because sales were usually made at the landing site as well as the fish auction place in Kaima Village.

2.2. Fisherman Income Level

Carrying out fishing activities such as catching Nike Lake Tondano fish, as the Head of the Household, it is an obligation to play a role to support his family. Family needs, whether as husband or wife, especially children have different needs. Husbands and wives as parents are supposed to work in meeting every need that is needed by their family members. Her husband as a fisherman who works catching fish, especially catching Nike Fish in Lake Tondano, has an income, this result can be seen in Table 2 below.

Table 2. Income Level of Nike Fishing Fishermen per Month.

No	Income Level (Rp)	Fish Season		Not Fish Season	
		Total (org)	Percentage (%)	Total (org)	Percentage (%)
1.	1.000.000 – 2.000.000	0	0	0	0
2.	2.025.000 – 3.000.000	0	0	6	60
3.	3.025.000 – 4.000.000	2	20	4	40
4.	4.025.000 – 5.000.000	6	60	0	0
5.	> 5.000.000	2	20	0	0
	Total	10	100	10	100

Source: Research data, April 2021

The results of the study in Table 2 show that for the fishing season 60% of fishermen have an income of between Rp. 4,025,000 - Rp. 5,000,000, this is the fisherman with this income usually catching in the same area so that all the catches regardless of the results. will be divided equally, so that their income amounts to the same. The lowest 20% of income is IDR 3,025,000 – IDR 4,000,000 only doing activities based on whether or not they want to catch or not, while the highest 20% of income is due to the addition of fishing days than what fishermen usually do in Kaima Village. For non-season fish, the income does not vary much, because when catching fish, fishermen just surrender to the situation, because this season, Nike fish are difficult to catch.

2.3. Fisherman's Wife's Income

Fishing activities carried out by husbands who do not meet household needs, ultimately encourage other family members, such as wives, to carry out activities to support the family's economic life. The Fisherman's Wife contribution is very helpful in terms of house date income. This can be seen in the activities of Fishermen's Wives in the fishery business and outside the fishery business in Table 3 below.

Table 3. Income Level of Fishermen's Wives per Month.

No	Income Level (Rp)	Fishery Business		Not Fishery Business	
		Total (Person)	**Percentage (%)**	**Total (Person)**	**Percentage (%)**
1.	≤1.000.000	-	-	3	30
2.	1.100.000 – 1.500.000	4	40	4	40
3.	> 1.500.000	3	30	3	30
	Total	7	70	10	100

Source: Research data, April 2021

The results of the study on the income level of the wife of a fisherman who catches Nike Lake Tondano in Kaima Village as Table 3 shows that in the fishing business there are only 70% who carry out activities in terms of increasing the income of another 30%. take care of grandchildren at home. The results of observations of wives who do not carry out activities in the field of fishery business, they do not carry out activities in the field of fishery business.

Fishermen's wives who work in non-fishing businesses (30%) actually work to help neighboring families take care of children, cook family meals and other household chores, because these families work as civil servants or private employees. There is also a fisherman's wife who only works for another neighbor and does this work twice, so that the result of her work, the fisherman's wife receives wages every week as additional family income.

Income between IDR 1,000,000 - 1,500,000 is obtained from selling food such as Manado Porridge (Tinutuan), Skipjack noodle soup and Skipjck fried noodles and mixed rice where a plate of rice plus side dishes costs IDR 12,500. Another fisherman's wife (30%) opens a shop at her house by selling various ingredients and cooking spices as well as other basic needs such as sugar, coffee, tea and children's snacks.

2.4. Nike Fisherman's Household Expenditure Rate

The expenditures for the Nike Lake Tondano fisherman in Kaima Village, Remboken District, include basic household needs, there are also expenditures for children's education, expenditures for health, if a family member is sick. Usually the illness suffered by the Head of the Family is gout and for other family members such as influenza, fever, and others. To make it clearer how much each respondent of the Nike fishing household spends, it can be seen in Table 4 below.

Table 4. The level of expenditure per month of the Nike Lake Tondano fisherman household in Kaima Village

No	Expenditure Rate (Rp)	Amount Household	Percentage (%)
1.	300.000 – 600.000	5	50
2.	601.000 – 900.000	2	20
3.	901.000 – 1.200.000	2	20
4.	> 1.200.000	1	10
	Total	10	100

Source: Research data, April 2021

The results of the study in Table 4 can be seen that there are 50% of fishing households that catch Nike Lake Tondano, spending the lowest cost, which is Rp. 600,000 per month. This expenditure is only to buy medicine for fever and other costs to buy basic commodities such as rice, cooking oil, sugar, coffee, tea. The fish dishes are readily available and other kitchen spices are not purchased because they are available in the yard, only occasionally they buy cooking spices to the market if they are pressed for a family event at their house. Table 4 above also shows that 1 household (10%) spends the highest cost, which is above IDR 1,200,000 per month. It is known that the highest expenditure of the fishermen's household is the total expenditure of Rp. 1,765,000 per month and this includes the cost of education because it pays for children who are already studying at the University.

2.5 Net Income of Fisherman's Household

The net income of the Nike Lake Tondano fisherman's household in Kaima Village can be calculated by subtracting the total income of all family members by the total expenditure of the fishermen's household. The net income found in the fishermen's household in Kaima Village, turns out to be only from the income of the wife and the husband's income, because of all respondents from other family members, namely children who have not earned because they are still small and young people who are still in school, there is one family whose son is married. So it can be calculated in net income.

Fishermen's total income is Rp.36,737,500 per month multiplied by 10 months, the result is Rp.367,375,000, the average per fisherman is Rp.367,375,000 divided by 10, the result is Rp.36,373,000 per year per fisherman.

The total income of the Fisherman's Wife is Rp. 25,520,000 per month multiplied by 10 months (1 year)

the result is Rp. 255,200,000 per year per person, with an average yield of Rp. person.

The total household expenditure is Rp. 11,965,000 per month multiplied by 10 months (1 year) the result is Rp.119,650,000 per year, the average for 1 household is Rp.119,650,000: 10 people the result is Rp.11,965,000 per year per family.

The net income of the Fisherman household per year is:

The total income of the fishermen's household is reduced by the total expenditure with the result (per year):

Rp.25,520,000 + Rp.36,737,000 = Rp.62,257,000

Rp.62,257,000 – Rp.11,965,000 = Rp.50,292,000

3. Income Contribution of Fisherman's Wife

The contribution given by the wife of a fisherman who catches Nike Lake Tondano in Kaima Village, Remboken District can be calculated from the total family income multiplied by 100%, so the results are:

(Rp 25,520,000)/(Rp 50,292,000) X 100% = 50.7%

The results of the calculation of the results of the study to determine the contribution of the wives of fishermen to Nike Lake Tondano's fisherman's income turned out to be 50.7%. This shows that as a wife has a large income contribution to family income because it illustrates that the wife has a high contribution to the household economy.

IV. CONCLUSION

1. The contribution given by the wife of the fisherman who catches Nike Lake Tondano in Kaima Village, Remboken District is 50.7%
2. The wife of a fisherman who catches Nike Lake Tondano in Kaima Village, Remboken District, has a high contribution to the household economy.

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Effect of Intra Row Spacing on the Growth and Yield of Maize (Zea Mays L.) Varieties in Southern Guinea Savanna of Nigeria

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Abstract— *This study was carried out at the Research Farm of the Faculty of Agriculture, University of Abuja on latitude 6°45' and 7°39' East and Longitude 8°25' and 9°20' North in the Southern Guinea Savanna Zone of Nigeria, during the cropping season of 2018 to evaluate the effects of intra-row spacing on growth and yield of maize varieties. Two hybrid maize varieties Dekalb920 and Dekalb 818 sourced from National Seed Council of Nigeria were evaluated under six different intra-row spacing's of 14cm, 18,21,25,30,and 37cm, with gross plot of 15m and net plot 0f 9m for such Parameters as plant height, number of leaves, leaf area/index, stem diameter and grain yield. It was a factorial combination experiment laid out in a Randomized Complete Block Design (RCBD) with three replicates. The results obtained during the 12th week after sowing indicated that hybrid variety Dekalb920 which had mean plant height of 273.06cm, number of leaves of 16, leaf area of 734cm2 and grain yield of 8,0t/ha among other parameters was superior to Dekalb818 variety investigated. With respect to intra-row spacing, plants sown on 14 cm had higher mean plant height and number of leaves of 287.7 cm and 16, respectively while plants sown on intra-row spacing of 37 cm had mean plant height of 251.7 cm2 and number of leaves up to 15 respectively. Therefore, it can be concluded that optimum intra row spacing and Varietal difference combination in the study area for the maximum grain yield was 25 cm and 14cm for Dekalb 920 variety under adequate amount and regular distribution of rainfall. Results showed that variety and spacing were significantly (P<0.05) different.*

Keywords— ***Maize, growth, yield, spacing, varieties, savanna.***

I. INTRODUCTION

Maize (*Zea mays* L) is one of the major cereal crops grown in the humid tropics and Sub-Saharan Africa. It is a versatile crop and ranks third following wheat and rice in world production, (FAO, 2002) Maize is an annual crop of the family *Poaceae*, commonly known as the grass family and is a key source of food and livelihood for millions of people in many countries of the world.(Adeniyan,2014). The corn plant possesses a simple stem of nodes and internodes. A pair of large leaves extends off of each internode and the leaves total 8–21 per plant. The leaves are linear or lanceolate (lance-like) with an obvious midrib and can grow from 30 to 100 cm (11.8–39.4 in) in length. The male and female inflorescences (flower bearing region of the plant) are positioned separately on the plant. The male inflorescence is known as the 'tassel' while the female inflorescence is the 'ear'. The ear of the corn is a modified spike and there may be 1–3 per plant. The corn grains, or 'kernels', are encased in husks and total 30–1000 per ear. The kernels can be white, yellow, red, purple or black in color. Corn is an annual plant, surviving for only one growing season prior to harvest and can reach 2–3 m (7–10 ft) in height. The term maize is derived from the Spanish form of the Arawak Native American term for the plant. However, it is popularly called corn in the United States, Canada, New Zealand, and Australia. Corn may also be

referred to as maize or Indian corn and is believed to originate from Mexico and Central America. According to the FAO reports (*2008*). Cultural practice improvements like fertilization, using higher yielding varieties and higher plant densities will lead to rise in sweet corn yield (Akman, *2002*). The importance of the crop arises from its great productivity over an extremely wide range of environmental conditions. It is grown mainly in the tropics, subtropics, and temperate climates, but the best maize regions are those which receive an annual precipitation of 600 to 1000mm, except where the crop is irrigated (Sprague and Dudley, 1988).

It is mainly used as a food source and now has become the most important raw material for animal feed (Dutt, 2005) and one of the main sources of cereals for food, forage and processed industrial products. It is produced extensively in Nigeria, where it is consumed roasted, baked, fried, pounded or fermented. Human consumption of corn and corn meal constitutes a staple food in many regions of the world. In advanced countries, it is an important source of many industrial products such as corn sugar, corn oil, corn flour, starch, syrup, brewer's grit and alcohol and the grain is extensively used for the preparation of corn starch, dextrose, corn -flakes, gluten, grain cake, lactic acid and acetone which are used by various industries such as textile, foundry, fermentation and food industries. Corn oil is used for salad, soap-making and lubrications (Dutt, 2005).

World production of maize is around 790 million tones (Chiezey, 2010). By 2050 demand for maize will double its presence demand in the developing world and the crop is predicted to become the crop with the greatest production globally and in the developing world by 2025(Abuzar et al., *2011*) and the world area of maize production was 176 million ha while that of wheat was 216 million ha and rice at 184 million ha as reported in in 2017. About 70% of the world maize production area is found in the developing countries. However, these countries contribute to only 49% of the world's maize production (FAOSTAT, 2016).

Global maize consumption is projected to increase by 1.3% per annum over the projection period, a slower pace compared to 3.3% p.a . in the previous decade. This increase is principally driven by higher feed demand, which holds the largest share of total utilization, rising from 56% in the base period to around 58% in 2027. Developing countries account for over three quarters of the increase in feed consumption due to fast expanding livestock and poultry sectors. Feed demand is expected to rise 120 metric tons (mt) to 699 mt, and major countries that account for the increase are China (+32 mt), the United States (+20 mt), Argentina (+5 mt), Indonesia (+5 mt) and Viet Nam (+5 mt). Production in Viet Nam and Thailand, in particular, will grow due to fast-expanding poultry industries. Food use of maize is expected to expand mostly in developing countries due to growing populations and maize is becoming increasingly important in diets, especially white maize. Maize will remain an important staple for Sub-Saharan Africa, where consumption of white maize is expanding and where maize accounts for about a quarter of total caloric intake. Overall, African countries show the strongest growth in maize consumption for food among all developing countries at about 3% per annum(p.a.)

Among all the factors which affect the corn yield remarkably, plant Density is one of the most important ones and due to its genetic potential, corn yield is different under various plant populations (Abuzar et al., *2011*). It was reported that new maize cultivars cannot tiller considerably and usually produce one ear/plant at low plant population and interplant competition rows which affected the total yield negatively since it instigates apical dominance, impels barrenness and finally reduce the number of ears/plant and grain/ear (Abuzar et al., *2011*). According to the Sangoi (*2001*) there is no specific optimum population density for all the weather conditions since it differs based on environmental and controlled conditions Low plant density causes minimum corn yield due to the leaf area's little plasticity in each plant (Lashkari et al., *2011*).

Therefore, the objectives of the experiment include;

- ✓ to compare the performance of the varieties of maize used in this trial as influenced by the different intra- row spacing
- ✓ to determine best intra-row spacing for optimum growth and yield for maize hybrids production in Nigeria
- ✓ to determine the best hybrid maize variety that will give optimum growth and yield of the Crop

II. METHODS

Location of the study

The field experiment was conducted at the Research Farm of the Faculty of Agriculture, University of Abuja, in the Southern Guinea Savanna Zone of Nigeria, during the cropping season of 2018.It has boundary with Kogi state in the south, Kaduna in the north, Nasarawa in the east and Niger state by the west. Its total area is about 724,473.9 hectares. The major food crops are mainly corn, yams, millet, beans, cassava, garden eggs, soya beans, melon, okra, groundnuts and vegetables. The major occupation

among the people within the study area is farming. The farm lies on latitude 6°45'N and 7°39' E and Longitude 8°25' and 9°20' North, 300m altitude. The average temperature is between 28°C to 30°C in the dry season and can be as high as 40°C or more especially in Gwagwalada area, with sandy loamy soil. The rainy season begins from April and ends in October with mean annual rainfall of 1,100mm to 1,800mm(Barnabas and Nwaka,2014), humidity of 14% at planting period and wind of 10km/h north east. During the rainy season, the area witnesses uneasy hot temperature beginning early in the morning, through the day and the night becomes warm. About 60% of the yearly rains fall from the months of July to September and the rest of the year is relatively cold and dry. The vegetation of Abuja is dominated by species of plants such as *Albizia zygia, Butrospermum paradocium, Parkia clappertoniana, Terminalia supera, Bombax buonepozenze* (Balogun,2001), The vegetation is very important to the people where 60% depend on it for household energy (fuel wood) and construction materials. The soil in the study area is deep and well drained Sandy Loam that constitute soil that result from the granite and migmatite as the principal rocks in the area. They have moderate runoff potential and the texture of the soil is typically loamy sand, clay loam and sandy clay. Fertility of soil is moderate with acidity rating of 5.60 to 5.80(Ishaya and Grace, 2007)

Land Preparation

The Experimental land was manually cleared and mechanically ploughed using a tractor mounted disc plough, and then harrowed twice to break the soil clods. Most of the debris was ploughed back into the soil then the experimental plots were laid out using measuring tape and pegs

Planting Material

The Planting materials used for the trial were two varieties of maize Dekalb 818 and 920. The maize varieties were obtained from National seed council of Nigeria. Abuja.

Dekalb818 and 920hybrids are extremely adaptable and give superior results all over the World; this comes from proved reliability in dramatically diverse climate conditions that are present during last several years, depending upon location and weather condition.

Dekalb920 is adaptable to a well drain sandy loam soil with average rainfall of 600-1000mm and can also grow under irrigation condition, It mature between 80 to 90 days after sowing and is high yielding hybrid with excellent standard ability. Dekalb818 is high drought resistant hybrid, can tolerate poor soil and tolerant to folia disease and stalk rot. It mature within 90 days after sowing with long and cylindrical ears, good grain coloration and more row per cob.

These hybrids are designed for intensive agricultural production and application of all modern agro-technical measures in maize production. Dekalb hybrids had been commercialized since 2008.They arrived into our market with top quality hybrids, from wide European offer, characterized by high seed quality for which were shortly recognized by agricultural producers as the right choice for their fields. In 2010 offer for Africa market are the hybrids from all FAO groups significant for our area (FAO 200 – FAO 600).

Treatment and Experimental Design

The experimental treatments were 6 different intra-rows spacing of 14cm, 18cm, 21cm,25cm, 30cm and 37cm by 75cm inter row spacing and two maize varieties.

I. Maize varieties denoted as A which include Dekalb920 (A_1) and Dekalb 818(A_2)
II. Six different intra-rows spacing denoted as B, 14cm(B_1), 18cm(B_2), 21cm(B_3),25cm(B_4), 30cm(B_5) and 37cm(B_6)

Thus, a 2 x 6 factorial combination trial was used for the treatments and making a total of 12 treatment combinations.

Table 1: Treatment Combinations

Combination	Varieties	Intra-row spacing
A_1B_1	Dekalb 920	14cm
A_1B_2	Dekalb 920	18cm
A_1B_3	Dekalb 920	21cm
A_1B_4	Dekalb 920	25cm
A_1B_5	Dekalb 920	30cm
A_1B_6	Dekalb 920	37cm
A_2B_1	Dekalb 818	14cm
A_2B_2	Dekalb 818	18cm

A_2B_3	Dekalb 818	21cm
A_2B_4	Dekalb 818	25cm
A_2B_5	Dekalb 818	30cm
A_2B_6	Dekalb 818	37cm

Table. 2: Plant Population Per hectares and Per Plots

S/N	Spacing (cm)	Plant Population per hectare	Population per plot
1	14cm	95,238 plants/ha	143plants
2	18cm	74,074 plants/ha	112 plants
3	21cm	63,492 plants/ha	96 plants
4	25cm	53,333 plants/ha	80 plants
5	30cm	44,444 plants/ha	68 plants
6	37cm	36,036 plants/ha	56 plants

Experimental Design

Randomized Complete Block Design (RCBD) with 3replications was used in the study. Factorial arrangement was used in organizing the treatments which were fitted into the Design Each replicate contained 12 plots, of 3m×5m sizes giving 15m^2 gross plot and 9m^2 net plot and separated by 1m allay pathway. Thus a total of 36 plots were used for the experiment.

Sowing

Sowing of one seed per hole was done on the 22nd July 2018.The various plant spacing gave the different plant populations. Maize seeds were sown at depth of between 2cm -3cm in plots measuring 5m x 3m using six different spacing's listed above.

Fertilizer application

N: P::K 20:10:10 was applied 14 days after planting at recommended rate for maize at 200kg/ha and Urea 46%N at 150kg/ha.

Pest and disease control

There was incidence of Stem borer (*Busseola fusca*) and it was controlled by spraying the affected maize plant with Sharp shooter at the rate of 8.8kg ai/ha at 6WAS.

Weed control

Para force and Atrazine were applied as pre-emergence herbicide at the rate of 1.6kg ai/ha and later supplemented with manual weeding using hoe at 4WAS and 8WAS.

Data Collection

Growth Parameters

Plant Height (cm)

Six plants were chosen at random from the net plots at the center of the gross plot. Plant height for each plant was measured from the ground level of the plant to the tip of central spike tassel. The mean height of the six plants was recorded.

Number of Leaves per Plant

Six plants were randomly selected within the Net plot and number of leaves in each selected plant was counted. The mean number of leaves per plant was obtained.

Leaf Area Index

Six leaves from six randomly selected plants in the Area of the net plot were measured at the fourth leaf from the top of the plant, because it is fully expanded. Maximum length and width of the leaf were determined to obtain the leaf area. The Maize leaf area was estimated by multiplying the length and width of the leaf and the product was adjusted by a factor 0.75 according to Francis et al (1960). This was done for all the leaves taken and added up to give the leaf area per plant. The leaf area per plant was divided by area of ground cover to get the leaf area index (LAI)

LA = maximum length × maximum width × 0.75

Where 0.75 is a fixed factor

$$LAI = \frac{Leaf\ Area}{Area\ of\ Ground\ Covered}$$

Stem Diameter (cm)

Stem girth was measured on the selected plants using tape and was reported in millimeters.

Plant Stand Count

From each plot the number of stands was counted to determine the plant population per plot.

Grain Yields (tons/ha)

The grain yield was determined by using the dry grain weight after harvesting, threshing and winnowing in the air to remove chaff using a Mettle Toledo sensitive balance and converted to tons/ha.

Statistical Analysis

The data collected were subjected to Statistical analysis for Analysis of Variance (ANOVA) using SAS – Statistical Analytical Structure procedure and the means were separated using Duncan Multiple Range Test (DMRT)

III. RESULTS

Effect of intra- row spacing on the growth of maize varieties

Stand Count

The results showed that variety and spacing were significantly (P< 0.05) different throughout the period of the evaluation and positively affected the stand count. The effect of intra-row spacing on the stand count of two maize varieties Dekalb 920 and Dekalb 818 is shown in table 3. The two varieties gave statistically similar percent stand count in respect to the intra-row spacing however, varying intra-row spacing shows significant differences in emergence of two maize varieties. Spacing 14 cm gave the highest count stand while 37 cm gave the least stand count of maize

Table 3 Effect of Intra-row Spacing and Varietal difference on Stand Count/Plot of maize

Spacing (cm)	Variety 1	Variety 2
14	**138.67**	**139.67**
18	**107.00**	**108.00**
21	**94.00**	**92.33**
25	**75.33**	**76.00**
30	**64.67**	**63.00**
37	**52.33**	**53.00**
Grand Mean	**88.67**	**88.67**
LSD (α < 0.05)	**2.95**	**2.88**

Means with the same letter in a column of each factor are not significantly different at (P ≤ 0.05), LSD = Least Significant Difference, V1= Dekalb 920, V2 = Dekalb 818

Effects of Variety and Spacing and Varietal difference on Plant Height (cm) of Maize

The effects of variety and spacing on plant height of two maize varieties are shown in Table 4. There were significant differences also in the plant height of the maize varieties investigated. Variety 920 gave taller plants of 287.7 cm at 9th weeks after sowing, followed by 818 with height of 281.7cm. With respect to spacing, maize plants sown at 14 cm in 2018 gave taller than other plants spacing during the 9th weeks with height of 287.7 cm and 281.7cm for variety one and two respectively at 9thweeks. These were followed by plants sown at 18cm; 21cm and 25cm. Plants sown at37 cm were the shortest. The results showed that variety and spacing were significantly (P< 0.05) different throughout the period of the evaluation and positively affected plant height.

Table 4 Effect of Intra-row Spacing and Varietal difference on Plant Height (cm) of maize

Spacing (cm)	Variety 1	Variety 2
14	**287.7**	**281.7**
18	**275.3**	**275.7**
21	**272.7**	**271.0**
25	**275.0**	**266.0**
30	**268.0**	**260.3**
37	**259.7**	**251.7**
Grand Mean	**273.06**	**267.7**
LSD (α < 0.05)	**7.53**	**10.11**

Means with the same letter in a column of each factor are not significantly different at (P ≤ 0.05), LSD = Least Significant Difference, V1= Dekalb 920, V2 = Dekalb 818

Effects of intra-row Spacing and Variety on Number of Leaves per Plant of Maize

The results indicated that variety and spacing were significantly (P < 0.05) different and positively affected number of leaves of maize. The response of number of leaves of two maize varieties to different intra-row spacing is shown in Table 5. The mean number of leaves of maize variety Dekalb 920 and 818 were similar at 9 weeks, which indicated that the trend in the number of leaves did not change. Based on spacing, plants sown at 14 cm had the highest number of leaves at 9 weeks after sowing in the evaluation. There were significant differences in number of leaves of plants sown at both 14 cm and other spacing.

Table 5. Effect of Intra-row Spacing and Varietal difference on Number of Leaves/Plant of maize

Spacing (cm)	Variety 1	Variety 2
14	16.00	16.00
18	16.00	15.33
21	15.33	15.00
25	15.00	15.00
30	15.00	15.00
37	15.00	15.00
Grand Mean	**15.39**	**15.22**
LSD (α < 0.05)	**0.43**	**0.43**

Means with the same letter in a column of each factor are not significantly different at (P ≤ 0.05), LSD = Least Significant Difference, V1= Dekalb 920, V2 = Dekalb 818

Effect of intra-row Spacing and Varietal difference on Leaf Area per Plant of Maize

The response of leaf area of two maize varieties to different spacing is shown in Table 6. There were significant differences in leaf area of the two maize varieties evaluated. In the 9th week, maize variety 920 had the highest leaf area (763.30 cm), followed by 818 which had 758.3 cm. Dekalb 818 plants had the smallest leaf area (690.3 cm). Based on spacing, plants sown at 37 cm were superior in leaf area with values of 763.30 cm at 9WAS. The superiority in leaf area based on spacing was 37 cm > 30 cm > 25 cm> 21cm > 18cm > x 14cm. The results showed that variety and spacing were significantly (P < 0.05) different and positively affected leaf area of maize.

Table 6 Effect of Intra-row Spacing and Varietal difference on leaf area (cm) of maize

Spacing (cm)	Variety 1	Variety 2
14	**704.00**	**690.3**
18	**717.30**	**715.0**
21	**726.00**	**723.3**
25	**739.70**	**733.0**
30	**753.30**	**745.0**
37	**763.30**	**758.3**
Grand Mean	**733.9**	**727.5**
LSD (α < 0.05)	**8.96**	**20.35**

Means with the same letter in a column of each factor are not significantly different at (P ≤ 0.05), LSD = Least Significant Difference, V1= Dekalb 920, V2 = Dekalb 818.

Effect of Intra-row Spacing and Varietal difference on leaf Area Index of maize

Table 7 shows the effect of intra-row spacing on leaf area Index (LAI) of two maize varieties. Varietal differences did not result in significant differences in LAI in the evaluation. Varying spacing caused significant differences in LAI, with each decrease in spacing significantly increasing LAI in sampling period .The results showed that variety and spacing were significantly (P < 0.05) different and positively affected leaf area index of maize.

Table 7 Effect of Intra-row Spacing and Varietal difference on leaf Area Index of maize

Spacing (cm)	Variety 1	Variety 2
14	**14.03**	**13.77**
18	**13.07**	**13.00**
21	**12.50**	**12.33**
25	**12.20**	**12.13**
30	**11.57**	**11.40**
37	**11.27**	**11.10**
Grand Mean	**12.44**	**12.29**
LSD (α < 0.05)	**0.18**	**0.45**

Means with the same letter in a column of each factor are not significantly different at (P ≤ 0.05), LSD = Least Significant Difference V1= Dekalb 920, V2 = Dekalb 818

Effects of intra-row Spacing and Varietal difference on Stem Diameter of Maize

The effect of intra-row spacing on stem girth of maize is shown in Table 8.Variety 920 had the highest stem girth of 11.20cm, while variety 818 had 11.00cm at 9th week after sowing. Based on spacing, maize plants sown at spacing of 37 cm were superior in stem girth while plants sown at spacing of 14 cm had the smallest stem girths. The results showed that variety and spacing were significantly (P < 0.05) different and positively affected stem girth of maize.

Table 8 Effect of Intra-row Spacing and Varietal difference on Stem Diameter (cm) of maize

Spacing (cm)	Variety 1	Variety 2
14	**8.20**	**8.03**
18	**8.70**	**8.57**
21	**9.13**	**9.03**
25	**9.77**	**9.50**
30	**10.33**	**10.23**
37	**11.20**	**11.00**

Grand Mean	**9.56**	**9.39**
LSD (α < 0.05)	**3.07**	**2.63**

Means with the same letter in a column of each factor are not significantly different at (P ≤ 0.05), LSD = Least Significant Difference, V1= Dekalb 920, V2 = Dekalb 818

The analysis revealed that the effect of intra- row spacing, variety and the plant density was significant (P < 0.05) for grain yield as shown in (table 9). Mean comparisons showed that Dekalb 920 maize variety at 25cm spacing had the highest yield of (8.00ton/ha average) and was followed by 14cm spacing which had (7.70 ton/ha average) which was due to grain weight. Also, Dekalb 818 variety at 14cm spacing had the third highest yield (7.33ton/ha average) and was followed by 18cm spacing which had (7.27ton/ha average). The result shows that the two varieties at 14cm intra- row spacing had a higher grain yield compared to the 37cm intra- row spacing which means that the trend of changes in the two maize varieties to row spacing was similar and positively affected grain yield of maize.

Table 9 Effect of Intra-row Spacing and Varietal difference on Grain Yield (t/ha) of maize

Spacing (cm)	Variety 1	Variety 2
14	**7.70**	**7.33**
18	**6.30**	**7.27**
21	**6.63**	**6.27**
25	**8.00**	**7.13**
30	**6.03**	**5.33**
37	**5.97**	**6.30**
Grand Mean	**6.77**	**6.61**
LSD (α < 0.05)	**1.12**	**0.94**

Means with the same letter in a column of each factor are not significantly different at (P ≤ 0.05), LSD = Least Significant Difference V1= Dekalb920, V2 = Dekalb 818

IV. DISCUSSION

Effect of Intra-row Spacing and Varietal Difference on the Growth and Yield of Maize

The differential growth with respect to plant height observed among the two varieties may be attributed to differences in genetic characteristics of the individual varieties, including rapid growth rates, tallness or shortness of the varieties. This is similar to the findings of Majambu *et al.*(1996) and Ibrahim *et al.*(2000) that attributed the differences in growth indices of crops to genetic constitution. Maize plants spaced 14cm intra-row spacing were taller than other plants possibly because of increased competition for space, sunlight and available nutrients. This is similar to the findings of Teasdale (1995), Widdicombe and Thelen (2002), and Dalley *et al.* (2006) who attributed the increased growth rates and earlier canopy closure of narrow row spaced crops to quest for increased light interception as well as increased availability of soil moisture because of equidistant distribution of crop plants. It is also consistent with the reports of Al-Rudha and Al-Youmis (1998) that maize sown at 15cm had the highest plant height compared with their counterparts sown at wider intra-row spacing.

The increase in plant population with the decrease of plant spacing obtained in this study is obvious since plant spacing is used as a tool to increase or decrease plant density. These results were supported by Roy and Quazem (1987).Roy and Biswas (1992) and Larson and Hanway (1977) who reported that, narrow plant spacing had resulted in high plant population. On the other hand, plant population is always a function of seed germination percentage and will not be affected when the plant is sown on the top of the ridge or on the bottom of the ridge, unless the soil is saline. Salt accumulation on the top of the ridge may result in poor seed germination and consequently low plant population. Therefore, these significant differences in plant population between the two varieties and the intra-row spacing between plants in this study might probably be due to the fact that seedlings emergence and establishment of the two varieties were similar.

The similarity observed in the number of leaves of the two maize varieties may be attributed to growth characters which are being influenced by genetic make-up of the plants. This is similar to the findings of Sajjan *et al.*, (2002) who reported that, growth characters of crops are based on their genetic make-up. Maize plant sown on 14cm spacing had higher number of leaves than their counterparts which were sown at wider spacing possibly because of increased growth rate in search for space, sunlight and other environmental resources. This is consistent with the findings of Al-Rudha and Al-Youmis (1998) and Ali *et al.* (2003) that made similar reports on 15cm-spaced maize plants.

The differences observed in leaf area of the two varieties of maize sown could be attributed to the differences photosynthetic activities of leaves, differences in chlorophyll content and activity of photosynthetic enzymes. This is similar to the findings of Gwizdek (1989) who attributed the differences between the leaf area and

other growth characters of maize genotypes to differences in photosynthetic activity of leaves, chlorophyll content, stomata conductance value and activity of photosynthetic enzymes. The differences observed in leaf area is also similar to the findings of Akinfoesoye *et al.,* (1997); Odeleye and Odeleye (2001) who suggested that, since maize varieties differ in leaf area, other growth characters as well as in yield and its components, breeders must select most promising combiners in their breeding programs. Increased intra-row spacing resulted in larger leaf area possibly because there was a reduction in competition for space, sunlight and nutrients within the wider spaced plants. This is similar to the findings of Ali *et al.,* (2003) who reported that, competition between maize plants for light, soil fertility and other environmental factors were markedly increased with highest population but decreased with lower plant population.

Leaf area index is an important parameter of maize. In our research, increase in LAI explains the general crop trends that increasing plant density increases leaf area index on account of more area occupied by green canopy of plants per unit area. On the other hand, increasing leaf area index is one of the ways of increasing the capture of solar radiation within the canopy and accumulation of dry matter. Responses of dry matter accumulation and leaf area index were similar when plant density was increased or intra-row spacing was decreased. These results are in close conformity with the results obtained by Winter and Ohleroch (1999) who found that LAI in maize increases with increase in plant density. The increasing of LAI was attributed to the rise in total leaf area/plant (Alam, Haider, 2006; Yasari, Patwardhan, 2006).

The highest leaf area index obtained in 14cm intra-row spacing by the two varieties in this study could be due to variation in environmental factors, influenced by genotype, plant population, climatic condition and soil fertility. Higher plant population produced which offset the effect of large leaf area produced at 37cm intra-row spacing. This result is supported by Winter and Ohlrogge (1973) and Scarbook and Doss (1973) who reported that leaf area index increased with the increase of plant population. The significant differences in leaf area and leaf area index between plant spacing were reported.

The superiority of Dekalb 920 maize variety over 818 maize variety with respect to stem diameter may be attributed to the special qualities credited to hybrids. This was probably due to the fertility of the soil used for the experiment which resulted in an equal ratio between the leaves and the stems of the two varieties. This is similar to the findings of Obi (1999), Kim (1997), Olakojo *et al.* (1998) and Udoh (2005) who reported that some hybrid maize varieties have yield advantage over other maize varieties because they possess special qualities as high yield, disease resistance, and early maturity, uniformity in flowering and ear placement, and ease of harvesting using combined harvester. Maize plants sown at spacing of 37cm were superior in stem girth over those sown at narrower or smaller spacing possibly because the plants obtained more soil moisture and nutrients than narrower-spaced plants. This is similar to the findings of Barbier *et al.* (2000); Hamayan (2003); Dalley *et al.* (2006) and Azam *et al.* (2007) who reported that, wider-spaced maize plants obtained more soil moisture and nutrients than narrower plants. Also, Kunuskan, O, (2000) reported that, stem diameter were lower in higher plant densities as a consequence of interplant competitions.

Effect of Intra-row Spacing and Varietal Differences on Yield of Maize

The superiority of Dekalb 920 maize variety over 818 maize with respect to grain yield may be attributed to the special qualities credited to the hybrids, including disease resistance, early maturity, uniformity in flowering and ear-placement, and very high yield and the genetic make-up of the Variety. This is similar to the findings of Obi (1999), Kim (1997), Olakojo *et al.* (1998) and Udoh (2005) who reported that, some hybrid maize varieties have yield advantage over other maize varieties because they possess such special qualities as high yield, disease resistance, and early maturity, uniformity in flowering and ear placement. Maize plants sown at spacing of 25cm were superior in grain yield and closely followed by maize sown at spacing 14cm. However, the higher grain yield obtained at closer intra-row spacing (25cm and 14cm), could be attributed to higher number of plants and harvestable cobs at optimum spacing. This result was in conformity with the findings of Okan*et.al.* (2004) that obtained highest grain yield from closest intra-row spacing of 20cm.There was no significant differences in yield ha-1 between the two varieties. This finding confirmed the result obtained by Mani *et al.* (2002), who observed a non-significant increase in grain yield ha-1 between maize varieties of Dekalb. The higher cob and grain yields so obtained at 25 cm and 14cm could also be due to fact that more cobs are harvested under this population (Mani *et al.,* 2002, Iqtas and Acar, 2006, Valentinus and Tollenaar, 2006, Onyango, 2009). . Optimum plant density for maximum grain yield per unit area may differ from hybrid to hybrid on account of significant interactions between hybrids and densities (Farnham, 2001; Widdicombe and Thelen, 2002; Tokatlidis *et al.,* 2005) Therefore from the result so obtained from this study it is therefore eminent that higher grain yield for the newly developed extra-early maize variety is possible at 25 cm and 14cm intra-row spacing**.**

V. CONCLUSION

The result of present study showed that Intra-row spacing and Variety had significant influences on most of the growth parameters, yield and yield components of maize. The result also indicated that variety Dekalb 920 was the more suitable of the two maize varieties tested, and 14cm and 25cm intra-row spacing was better to achieve optimum yield.

VI. RECOMMENDATIONS

Therefore, from the result so obtained from this study it is eminent that higher grain yield for the Dekalb 920 maize variety is possible at 14 cm and 25cm intra-row spacing x 1 plants per hole which can be adopted by farmers

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Nano remediation of Hormonal Endocrine Disrupting Chemicals from Water by *Acalypha indica* Silver Nanoparticles

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Abstract— *Endocrine disrupting chemicals are natural or synthetic hormones and can be derived from plastics and pharmaceuticals. They have been found in trace amounts (ppt to ppb) in our water supplies and treated wastewater. They are therefore difficult to remove. We have synthesized silver nanoparticles (AgNP's) using Acalypha indica (A. indica) leaf extracts and used it to treat water samples spiked with hormones to determine efficiency of removal. AgNP's were synthesized and characterized by UV-Vis spectrophotometer, Zetasizer for particle size and zeta potential. To investigate the effects of the AgNP's on the removal of hormonal compounds from water, deionized water was spiked with 6 selected hormones, incubated with synthesized AgNP's and agitation overnight. The effects AgNP's on removal of the hormones was determined by HPLC UV-Vis. Results indicated that the AgNP's synthesized showed absorption spectrum at about 400 nm, confirming formation of AgNP's. The average size and zeta potential analyzed by dynamic light scattering techniques showed the sized to be 132.6 nm and the Zeta potential as -ve 61.8mV suggesting higher stability of AgNP's. Nano remediation resulted in changes in peak areas of HPLC chromatograms. Among the selected hormonal compounds, there is a huge reduction of peak areas in the 1 and 5 ppm Diethylstilbestrol (Rt =10.6) by 36.68% and 15.46%; D Norgestrel (Rt = 11.6) by 7.97% and 27.95%; 19 Norethindrone (Rt = 8.5) by 10.17% and 0.64%; respectively. There was peak reduction observed for 5 ppm of 17 Alpha ethynyl estradiol (Rt = 8.6) by 2.33%, 1 ppm Beta Estradiol (Rt= 7.6) by 10.35 % and Estrone (Rt= 9.4) by 6.47 %. Results indicate that we successfully synthesized AgNP's and demonstrated nano remediation of hormonal compounds from water. This is a novel effort that need further investigation for broader application in water treatment processes and possible scale up production A. indica AgNP's.*

Keywords— ***Hormonal Compounds, Endocrine Disruptors, Acalypha indica, Silver Nanoparticles.***

I. INTRODUCTION

The unbounded presence of hormonal compounds/endocrine disruptor compounds (EDCs) in wastewater has emerged as a threat to aquatic species and humans. The presence of EDC in drinking water has resulted in substantial concerns due to possible toxicological and ecological impacts, with the possibility of introduction of these EDCs into our food chain at trace concentrations. Unfortunately, the conventional wastewater treatment protocols are not able of completely removing these EDCs from water. There is therefore the need to develop effective means to remove them from our environment [Chauhan A, et al 2019]. Remediation is the process of removal of contaminants or pollutants from environment such as soil, water, groundwater, sediments, or air for the general protection of human health and the environment [Ingle AP et al., 2014]. Currently, Environmental remediation is achieved by the use of existing methods like physical, chemical and biological remediation but may not be effective in completely

removing (100%) of toxicants from the environmental media. An alternative approach needs to be developed to be used to have a clean environment. Now the focus has shifted towards nanotechnology for the successful remediation of these persistent pollutants as such provide ample opportunity for effective removal of EDC from environmental matrices [Ingle AP et al., 2014].

Nanotechnology is an emerging technology with many applications. It involves the synthesis and application of materials with dimensions in the range of 1-100 nm [Bar et al., 2009; Rassaei et al., 2008]. Due to the exceptional properties of nanoparticles which possess large surface areas, are mechanically strong, optically active, and are chemically reactive and can be used to trap chemical contaminants. These make them unique and suitable candidates for different applications e.g., nanodevices for use in not only for environmental cleanup but also in biological, physical, biomedical and pharmaceutics.

Plants have been a source of filter for our water resources- they line our streams and rivers, populate the riverbanks and spread over the water sheds which serves as reservoir and collection points for run offs and storm water before they are discharged into other bodies of water. The waters from these rivers/streams serves as drinking water for several communities. Plants are rich in several biological resources which make them natural resources for several purposes. Apart from providing shades reducing water evaporation, plants help in filtration processes removing impurities from the water bodies. This is made possible because of the versatility of plant constituents. Plant extract produce a wide range of constituents and chemical compounds with varied properties. These phytochemicals present in plants have been identified to contain such constituents as tannins, saponins, alkaloids, glycosides, steroids, polyphenols etc. [Zhao et al., 2015]. These natural compounds are highly functionalized which make them capable of reacting with other chemicals possibly neutralize and remove impurities. These properties are being exploited in alternative medicine where they have been shown to have the ability of reducing oxidative stress through antioxidant activity [Das RK et al., 2017]. Therefore, these properties could be useful for use in the removal of environmental chemicals and toxicants from water in remediation process. We have synthesized AgNP's using herbal plant extracts. The resulting nanoparticles were used to treat water samples spiked with known concentrations of EDCs and evaluated for effectiveness in their removal. Many novel nanomaterial adsorbents have been developed for increasing the efficiency and adsorption capacities of removing contaminants from wastewater [Sadegh H et al 2017].

A wide variety of physical- chemical approaches are being used for the synthesis of nanoparticles, most of which involve use of harsh chemicals at high temperatures. However, biogenic reduction of metal precursors to produce corresponding synthesis of nanoparticles is eco-friendly, less expensive, free of chemical contaminants for medical and biological application where purity of synthesis of nanoparticles is of major concern [Gurunathan S. et al 2009; Pal S. et al., 2007; Bae E. et al. 2010].

So, in the current study, we synthesized AgNP's by the reduction of silver ions present in the solution of $AgNO_3$ using the leaf extract (aqueous) of *Acalypha indica*, characterized by DLS and UV-Vis spectrum. The efficacy for nano remediation was evaluated by treatment of water samples spiked with EDCs to determine the ability of the synthesized nano particle in the removal of these compounds.

II. MATERIALS AND METHODS

Materials:

Six hormonal compounds: 17 alpha ethynyl estradiol, beta estradiol, estrone, diethylstilbestrol, 19 norethindrone, and D norgestrel were purchased from Sigma Aldrich, St Louis Missouri, Silver Nitrate 99% ACS reagent purchased from Acros Organics, Thermo Fisher Scientific New Jersey – US. *Acalypha indica* leaves was freshly collected (Andhra Pradesh, India) and washed individually under the running water and then with Milli-Q (double distilled) water, it was then air dried and milled into fine powder.

A. indica leaf extraction:

Approximately 10 grams of air-dried *A. indica* plant extract was boiled in distilled water for15 min. The extract obtained was filtered through Whatman #1 filter paper and the filtrate was stored at 2-4℃ in the refrigerator for further use.

Synthesis of Ag nanoparticles:

To a baker containing 43ml of 0.01M $AgNO_3$, 7ml of *A. indica* extract was added and heat at 80^0C for 1 hour. The change from pale yellow to dark brown indicated the formation of colloidal silver nanoparticles. Reaction mixture was centrifuged at 14,000 rpm for 15 min [**Fig. 1]**. Precipitate was washed with distilled water thrice and then with 100% ethanol twice. Pellet collected and air dried, stored for further experiment and characterization.

Characterization of the synthesized AgNP's:

Synthesized AgNP's were characterized by various instrumental analyses. The formation of AgNP's (the bio reduction of the Ag) was measured by UV-Vis spectrophotometer. The samples were scanned with a

wavelength of range of 400–600 nm operated at a resolution of 1 nm at room temperature (25 C). Zetasizer (ZS 90, Malvern, UK) was employed to measure the particle size and zeta potential of the AgNP's. The synthesized AgNP's were diluted with PBS (0.15 M, pH 7.2). The aliquots were then sampled in dynamic light scattering (DLS) and nanoparticles were then examined for equivalent diameters, size distribution and zeta potential.

Treatment (Rx) of water samples with known concentration of EDCs and removal by synthesized nanoparticles:

Method:

Hormonal compounds selected and used were 17 alpha ethynyl estradiol, beta estradiol, estrone, diethylstilbestrol, 19 norethindrone, and D norgestrel. Stock concentrations of these hormones were prepared by dissolving 10 mg in 10 ml methanol. Each hormonal compound 1 and 5 ppm were added to deionized water and were subjected to treatment by simple conventional overnight incubation on a shaker along with the synthesized silver nanoparticles of *A. indica* (10 ppm) to check the remediation efficacy on hormones in water. After incubation (continuous shaking), samples were subjected to centrifugation for 10 minutes at 1500 rpm, supernatant collected and filtered through 0.25 µm filters into separatory funnel. The filtrate was extracted with 1:1 ratio of dichloromethane and allowed evaporate to under the hood. The residue was reconstituted with 1 ml methanol and analyzed using HPLC UV-Vis.

Instrument:

Dionex Ultimate 3000 High Performance Liquid Chromatography with UV detector - Ultimate 3000 HPLC comes with an assembly of binary pump, degasser, autosampler and a UV visible detector that can detect any of the four selected wavelengths from 190nm to 400nm at a time. The binary pump can work in isocratic and gradient mode. Isocratic mode is single solvent line flowing through the column, gradient mode enables to run a combination of two solvents in a programmed time frame. The degasser equipped to the pump helps in purging out air gaps in the solvent line generated during solvent mixing. Ultimate 3000 is suitable for analytical separations of various chemical compounds.

HPLC UV-Vis Conditions:

Mobile phase: Water: Acetonitrile 50:50

Flow rate: 0.8ml/min

Wavelength: 230 nm

Column: Supelco Discovery C18, 250 x 4.6mm, 5.0 µ

Diluent: Methanol

III. SUMMARY OF RESULTS AND DISCUSSION

The average size and zeta potential were analyzed by dynamic light scattering techniques (DLS) and the size values were shown to be 132.6 nm [**Fig. 2]**. Zeta potential provides the information about the stability of nanoparticles and surface charge. Zeta potential of the synthesized AgNP's is pictured [**Fig. 3],** with -ve 61.8 mV charge suggesting higher stability of AgNP's.

UV–vis spectroscopy is commonly used to confirm metallic nanoparticle formation by studying the optical properties which depend particularly on size effect. Basically, the silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [Krishnaraj et al.,2010]. **Fig 4** Shows the UV–Vis spectra recorded from the reaction medium after heating the solution to 80^0 C for 1 hour. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak near 400 nm, which confirmed the formation of silver nanoparticles. This is consistent with others who had synthesized silver nanoparticles with *A. indica* [Krishnaraj et al.,2010]

Treatment of water samples with AgNP's of *A. indica* at 10 ppm concentration by conventional incubation (due to nanoparticles larger surface and adsorption capacities of removing contaminants from wastewater [Sadegh H et al., 2017] resulted in changes in the peak areas (The size of the peak is proportional to the concentration of the analyte) [Corradini D et al., 1998]as indicated on the chromatograph identified by HPLC analysis (**Fig. 5-16)**. After treatment there is a significant reduction in the peak areas observed in samples treated with *A. indica* AgNP's. Among the selected hormonal compounds, there is a huge reduction of peak areas of 1 ppm and 5 ppm Diethylstilbestrol (Rt=10.6) by 36.68% and 15.46% respectively following treatment with 10 ppm AgNP's. After treatment peak areas of 1 ppm and 5 ppm of D-norgestrel (Rt= 11.6) were reduced by 7.97% and 27.95% respectively. Treatment reduced peak areas of 1 ppm and 5 ppm 19 norethindrone (Rt= 8.5) by 10.17% and 0.64% respectively. There is also a peak area reduction observed for 5 ppm 17 alpha ethynyl estradiol (Rt= 8.6) by 2.33%, 1 ppm beta estradiol (Rt= 7.6) by 10.35 % and estrone (Rt= 9.4) by 6.47 %. These results are summarized in **Table.1.**

IV. CONCLUSION

In this study we have successfully synthesized *A. indica* AgNP's using green synthesis method. Characterization

confirms the synthesis and shows a stable compound with potential for the use in possible remediation as our results shows that treatment of water samples spiked with hormones resulted in significant reduction in the peak area (The size of the peak is proportional to the concentration of the analyte) [Corradini D et al., 1998]. Our results suggest a potential for the use of AgNP's of *A. indica* in possible remediation to remove low concentration of hormones and other chemicals from the contaminated environment. However, this needs further studies using samples from wastewater treatment plants for example.

ACKNOWLEDGMENTS

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Fig. 1: Visual identification of silver nanoparticles A) 0.01 M silver B) silver nitrate solution added with plant extract C) brown color change-formation of Ag NP's

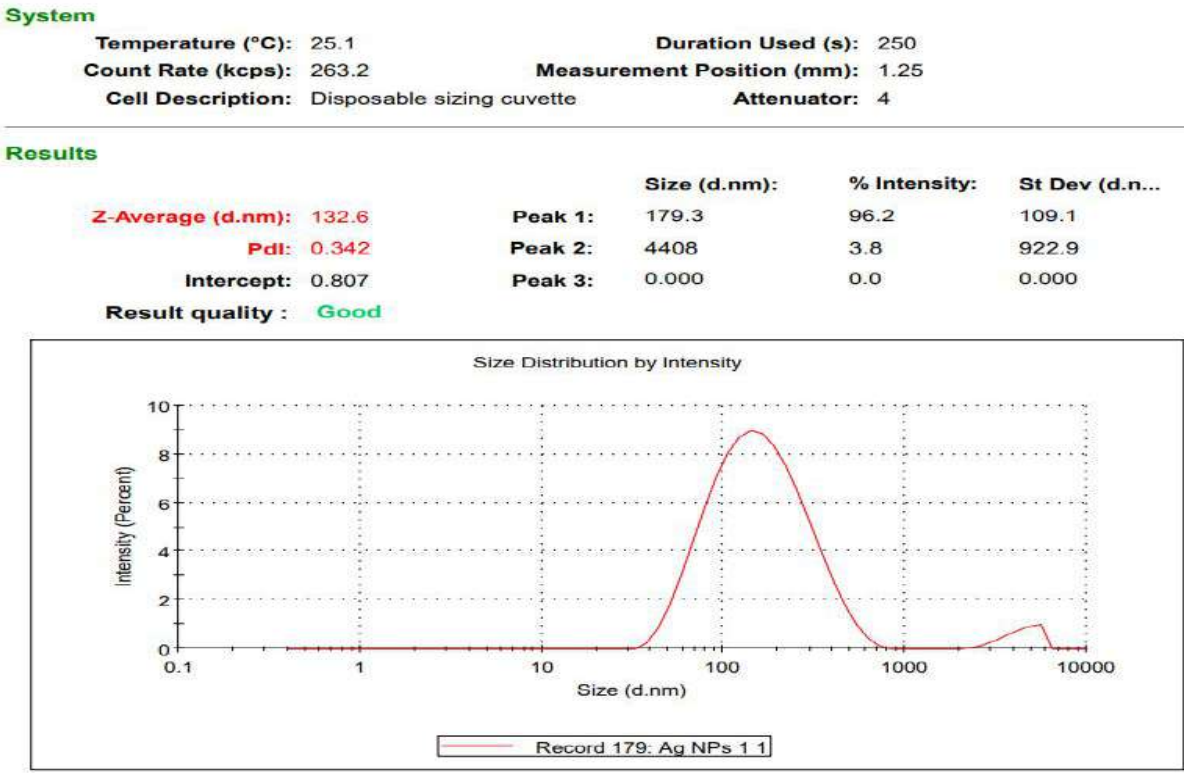

Fig. 2: Size distribution of AgNP's of A. indica measured by the DLS technique

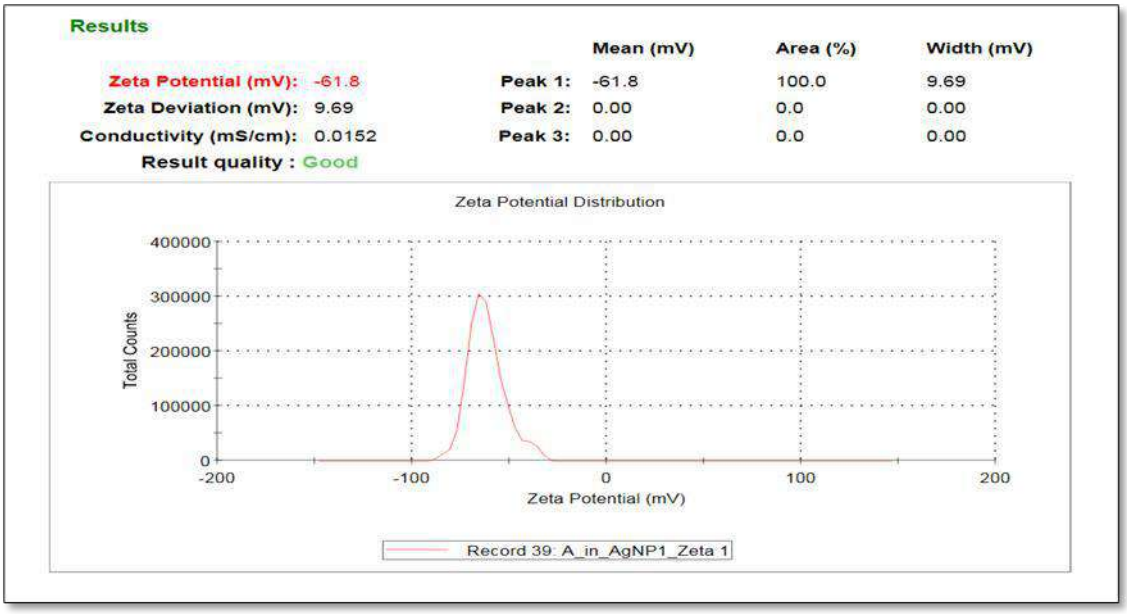

Fig. 3: Zeta potential of AgNP's of A. indica

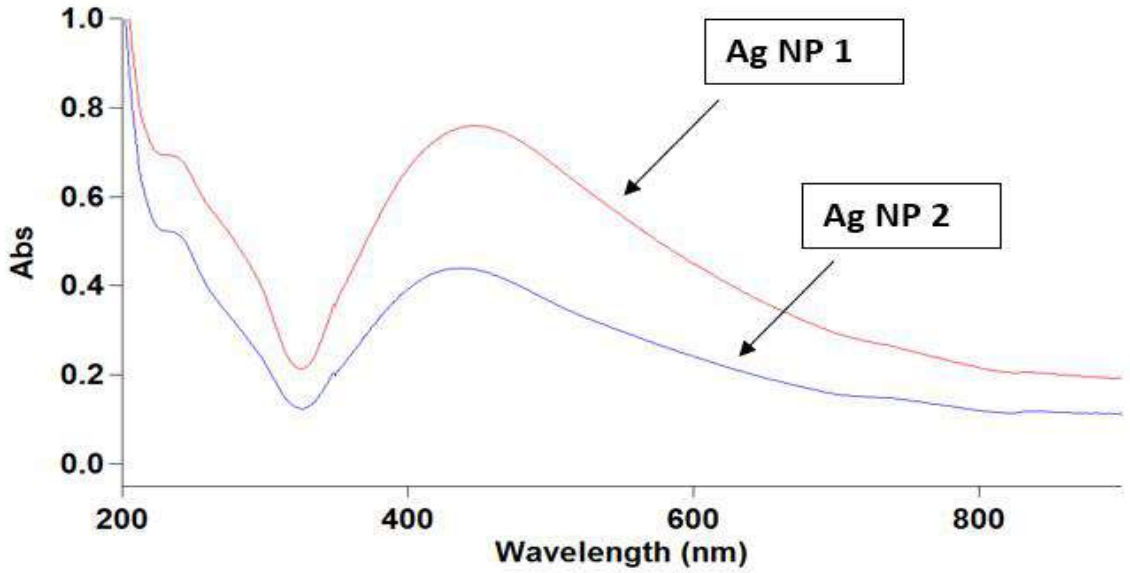

Fig. 4: UV–visible Absorption spectrum of silver nanoparticles AgNP1 (Sample 1) and AgNP2 (Sample2)

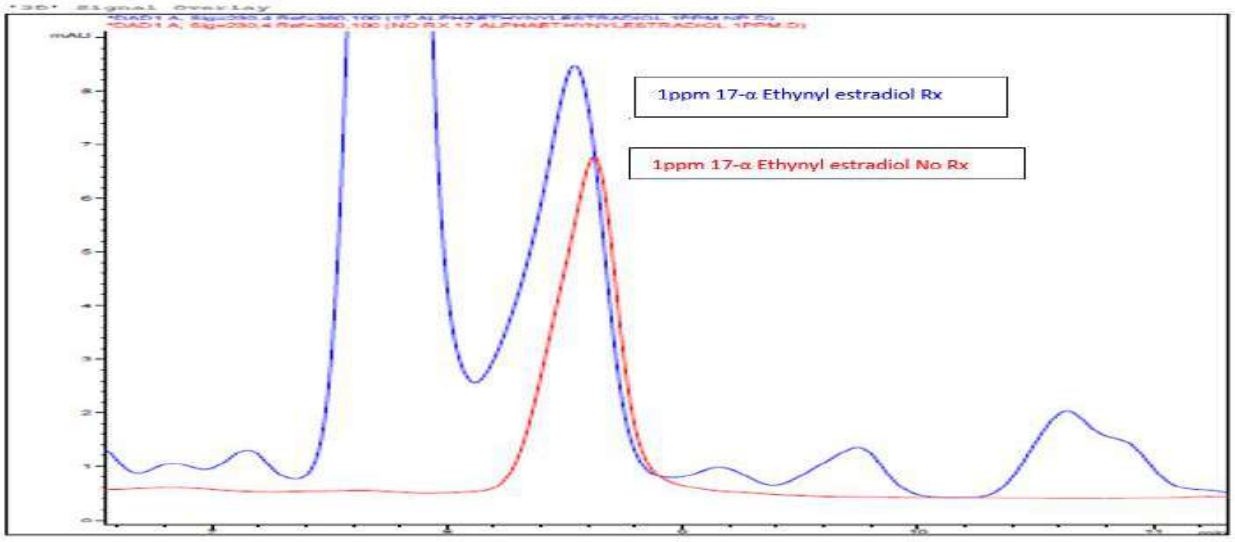

Fig. 5: Overlaid Chromatogram of 1 ppm 17-α Ethynyl estradiol shows changes in peak area with treatment of AgNP's

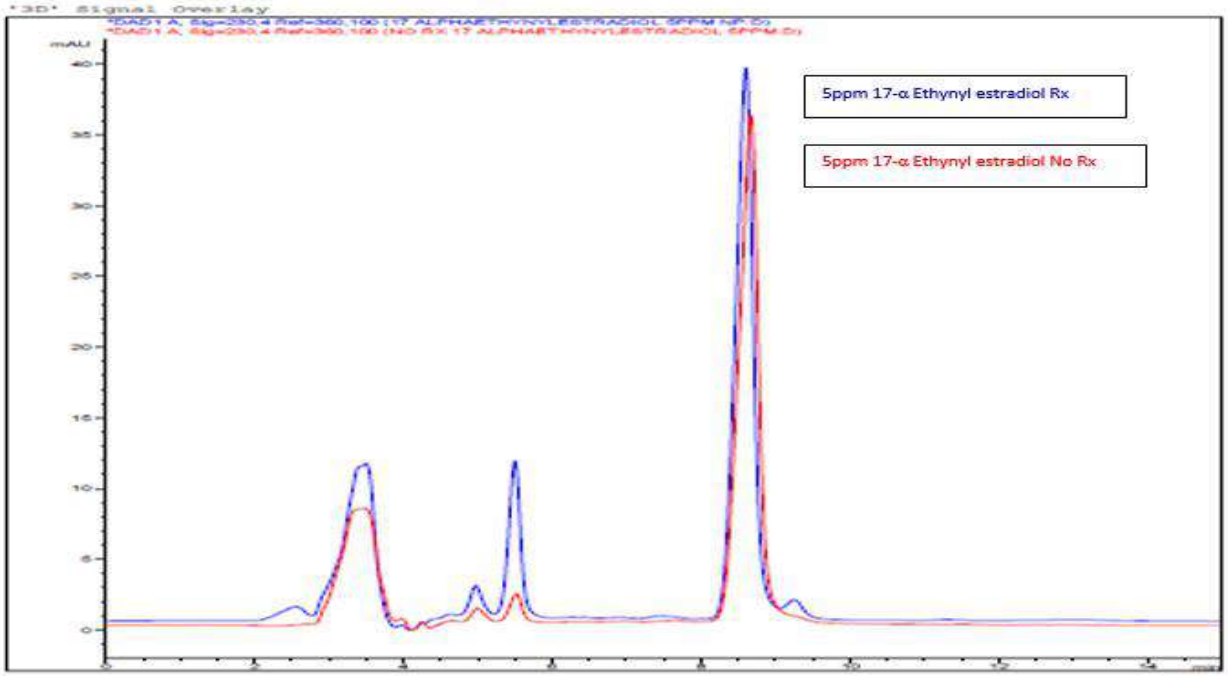

Fig. 6: Overlaid Chromatogram of 5 ppm 17-α Ethynyl estradiol shows changes in peak area with treatment of AgNP's

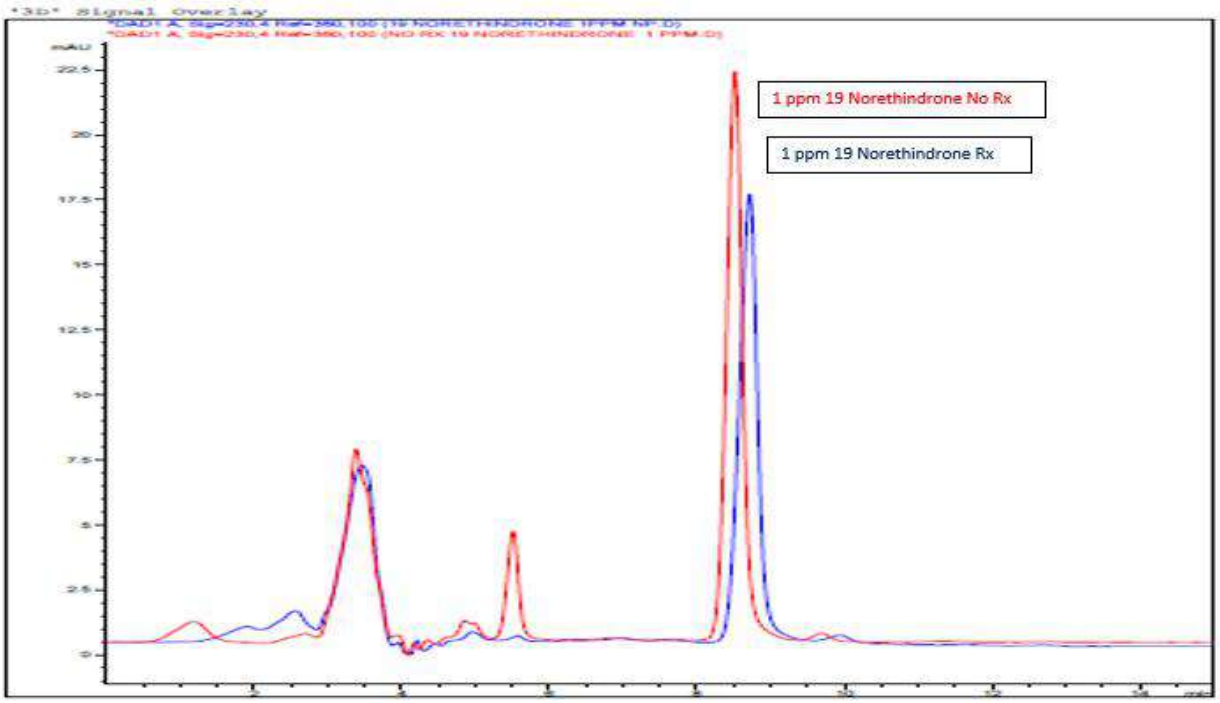

Fig. 7: Overlaid Chromatogram of 1 ppm 19 Norethindrone shows changes in peak area with treatment of AgNP's

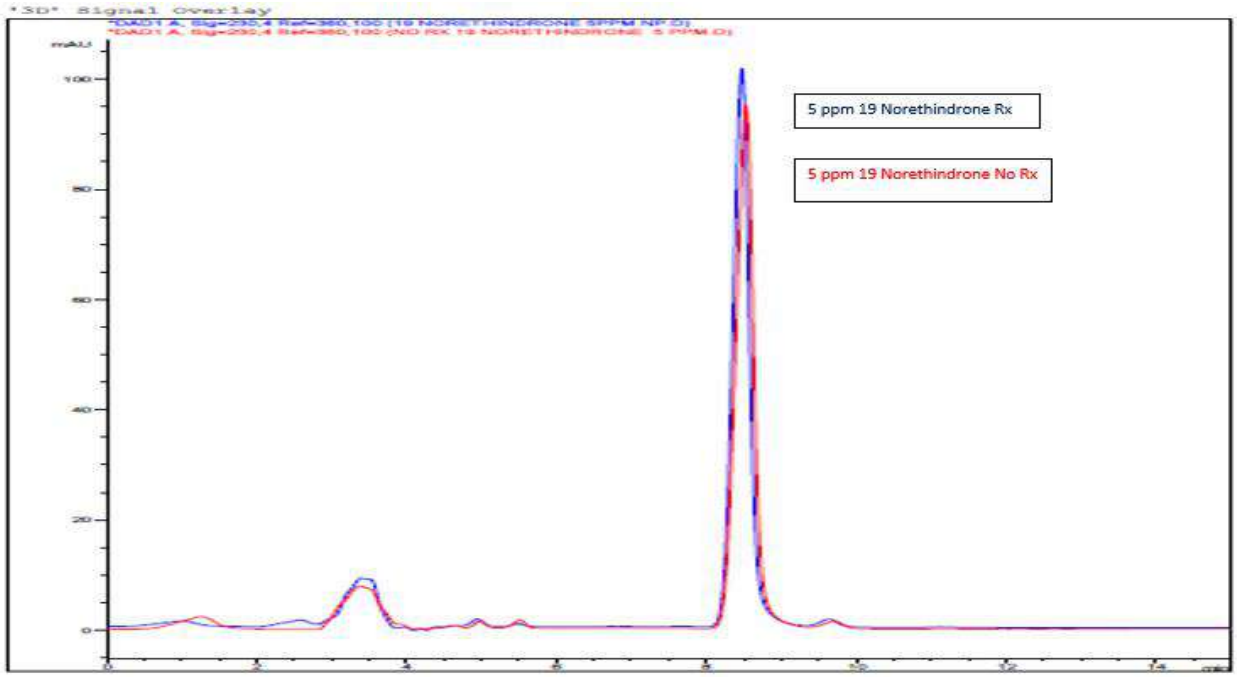

Fig. 8: Overlaid Chromatogram of 5 ppm 19 Norethindrone shows changes in peak area with treatment of AgNP's

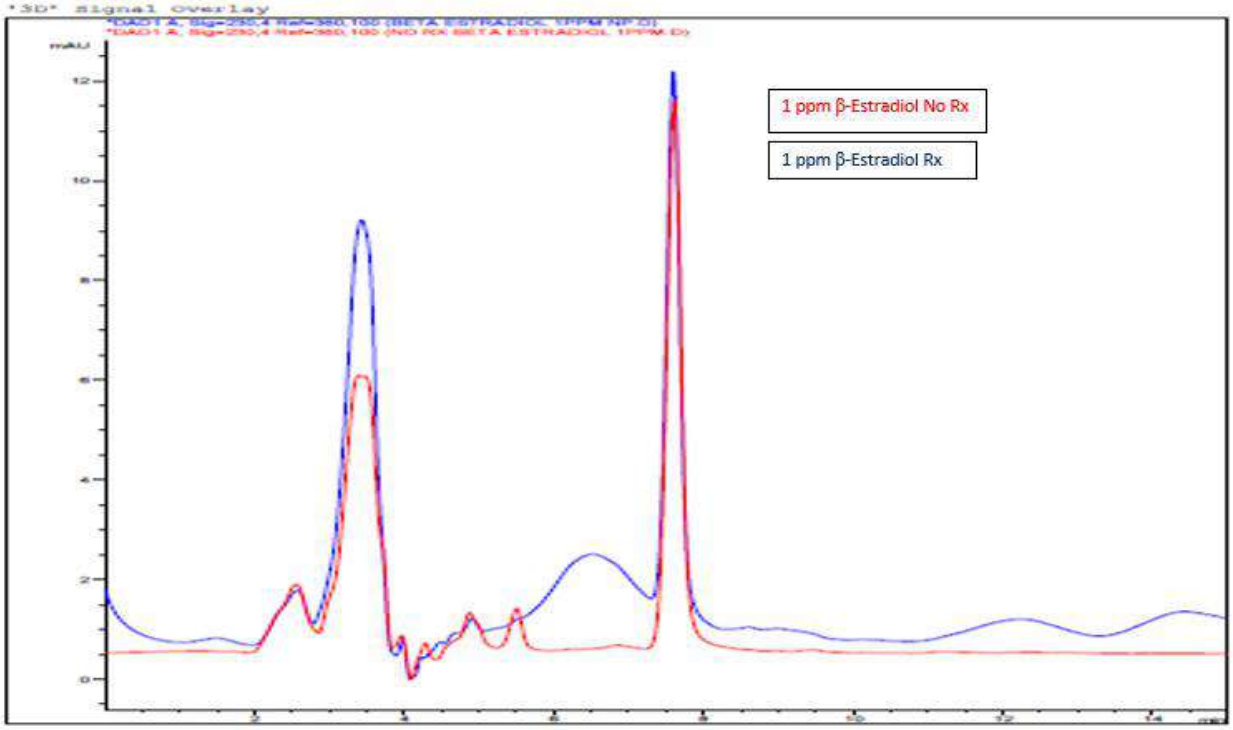

Fig. 9: Overlaid Chromatogram of 1 ppm β-Estradiol shows changes in peak area with treatment of AgNP's

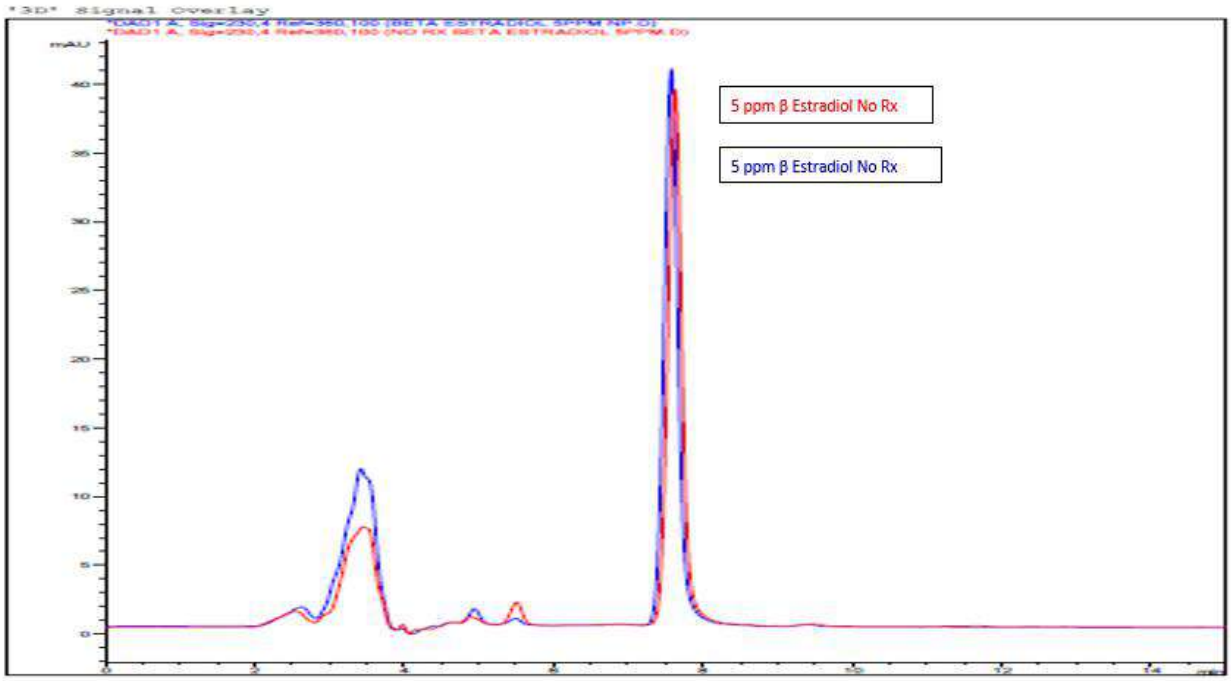

Fig. 10: Overlaid Chromatogram of 5 ppm β-Estradiol shows changes in peak area with treatment of AgNP's

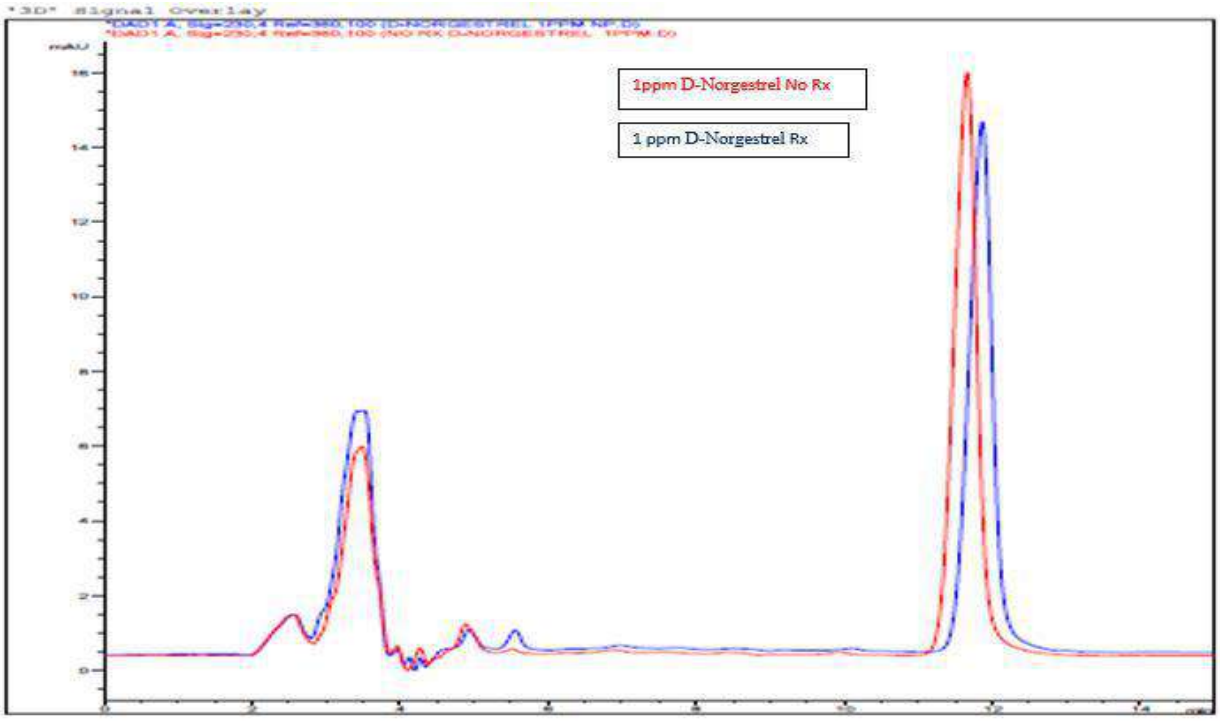

Fig. 11: Overlaid Chromatogram of 1ppm D-Norgestrel shows changes in peak area with treatment of AgNP's

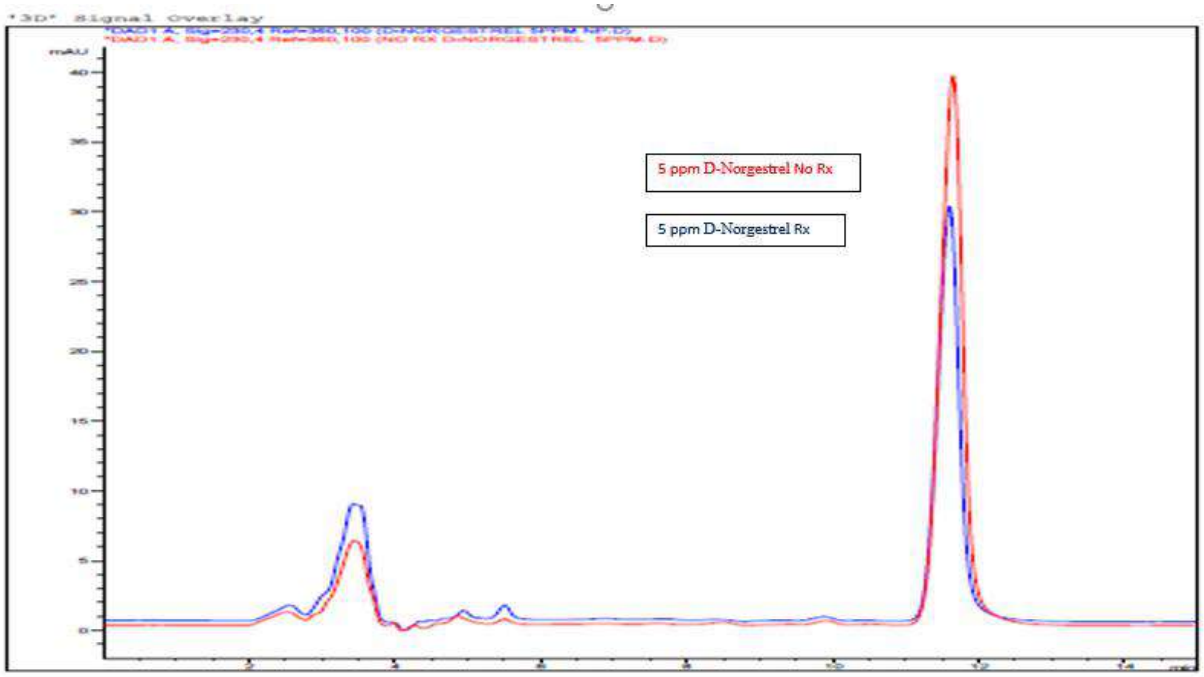

Fig. 12: Overlaid Chromatogram of 5 ppm D-Norgestrel shows changes in peak area with treatment of AgNP's

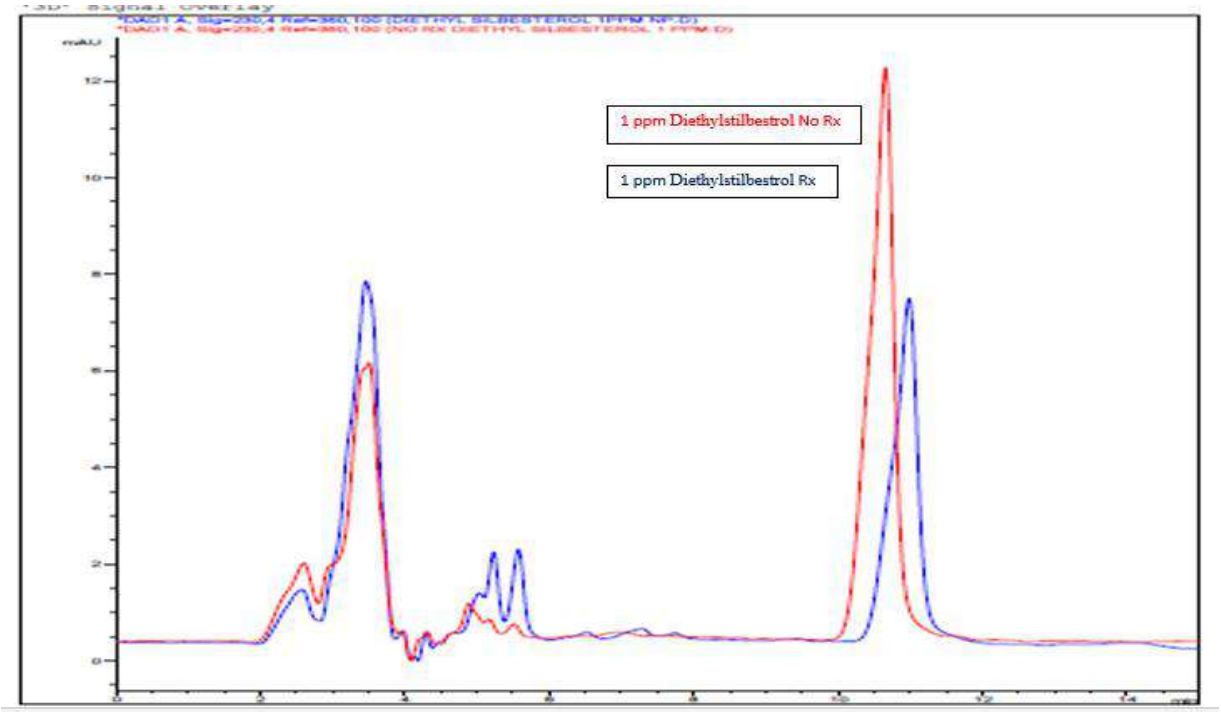

Fig. 13: Overlaid Chromatogram of 1ppm Diethylstilbestrol shows changes in peak area with treatment of AgNP's

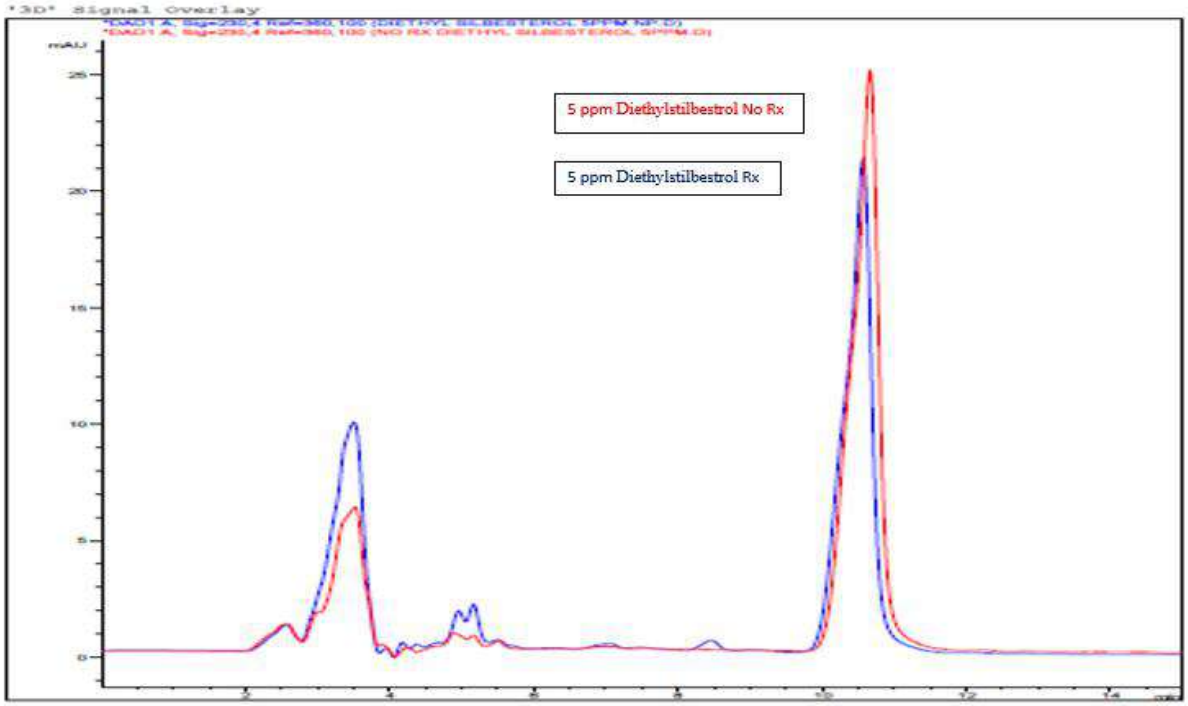

Fig. 14: Overlaid Chromatogram of 5 ppm Diethylstilbestrol shows changes in peak area with treatment of AgNP's

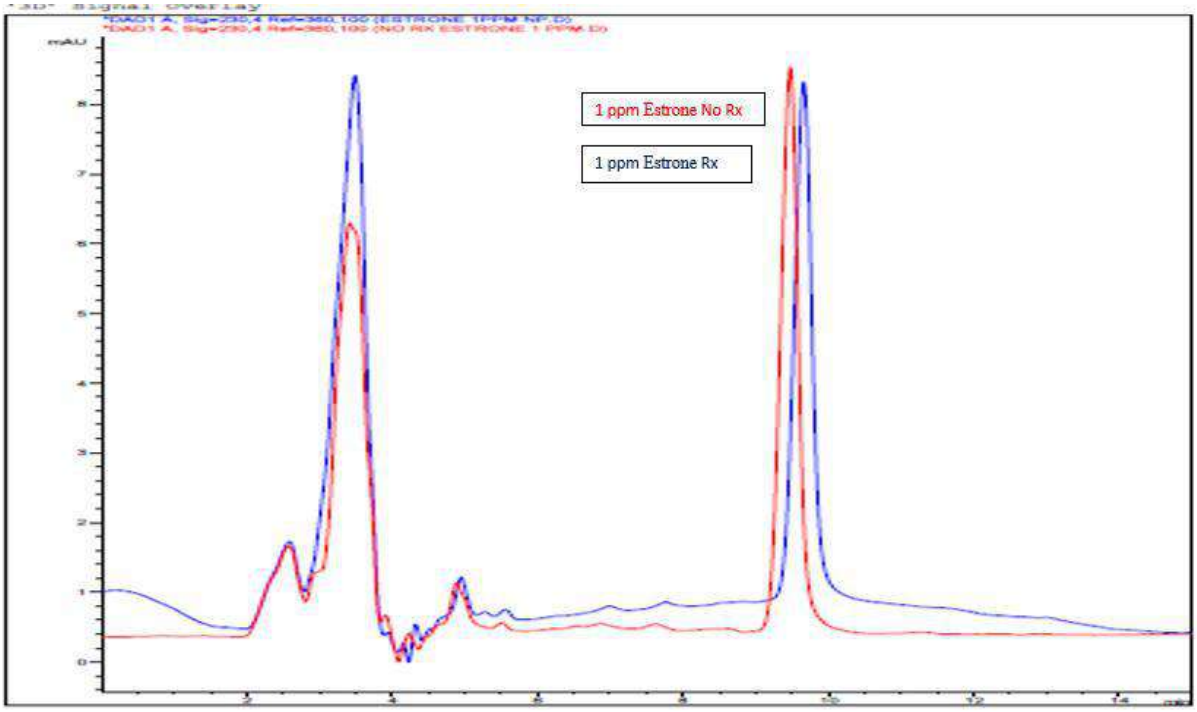

Fig. 15: Overlaid Chromatogram of 1ppm Estrone shows changes in peak area with treatment of AgNP's

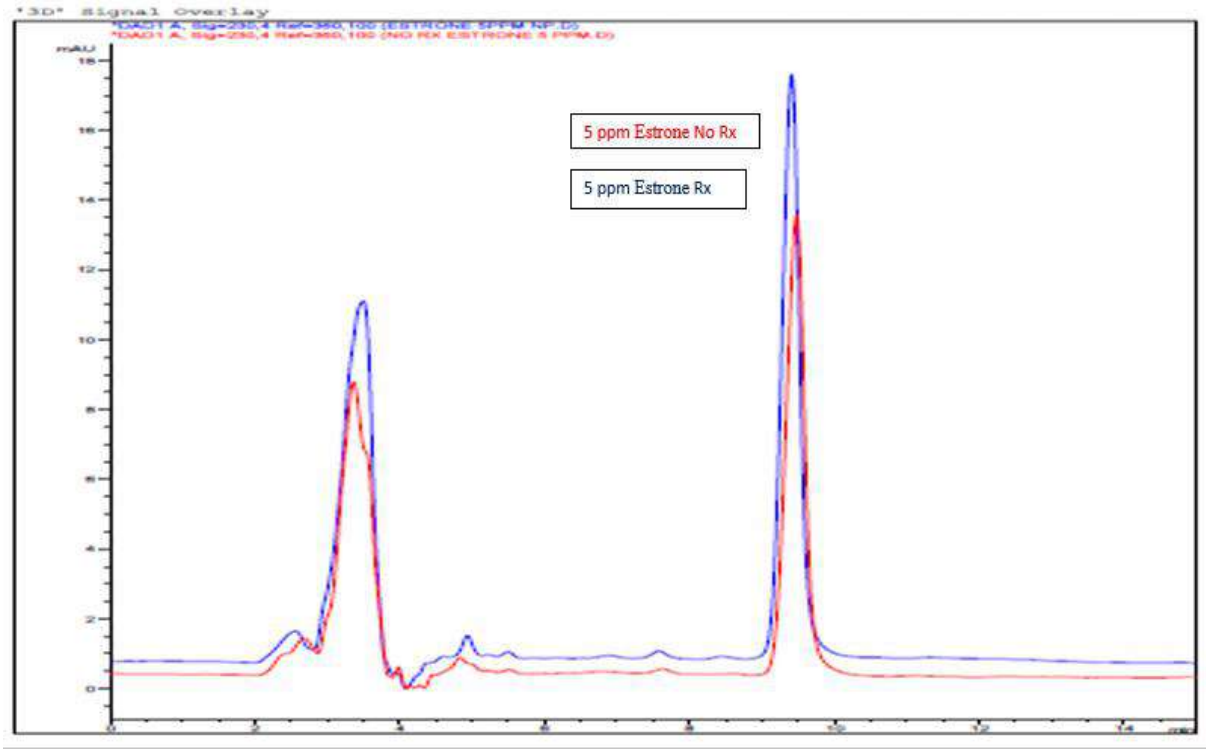

Fig. 16: Overlaid Chromatogram of 5ppm Estrone shows changes in peak area with treatment of AgNP's

Table. 1: Summary of changes in peak areas for selected hormones before and after treatment with AgNP's (-ve sign indicates decrease in peak area (percentage))

S.NO	Compound Name	1 ppm (Area)	5 ppm (Area)	1 ppm EDC Rx with 10 PPM NP's (Area)	5 ppm EDC Rx with 10 PPM NP's (Area)
1	17 Alpha ethynyl estradiol (Rt= 8.6)	116	660	133	644 (-2.3%)
2	Beta Estradiol (Rt= 7.6)	153	519	138 (-10.4 %)	539
3	Estrone (Rt= 9.4)	138	244	129 (-6.5 %)	292
4	Diethylstilbestrol (Rt= 10.6)	280	653	177 (-36.7%)	552 (-15.5%)
5	19 Norethindrone (Rt= 8.5)	334	1,597	300 (-10.2%)	1,586 (-0.64%)
6	D Norgestrel (Rt= 11.6)	344	874	316(-8%)	630 (-28%)

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Supply Chain Analysis of Small Industry Smoke Roa Fish processing in Kinabuhutan Village, West Likupang District North Minahasa Regency

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Abstract— *Distribution is an integral part of the supply chain of a product. The role of distribution greatly determines the distribution of a product to reach consumers. An efficient process is needed that can ensure that the product reaches consumers widely and at the lowest possible cost. A business should have an efficient distribution channel, so that the business can dominate the market.*

The purpose of this study was to determine the system of cooperation between roa fishing fishermen and smoked roa fish processors in Kinabuhutan Village and to determine the amount of marketing efficiency at each raw material supply node in Kinabuhutan Village. The population in this study were roa fishers, fish processors, middlemen and retailers. Data were collected using purposive sampling. The data collected in the form of primary data and secondary data. Primary data collection techniques with observation and interviews. The data obtained were analyzed using quantitative descriptive analysis and qualitative descriptive analysis.

Based on the results of the study, there are five main components or actors in supply chain analysis, namely fishermen, smoked roa fish processors, middlemen, retailers and final consumers. In addition, there are 3 types of flow in the supply chain, namely product flow, financial flow and information flow. The flow of smoked roa fish products flows from upstream to downstream or from fishermen to final consumers. Meanwhile, financial flows flow from downstream (downstream) to upstream (upstream) or from consumers to fishermen and the flow of information is a flow that flows from two directions, namely from upstream to downstream (downstream) or from fishermen to consumers and from downstream. (downstream) to upstream (upstream) or from consumers to fishermen. From the results of the analysis, it is also known that the marketing of smoked roa fish products is very efficient because all nodes have an efficiency value < 1, but the most efficient is at the fishermen's node because the value is equal to 0.

Keywords— ***Supply Chain, Smoke Roa, Kinabuhutan.***

I. INTRODUCTION

Distribution is an integral part of the supply chain of a product. The role of distribution greatly determines the distribution of a product to reach consumers. An efficient process is needed that can ensure that the product reaches consumers widely and at the lowest possible cost. A business should have an efficient distribution channel, so that the business can dominate the market. Efficient marketing distribution can be done by reducing the number of distributors, reducing distribution costs, reducing promotional costs, increasing sales from each distributor, increasing sales quantity for each customer, increasing profits from distributors and also from customers (Suseno, 2008).

Kinabuhutan Village is one of the coastal villages located on a small island called Kinabuhutan Island and is included in the Likupang Barat sub-district, North Minahasa Regency. The average population of

Kinabuhutan Village works as a fisherman and most of them are traditional fishermen. One of the catches of fishermen in Kinabuhutan Village is roa fish or julung-julung which is the raw material for the smoked roa fish processing industry. Previously, the processing of smoked roa fish only utilized the excess catches of local fishermen so that they were not damaged and could be stored longer. However, now it is a commercial business, the produce in the form of smoked roa fish has been marketed outside the village or Kinabuhutan Island.

The problem is that the sustainability of the smoked roa fish processing business is very dependent on the continuity of the supply of fresh roa fish as the main raw material. For this reason, cooperation is needed between various parties involved in the smoked roa fish processing industry, both business actors and the government in maintaining the availability of raw material supplies to the hands of smoked roa fish processors and end consumers. According to Vermulen et al. (2008), value chain analysis is used to assess all business activities and their relationships in the supply chain. This value chain analysis aims to overcome inefficiency constraints such as variability, reduce vulnerability and increase capacity to adapt to change.

This study aims to determine the system of cooperation between roa fishing fishermen and smoked roa fish processors in Kinabuhutan Village and to determine the amount of marketing efficiency at each supply node of raw materials in Kinabuhutan Village. This research is expected to increase the awareness of fishermen and smoked roa fish processors on the importance of mutually beneficial cooperation for the sustainability of their respective businesses. Fishermen and smoked roa fish processors have an interdependent relationship, fishermen need smoked roa fish processors as potential consumers and smoked roa fish processors need fishermen as suppliers of industrial raw materials. The greater the percentage at each supply node, the more efficient the existing supply chain, so that it can result in an increase in fishermen's income which will be followed by an increase in the welfare of the community in Kinabuhutan Village in general and fishermen in particular.

II. RESEARCH METHODS

This type of research is descriptive research. Descriptive research is a method that is intended to describe existing phenomena, both present and past. This research does not manipulate or change the independent variables, but describes the conditions as they are. Descriptive research can describe a situation only, but can also describe the situation in its stages of development

The population in this study was divided into 4 groups, namely roa fishermen, smoked roa fish processors, middlemen and retailers. The total population of fishermen and smoked roa fish processors can be said to be homogeneous so that the sampling for each group is enough for 1 person, for the marketing area there are 4 marketing areas namely Bitung, Airmadidi, Manado and Tomohon, 1 middleman and 1 retailer are taken each. . With such a number of samples in this study there were 10 people, namely 1 fisherman, 1 smoked roa fish producer and 4 intermediary traders with different marketing areas and 4 retailers, which were taken by purposive sampling. Primary data collection was carried out by observation, interviews guided by questionnaires and documentation. The data analysis used is quantitative descriptive analysis and qualitative descriptive analysis. Qualitative descriptive analysis is data processing that is carried out through logical considerations with systematic author languages. The analyzed data is then interpreted with logical considerations using the author's systematic languages by referring to references related to research results, journals (Fathoni, 2006). The data that has been collected is analyzed using steps that are often called triangulation, proposed by Miles and Huberman (1994) in Sugiyono (2010), namely: (1) Data reduction; (2) Data Display; and (3) Conclusion, as shown in Figure 1

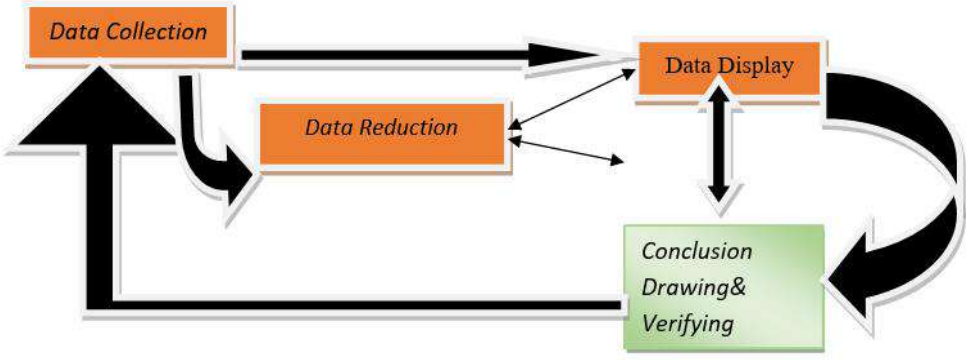

Fig.1: Interactive Model Data Analysis

III. RESULTS AND DISCUSSION

State of the research area

Kinabuhutan Village is one of the coastal villages located on a small island called Kinabuhutan Island and is included in the Likupang Barat sub-district, North Minahasa Regency. The average population of Kinabuhutan Village works as a fisherman and most of them are traditional fishermen. One of the catches of fishermen in Kinabuhutan Village is Julung-Julung fish or in the local language called Roa fish. The catch obtained by fishermen must be marketed as soon as possible

Fresh Roa Fish Supply Fisherman

The catch of roa fish in Kinabuhutan Village is only marketed in Kinabuhutan Village, because it is constrained by the perishable nature of the fish and the small and traditional production scale of the catch and the limited financial position of fishermen. The catch of roa fish in Kinabuhutan Village exceeds the amount of demand, especially when it is the fish season so that there is an excess of production or supply. Roa fish that are not sold will be damaged if it is not processed, if it has to be sold outside Kinabuhutan Island it takes time for transportation which can reduce the quality or quality of the fish.

Smoked Roa Fish Industry

Smoking fish is one way of preserving fish which is a combination of the process of salting, drying, and giving smoke. Smoking has several advantages, namely providing a preservation effect, affecting taste, taking advantage of excess catch when the catch is abundant, this allows fish to be stored longer until the lean season, and increases the availability of protein for the community throughout the year. Smoked fish becomes durable because of the reduction in water content as a result of the heating process and the presence of chemical compounds in the smoke such as the phenol group which can inhibit the growth of microorganisms and act as antioxidants.)

In Kinabuhutan Village, there are 3 entrepreneurs who process smoked roa fish. Thus the problem of excess roa catches in Kinabuhutan Village has been resolved because all roa catches can be accommodated and purchased as raw material for smoked roa fish with the same selling price of Rp. 500,- per fish from fishermen. This is very helpful for fishermen in marketing their catch.

Based on the results of the interview, it is known that the costs incurred in the Smoked Roa Fish Processing Business per production with an average production of 150 pins are Rp. 1.789.250, -. Thus the production cost of smoked roa fish per pin is Rp. 12,000,- The production in the form of smoked roa fish is sold for Rp. 20,000, - per pin and marketing costs in the form of sea transportation to Likupang are Rp. 100,000, - and eat and drink coffee and the cake is Rp. 30,000, - so that the total marketing costs incurred by the producer are Rp. 130.000,-. In one marketing, an average of 1000 pins of smoked roa fish are brought in. Thus, the marketing cost per pinch spent by smoked roa fish producers is Rp. 130, - so that the profit or margin obtained by smoked roa fish producers is Rp. 7,870, - per pin.

Intermediary Trader

The production of smoked roa fish originating from Kinabuhutan Village is quite large so that the marketing area is not only in Kinabuhutan Village but spreads to Likupang, Bitung, Manado and Tomohon through intermediary traders. This middleman picks up roa fish from Kinabuhutan Village directly at the Likupang port where transportation ships from Kinabuhutan dock. The middleman buys smoked roa fish from Kinabuhutan Village for Rp. 200,000,- per bunch containing 10 pins or Rp. 20,000,- per pin or gepe. Then these middlemen supply smoked roa fish to retailers in markets in Manado, Bitung, Tomohon and Airmadidi.

The costs incurred at the intermediary level vary depending on the marketing area, in general the brokerage fees incurred are only for vehicle rental costs and meals. \Based on the results of interviews with intermediary informants from Bitung and Manado, the cost for renting a pick-up vehicle is the same, namely Rp. 250,000 and a meal of Rp. 25,000, with a load of 300 flops for those in Bitung and 500 flops for those in Manado. Thus the cost at the intermediary level per pin for the Bitung marketing area is Rp. 915, -, while for the Manado marketing area it is Rp. 550, -. For intermediary traders in Airmadidi only use 2-wheeled vehicles so that the costs incurred are only for gasoline costs of Rp. 25,000, - and only drinking coffee and cakes for Rp. 10,000, - so a total of Rp. 35,000, -. The smoked roa fish that is transported is not much, only 100 pins, so the cost per pin is Rp. 350,. Lastly, for middlemen in the marketing area of Tomohon, the cost of renting a vehicle is up to Rp. 400,000, - and eating 2 people is Rp. 50,000, - so the total cost is Rp. 450,000, - while the smoked roa fish transported reached 500 flops. Thus the cost per pin is Rp. 900, -

Retailer

Retailers who are used as informants are retailers who are marketed in their marketing areas. The costs incurred by retailers who are in the market are generally only costs for eating and drinking because smoked roa fish are delivered by intermediary traders directly based on orders. The cost for eating and drinking coffee and cake per day for retailers in the market is the same in all marketing areas, which is Rp. 30,000, - because that is the general standard

of their costs while in the market. However, the price at each retailer varies according to the marketing area. The average smoked roa retailer in the market not only sells smoked roa but also sells almost all kinds of salted fish so that the marketing costs cannot be charged to the sale of smoked roa only but share with other types of processed fish. Smoked roa fish retailers sell about 20 types of salted fish from various types of anchovies to various types of salted reef fish with various levels of selling prices. Therefore, in analyzing marketing costs by retailers per type of fish, it is obtained that Rp. 30,000, - is divided by 20 types of fish sold so that per type is charged a fee of Rp. 1,500, - Likewise, Smoked Roa Fish which is successfully sold by each retailer is not same, this will affect the marketing cost per pin. The selling price per pin for marketing the city of Bitung is Rp. 30,000 with a capital of Rp. 26,075, - so that retailers in Bitung earn a profit of Rp. 3.925. The selling price per pin for marketing Airmadidi is Rp.28,500,- with a capital of Rp.25,150,- so that retailers in Airmadidi earn a profit of Rp.3,350,-. The selling price per pin for marketing in Manado City is Rp. 30,000, - with a capital of Rp. 26,075, - so that retailers in Manado City earn a profit of Rp. 3,925, -. The selling price per pin for marketing in Tomohon City is Rp. 32,000, - with a capital of Rp. 27,100, - so that retailers in Tomohon City get a profit of Rp. 4,900, -.

Supply Chain Analysis of Smoked Roa Fish Industry

According to Assauri (2011) supply chain analysis or supply chain analysis involves continuous relationships regarding goods, money and information. Goods generally flow upstream to downstream, money flows from downstream to upstream, while information flows both from upstream to downstream and from downstream to upstream. Viewed horizontally, there are five main components or actors in the supply chain, namely suppliers (suppliers in this study are fishermen), manufacture (factories that make goods in this study are the smoked roa fish processing industry), distributors (intermediary traders), retailers (traders) retailer), customer (customer). Vertically, there are five main components of the supply chain, namely buyer (buyer), transporter (transporter), warehouse (storage), seller (seller) and so on.

Based on the results of interviews and direct observations that researchers did during the research process on the smoked trout processing industry in Kinabuhutan Village, a model was obtained that describes the supply chain flow of the smoked roa fish processing industry in Kinabuhutan Village, as can be seen in the following figure:

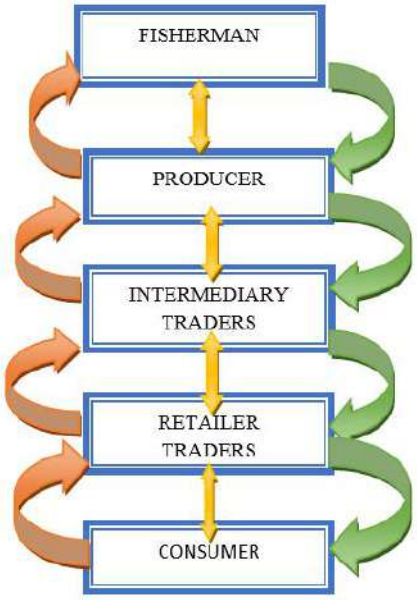

Fig.2: Supply Chain of Smoked Roa Fish Processing Industry in Kinabuhutan Village

Information:

= Product Flow

= Financial Flow

= Information Flow

In general, the supply chain of smoked roa fish processing industry in Kinabuhutan Village is as shown in Figure 1. Supply chain actors consist of fishermen as suppliers of raw materials in the form of fresh roa fish, then smoked roa fish processing producers, intermediary traders, retailers and final consumers. There are 3 types of flow in the supply chain, namely product flow, financial flow and information flow.

The product flow in the supply chain of smoked trout in Kinabuhutan Village is a channel that flows from upstream to downstream. The catch of fishermen in the form of fresh roa fish is the raw material for the smoked roa fish processing industry. The excess catches of fishermen have led to the idea of preserving the catches of fishermen by means of smoke so that they can last up to several months. This idea was followed by several people because if only one person has a smoked roa fish processing business, then all of the excess catches of fishermen have not been accommodated, after 3 people who have a smoked roa fish processing industry business, all the catches of fishermen are absorbed, so the problems

faced by fishermen in Kinabuhutan Village can be resolved.

The flow of fresh roa fish flows into the smoked roa fish processing industry, after going through several stages in the production process of smoked roa fish then it is taken by boat to Likupang to be marketed, and there are waiting for middlemen who are ready to accommodate the flow of smoked roa fish products. The middlemen are then ready to supply smoked roa fish products to retailers in traditional markets in Bitung, Airmadidi, Manado and Tomohon. The final consumer buys smoked roa fish products at the market closest to where they live.

The financial flow in the supply chain of smoked roa fish processing industry in Kinabuhutan Village is a flow that flows from downstream (downstream) to upstream (upstream). Final consumers buy smoked roa fish products at different prices according to where they live in Bitung and Manado at a price of Rp. 30,000 per pin, for the Airmadidi area at a price of Rp. 28,500, - per pin and for the Tomohon area it is Rp. .32,000,- per clip. These retailers buy smoked roa fish products from intermediaries at different prices according to the marketing area. In Bitung and Manado the price is IDR 26,000 per pin, for the Airmadidi area it is IDR 25,000 per pin and for the Tomohon area it is IDR 27,000 per pin. Meanwhile, the middlemen all buy from the smoked roa fish processing industry at the same price, which is Rp. 20,000,-. The smoked roa fish processing industry buys raw materials from fishermen for Rp. 500,- per fish or raw materials per pinch for Rp. 10,000, - because each pinch consists of 20 fresh roa fish. The entire financial flow in the supply chain of smoked roa fish uses cash transactions because in Kinabuhutan Village itself there is no Automated Teller Machine (ATM), because electricity only turns on once every 2 days.

The flow of information in the supply chain of smoked trout fish processing industry in Kinabuhutan Village is a flow that flows from two directions, namely from upstream to downstream and from downstream to upstream. Among supply chain actors, the information provided is information about product prices, product quality, product inventory and the number of purchases.

Supply chain actors from upstream will provide information about product prices, product quality and product inventory while supply chain actors from downstream directions will provide feedback such as prices, appreciation or complaints about product quality and the number of orders and purchases. This information is all done through the internet using a cellphone.

The Relationship between Fishermen and the Smoked Roa Fish Processing Industry

The relationship that exists between fishermen as suppliers of raw materials for smoked roa fish production and the smoked roa fish processing industry is very good because they realize that their businesses are interdependent with each other. Fishermen are very dependent on the smoked fish processing industry to accommodate their catch, without the smoked fish processing industry in Kinabuhutan Village, fishermen will find it very difficult to market their catch. Another alternative if the fishermen do not sell their catch to the smoked roa fish processing industry is that the fishermen must sell directly to the Likupang market which is about 1 hour away by boat, in addition to time consuming it will also increase transportation costs. The length of the trip will reduce the quality of the fish caught which results in a decrease in the selling price. This is very detrimental to fishermen, so the existence of smoked roa fish processing industry in Kinabuhutan Village is strongly supported by fishermen, so that the relationship between the two is very well established. Their relationship is the foundation for the sustainability of their respective businesses.

The smoked roa fish processing industry also feels that its business is very dependent on the catch of fishermen, because without fishermen, their business will stop because there are no raw materials to be processed. It is the awareness of this interdependence that causes a very good relationship between fishermen and the smoked roa fish processing industry in Kinabuhutan Village. Their communication and information can be done through cellphones or directly because Kinabuhutan Village is not large, it is only a small island that can be surrounded in just an hour on foot. Transportation in Kinabuhutan village is only by walking everywhere there is no public transportation.

Relationship between Smoked Roa Fish Processing Industry and Intermediary Traders

The relationship between the smoked roa fish processing industry and intermediary traders has existed for a long time because they always communicate via cellphones to discuss the flow of smoked roa fish products that will be sent from Kinabuhutan Village both regarding the price and the quantity available. The number of requests from intermediary traders is adjusted to the smoked roa fish products produced by the industry in Kinabuhutan Village. Delivery of smoked roa fish products from Kinabuhutan Village to Likupang if by chance there is no need for smoked roa fish processors to Likupang, it can be entrusted to the captain of the existing transportation ship with a transportation fee of Rp. 10,000, - per 1000 pins, as

well as the payment money can be deposited because they already have a relationship of mutual trust. The trust of smoked roa fish processors with intermediary traders or vice versa intermediary traders against smoked roa fish processors is the main capital in their relationship.

Relationship between Brokers and Retailers

Intermediaries supply smoked roa fish products to retailers located in several marketing areas. Their relationship is also based on trust, because sometimes middlemen supplying smoked roa fish products do not immediately get cash, sometimes they have to wait a few days before the smoked roa fish products are sold, but this only happens occasionally. Retailers also more often pay in cash to intermediaries, sometimes in debt, but there is still trust between middlemen and retailers or vice versa. This high level of trust that underlies their relationship in business continues to run smoothly

Efficiency of each node of the smoked roa fish supply chain

According to Downey and Erickson (1992) in Ismail et al (2008) that the marketing system is said to be efficient if the value of marketing efficiency is < 1. The value of marketing efficiency is a comparison of product value with marketing costs incurred at each marketing node. In table 5 it can be seen that the marketing of smoked roa fish products is very efficient because all nodes have an efficiency value of < 1, but the most prominent and most efficient is the fishermen's node because the value is equal to 0. This is because to sell their catch, fishermen do not require marketing costs. because from the sea the fishermen immediately docked behind the smoked roa fish processing industry which was on the beach in Kinabuhutan Village. This also makes the marketing of fresh roa fish very efficient. For more details, the value of the marketing efficiency of smoked roa fish products for each node can be seen in Table 1.

Table 1: Value of Marketing Efficiency of Smoked Roa Fish Products for Each Node

No.	Knot	Cost (Rp/pin)	Product Value (Rp/pin)	Eficiency Value (Rp/pin)
1	Fisherman	0	10.000	0
2	Processors	130	20.000	0,0065
3	Brokers			
	Bitung	915	26.000	0,0352
	Airmadidi	350	25.000	0,0140
	Manado	550	26.000	O,0212
	Tomohon	900	27.000	0,0333
4	Retailer Merchant			
	Bitung	75	30.000	0,0025
	Airmadidi	150	28.500	0,0053
	Manado	75	30.000	0,0025
	Tomohon	100	32.000	0,0031

Source: primary data processed, 2021.

In table 1 it can be seen that all the nodes in the marketing of smoked roa fish products are very efficient, but the most efficient are the fishermen's nodes, this is because there are no marketing costs incurred by fishermen.

IV. CONCLUSION

Based on the results of the study, there are five main components or actors in supply chain analysis, namely fishermen, smoked roa fish processors, middlemen, retailers and final consumers. In addition, there are 3 types of flow in the supply chain, namely product flow, financial flow and information flow. The flow of smoked roa fish products flows from upstream to downstream or from fishermen to final consumers. Meanwhile, financial flows flow from downstream (downstream) to upstream (upstream) or from consumers to fishermen and the flow of information is a flow that flows from two directions, namely from upstream to downstream (downstream) or from fishermen to consumers and from downstream. (downstream) to

upstream (upstream) or from consumers to fishermen. From the results of the analysis, it is also known that the marketing of smoked roa fish products is very efficient because all nodes have an efficiency value < 1, but the most efficient is at the fishermen's node because the value is equal to 0.

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In-Vitro Efficacy of Commercial Fungicides against *Bipolaris Sorokiniana*: Induced Spot Blotch Disease of Wheat

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Abstract— *Spot blotch caused by Bipolaris sorokiniana is an important disease of wheat. A laboratory experiment was conducted at Plant Pathology Division, Nepal Agriculture Research Council, Khumaltar, Nepal to evaluate the efficacy of commercially available fungicides viz, Saaf (Carbendazim 12%+ Mancozeb63%), Sectin (Fenamidone 10%+ Mancozeb 50%), Angel (Metalaxyl 8%+ Mancozeb 64%), Diathane M-45 (Mancozeb 75%), G-MIL(Cymoxanil 8%+ Mancozeb 64%) tested in two different concentration i.e. 50ppm and 100 ppm except Curex (Copper oxychloride 50%) tested in the concentration of 100ppm and 200ppm and Tilt (propiconazole 25%) tested in 15ppm and 30 ppm concentration with a control test by employing poisoned food technique against spot blotch pathogen of wheat Bipolaris sorokiniana. The experiment was laid out in Completely Randomized Design (CRD). The result revealed that all concentrations of different fungicides successfully inhibited the radial mycelial growth of the pathogen under in vitro condition. Based on the measurement of fungal radial growth, fungicide Tilt of 30ppm and 15 ppm concentration were the most effective followed by Sectin 100ppm, Diathane M-45 100ppm, Angel 100 ppm and Saaf 100ppm. G-MIL 50 ppm in poisoned food technique was the least effective. Use of safer and economical chemical fungicides can provide an effective and long-term solution against plant diseases in agricultural farming.*

Keywords— ***Spot blotch, Tilt, Trichoderma, efficacy, fungicides, pathogen.***

I. INTRODUCTION

After rice and maize, wheat (*Triticum aestivum*) is Nepal's third most significant cereal crop. Wheat is produced from the Terai to the high alpine regions, and wheat consumption is on the rise. However, the yield per unit is much lower than anticipated. The disparity between the maximum observed yield and the national average yield, as well as the declining yield trend, necessitate immediate research. The low yield is due to a number of factors, including the scarcity of improved varieties, the occurrence of various wheat diseases, a lack of reliable irrigation, inclement weather, a lack of improved technology, and biotic and abiotic stress factors, all of which result in significant yield losses each year (Joshi et al., 2007). Fungal diseases in wheat cultivation are more important among biotic stress factors because they produce a significant drop in yield as well as deterioration in grain quality. In the humid subtropics of South Asia, where the irrigated rice-wheat rotation covers more than 12 million ha, there is growing evidence that stress conditions are increasing the severity of foliar diseases (Dubin *et al.*, 1994) because rice serves as a host for the spot blotch fungi and rice stubble plays its role as a substrate for the fungi after rice harvest (Saari, 1998). Therefore, the fungal pathogen, *Bipolaris sorokiniana* (Sacc.) Shoemaker (teleomorph *Cochliobolus sativus*) induced spot blotch disease of wheat has emerged as one of the prime diseases for profitable wheat production in different zones of Nepal. Spot Blotch is caused by *Bipolaris sorokiniana* in Mega Environment 5A (ME5A), characterized by high temperature (coolest month greater than 17ºC) and high relative humidity (RH) (Dubin *et al.*, 1991; Rajaram *et al.*, 1993). It is a seed borne fungal. Spot blotch (oval to round brown blotch encircled by yellow halo) is the pathogen's principal symptom. The pathogen's continuing growth and

development results in increasing leaf and spike damage, leading in yield loss. The disease occurs every year in Nepal in moderate to severe form (Duveiller *et al.*, 2005). *Bipolaris sorokiniana* was also identified as a main contributing factor in a study undertaken in Nepal in 1996 to investigate and identity the national issue of "low germination in wheat' (Shrestha et al., 1997). The losses due to spot blotch in warm regions of Nepal ranged from 23 –40% depending on the genotypes and other environmental factors (Tripathi *et al.*, 2005; Sharma *et al.*, 2006).

To lessen the losses caused by the disease, a range of syste mic fungicides with various modes of action and targets ha ve been developed (Pasquer et al., 2005). However, in vitro evaluation against *Bipolaris sorokiniana* are barely sufficient and even accurate information on determining efficacy, sensitivity of different fungicides with minimum inhibitory concentrations have yet to be defined against *Bipolaris sorokiniana* (Iqbal, 2010). It is important to examine the effect of different concentration of fungicides commonly available in market to control this disease.

II. MATERIALS AND METHOD

Subculture of pathogen was made by transferring cells from a previous year pure culture to petri-dishes containing fresh growth medium (Potato Dextrose Agar) and incubating at 25ºC for 6 days to prepare the test pathogen (*Bipolaris sorokiniana*). The pure culture was isolated from infected leaves of wheat crop showing characteristic blight symptoms in the research field of Plant Pathology Division under Nepal Agriculture Research Council.

Seven fungicides with two different concentrations were evaluated for their efficacy to inhibit the mycelium growth of *B. sorokiniana* under in vitro condition following poisoned food technique. Five fungicides (Saaf, Sectin, Angel, Diathane M-45, G-MIL) with two concentration viz., 50 and 100 ppm, one fungicide (Curex) with two different concentration viz., 100 and 200 ppm and one fungicide (Tilt) with two different concentration viz., 15 and 30 ppm were evaluated. Concentration (PPM) of the fungicide was calculated based on active ingredients (a.i.) of the pesticide provided by the company on each packet. Stock solution of each fungicide was prepared in distilled water and incorporated into Potato dextrose agar medium and mixed thoroughly before autoclaving. After autoclaving the medium was poured aseptically in sterilized petri-plates of 9 cm size under laminar air flow and allowed to cool. Five mm mycelial circular discs of pathogen excised with sterile cork borer from a seven days old culture of test pathogen was placed at the centre of each petri-plate and incubated at 25ºC. PDA with water or without chemical served as control. The experiment was arranged in complete randomized design (CRD) and there were four replications for each (15) treatments. The plates were incubated at 25 ºC inside BOD incubator. Measurement of the colony diameter of pathogens was taken after 48 hours for 10 days (i.e 2^{nd}, 4^{th}, 6^{th}, 8^{th} day and 10^{th} day) after inoculation with the help of vernier caliper. Percent growth inhibition of the pathogen was calculated by using the following formula of Vincent (1947).

$$I\ \% = \frac{C-T}{C} \text{ X } 100$$

Where,

I= inhibition percentage

C= Colony diameter in control and T=Colony diameter in treatment

The data collection started from 48 hours after poisoned food technique i.e. 8th to 18^{th} March 2021. The data were recorded by measuring the growth of the test pathogen after each 24 hours by using Vernier caliper in mm. The data obtained from the experiment were analyzed using the software Gen Stat for the analysis of variance (ANOVA) to test the significance of treatments effect on mycelial growth of *Bipolaris sorokiniana.* Means of significant treatments at 5% level of significance were compared following Duncan's Multiple Range Test (DMRT) and Microsoft Excels.

Table 1 Commercial agrochemicals (treatments) used in the study

SN	Commercial Name	Common Name	Active Ingredients (a.i.)	50 ppm (mg)	100 ppm (mg)
1.	Saaf	Carbendazim 12% +Mancozeb 63% WP	75	6.67	13.33
2.	Sectin	Fenamidone 10% + Mancozeb 50% EC	60	8.33	16.67
3.	Angel	Metalaxyl 8% + Mancozeb 64% WP	72	6.94	13.89
4.	Diathane M-45	Mancozeb 75 % WP	75	6.67	13.33
5.	G-MIL	Cymoxanil 8% + Mancozeb 64% WP	72	6.94	13.89

SN	Commercial Name	Common Name	Active Ingredients (a.i.)	15 Ppm	30 Ppm
6.	Tilt	Propiconazole 25% EC	25	6	12
SN	**Commercial Name**	**Common Name**	**Active Ingredients (a.i.)**	**100 Ppm**	**200 Ppm**
7.	Curex	Copper oxychloride 50% WP	50	20	40
8.	Control	Water (Distilled)	0	0	0

III. RESULTS AND DISCUSSION

3.1 Effect of commercial fungicides on the radial growth of mycelium of *Bipolaris sorokiniana* by poisoned food method

Chemical fungicides viz, Saaf (Carbendazim 12%+ Mancozeb63%), Sectin (Fenamidone 10%+ Mancozeb 50%), Angel (Metalaxyl 8%+ Mancozeb 64%), Diathane M-45 (Mancozeb 75%), G-MIL(Cymoxanil 8%+ MAncozeb 64%) were tested in two different concentration i.e. 50ppm and 100 ppm except Curex (Copper oxychloride 50%) which was tested in concentration of 100ppm and 200ppm and Tilt (propiconazole 25%) tested in 15ppm and 30 ppm concentration against *Bipolaris sorokiniana*. In the table below, data were transformed to standard unit (cm) to avoid statistical complications during analysis and data were and rounded to hundredth decimal unit. From the result it was observed that poisoned food method showed all fungicides tested with different concentration inhibited the radial growth of *Bipolaris sorokiniana* as compared to untreated control.

Means with the different letters are significantly different at 5% level of significance using LSD. In (Table 2), different letters in a column signifies that the treatment means are significantly different with each other at P-value <0.001.

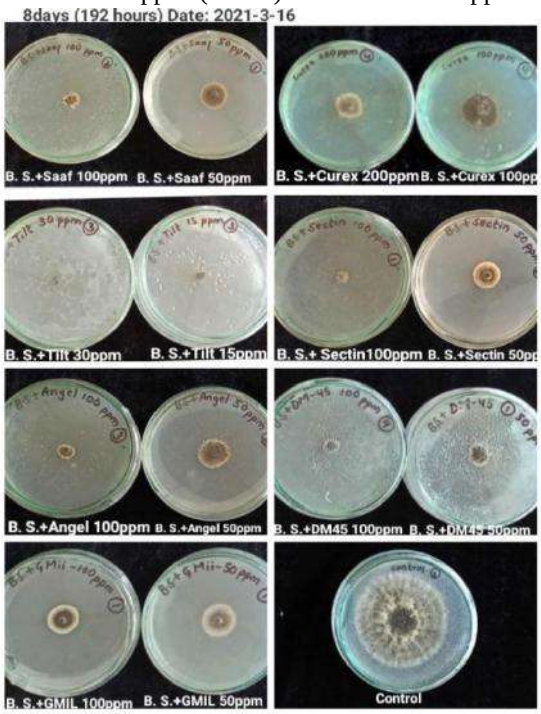
Table 2 Mean radial growth of mycelium of Bipolaris sorokiniana in presence of commercial fungicides

Fungicides	***Mean radial mycelial growth (cm)***				
	Day 2	*Day 4*	*Day 6*	*Day 8*	*Day 10*
Saaf 100ppm	0.00 ^{g}	0.99 ^{gh}	1.25 ^{f}	1.55 ^{f}	2.03 ^{hi}
Saaf 50ppm	0.90 ^{d}	1.46 ^{d}	2.07 ^{cd}	2.75 ^{bc}	3.37 ^{bc}
Sectin 100ppm	0.00 ^{g}	0.00 ^{i}	0.74 ^{g}	0.88 ^{g}	1.06 ^{k}
Sectin 50ppm	0.84 ^{de}	1.37 ^{de}	2.01 ^{cd}	2.34 ^{d}	2.60 ^{ef}
Angel 100ppm	0.63 ^{f}	0.85 ^{h}	1.08 ^{f}	1.59 ^{f}	1.82 ^{ij}
Angel 50ppm	0.86 ^{de}	1.45 ^{d}	2.10 ^{cd}	2.60 ^{c}	2.88 ^{de}
Diathane M-45 100ppm	0.69 ^{f}	0.83 ^{h}	1.09 ^{f}	1.35 ^{f}	1.54 ^{j}
Diathane M-45 50ppm	0.83 ^{de}	1.14 ^{fg}	1.73 ^{e}	2.05 ^{e}	2.44 ^{fg}
G-MIL 100ppm	0.74 ^{ef}	1.21 ^{ef}	1.94 ^{de}	2.67 ^{c}	3.20 ^{c}
G-MIL 50ppm	0.88 ^{d}	1.49 ^{d}	2.25 ^{c}	2.99 ^{b}	3.63 ^{b}
Curex 200ppm	1.52 ^{c}	1.93 ^{c}	1.95 ^{de}	2.18 ^{de}	2.24 ^{gh}
Curex 100ppm	1.76 ^{b}	2.50 ^{b}	2.62 ^{b}	2.81 ^{bc}	3.08 ^{cd}
Tilt 30ppm	0.00 ^{g}	0.00 ^{i}	0.00 ^{h}	0.00 ^{h}	0.00 ^{l}
Tilt 15ppm	0.00 ^{g}	0.00 ^{i}	0.00 ^{h}	0.00 ^{h}	0.00 ^{l}
Control	2.15 ^{a}	4.77 ^{a}	6.92 ^{a}	8.36 ^{a}	9.00 ^{a}
Grand Mean	0.78	1.33	1.85	2.27	2.59
LSD	0.1137	0.1636	0.2299	0.243	0.2827
CV	10.1%	8.6%	8.7%	7.5%	7.7%
SEm	0.0399	0.0574	0.0807	0.0853	0.0992

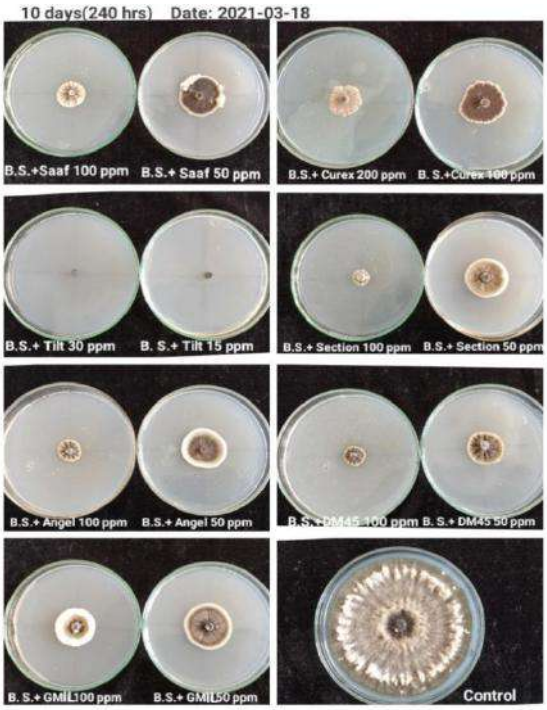
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
Sedm	0.0564	0.0812	0.1141	0.1206	0.1402

CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by Duncan's Multiple Range Test at 5% level of significance, SEm (±) indicates standard error of the mean, cm is centimeters

Data of Day 10 shows that the pathogen covered the entire petri-plate of 9cm in the control treatment. Tilt 15 ppm and Tilt 30 ppm made lowest or no growth of the pathogen. The lowest mean radial growth was observed in the treatment Sectin 100ppm (1.06cm) being the most effective fungicide treatment after Tilt 15 and 30 ppm. The radial growth of the pathogen was highly influenced by Tilt (30 and 15ppm) and Sectin 100ppm. While significant treatments, G-MIL 100ppm (3.2cm) and Curex 100 ppm (3.08cm) were least effective followed by significant treatments G-MIL50 ppm (3.63cm), Saaf 50ppm (3.37cm) as compared to other treatments. The results clearly demonstrated that the fungicide Tilt (Propiconazole 25%) of both 30 and 15 ppm concentration followed by Sectin (Fenamidone 10% + Mancozeb 50%) of 100 ppm were individually effective against the pathogen by maximum inhibiting the mycelia growth in all days of data collection.



Radial mycelial growth of pathogen on Day 8



Radial mycelial growth of pathogen on Day 10

Table 3 Effect of commercial fungicides on the inhibition percentage of Bipolaris sorokiniana by poisoned food method

Fungicides	**Mycelial growth inhibition (%)**				
	Day 2	**Day 4**	**Day 6**	**Day 7**	**Day 10**
Saaf 100ppm	100.00	79.21	81.88	81.49	77.48
Saaf 50ppm	58.24	69.24	70.04	67.12	62.50
Sectin 100ppm	100.00	100.00	89.29	89.38	88.13
Sectin 50ppm	60.90	71.29	70.93	72.06	71.11
Angel 100ppm	70.77	82.08	84.33	80.90	79.72
Angel 50ppm	60.09	69.66	69.63	68.85	68.00
Diathane M45 100ppm	67.98	82.61	84.14	83.80	82.83
Diathane M45 50ppm	61.60	76.05	75.05	75.49	72.91
G-MIL 100ppm	65.55	74.60	72.01	68.01	64.44
G-MIL 50ppm	59.16	68.73	67.44	64.22	59.67

Curex 200ppm	29.58	59.56	71.77	73.84	75.11
Curex 100ppm	18.21	47.61	62.21	66.38	65.78
Tilt 30ppm	100.00	100.00	100.00	100.00	100.00
Tilt 15ppm	100.00	100.00	100.00	100.00	100.00
Control	0.00	0.00	0.00	0.00	0.00

Irrespective of the low concentration, at the end of tenth day maximum inhibition of mycelial growth for the control of *Bipolaris sorokinina* was recorded in Tilt 30ppm (100%) and Tilt 15ppm (100%) which was significantly superior than other treatments followed by Sectin 100ppm (88.13%) , Diathane M-45 100ppm (82.83%), Angel 100ppm (79.72%), Saaf 100ppm (77.48%) and Curex 200ppm (75.10%), Diathane M-45 50ppm (72.90%) and Sectin 50ppm (71.10%). The mycelial growth inhibition percentage ranged between 59.6 to 100 % on tenth day of poisoned food experiment. The inhibitory effect of G-MIL50 ppm was the lowest i.e. 59.6%. Looking at the percentage inhibition between same fungicides of different concentrations, the results revealed that inhibition percentages increased with the increase in concentration of fungicides. Most of the reports also concluded increase in concentration to be directly proportional to the increase in inhibition potential (Maitlo *et al.,* 2014; Rafique *et al.,* 2016). Among the fungicides, Tilt (Propiconazole 25%) in almost all days of data collection was found to be significantly superior showing highest mycelial growth inhibition throughout the whole experiment.

Complete inhibition of radial mycelial growth and sporulation of *Bipolaris sorokiniana* was found with Tilt (Propiconazole), so it may be effective to completely control the pathogenic activity of *Bipolaris sorokiniana*. The result agrees with the results of various previous workers. Chattopadhyay *et al.,* (2013) found out that Tricyclazole inhibited the melanin production and reduced the sporulation, spore size and number of septa in conidia of *Bipolaris sorokiniana* under in vitro condition. Triazole fungicides (e.g., propiconazole and tebuconazole) inhibit the synthesis of sterols, which are building blocks of the membranes of fungal cells. Response of *Bipolaris sorokiniana* on media containing fungicide of Triazole group (e.g.- Tebuconazole and Propinazole) reduced the growth of fungus successfully (Pannu *et al.,* 2006; Sooväli and Koppel, 2009; Yamaguchi *et al.,* 2010; Acharya *et al.,* 2011; Rahman *et al.,* 2013). Singh and Gupta (2000) studied the bioassay of fungicides against *Dreschlera sativus* causing leaf blight of wheat and the result recordes tilt to be the most effective fungicide in inhibiting the mycelial growth.

Diathane M-45 (Mancozeb) 100ppm (82.83%) also successfully inhibited the mycelial growth. Giri *et al.* (2001) also demonstrated effectiveness of mancozeb (90.5%) to control infection of seeds caused by *Bipolaris sorokiniana*. Mancozeb, belonging to the dithiocarbamate family, disrupt the metabolism of fungi by inhibiting either glucose oxidation, or nucleic acid synthesis, or by degradation of fatty acids (Angdembe *et al.,* 2019). Mancozeb has direct effect upon the core biochemical processes within the fungus which results in inhibition of spore germination (Wong and Wilcox, 2001). Variation in the inhibition percentage of *Bipolaris sorokiniana* at different concentrations of mancozeb was reported in different experiments. There was higher (82.83%) percent inhibition at 100 ppm in our experiment whereas only 70 percent inhibition even at higher concentration of 400 ppm (Hasan *et al.,* 2012) and 34 percent inhibition at 300 ppm (Samia *et al.,* 2015) are also reported. These differences in inhibition rates may be due to different strains of *Bipolaris sorokiniana* and different quality of mancozeb used in these experiments. Sharma (2006) findings explored effectiveness of Mancozeb (Dithane M-45) against rot of *Coccinia indica* caused by *Bipolaris tetramera.*

Mancozeb alone highly inhibited the growth of *Bipolaris sorokiniana*. However, it showed variations when used as combination fungicides. Mancozeb with Fenamidone inhibited the mycelial growth by 88.13 % at 100 ppm. Mancozeb with carbendazim at 100ppm also showed better results i.e. 77.48% mycelial inhibition. Likewise Mancozeb with metalalxyl at 100 ppm showed 79.72% mycelial growth inhibition. But Mancozeb with Cymoxanil at 100 ppm (64.4%) and 50 ppm (59.6%) was lower as compared to other combinations.

It was reported that copper oxychloride showed 75.1% mycelial growth inhibition at 200 ppm and 65.7% at 100ppm. The mode-of-action of copper fungicides is the nonspecific denaturation of cellular proteins. It disrupts the function of proteins and enzymes after absorption and results in cell damage and membrane leakage (Husak, 2015). Samia *et al.* (2015) also reported 70-80% mycelial growth inhibition in isolates of *Bipolaris sorokiniana*, collected from different region of Bangladesh, at 300 ppm concentration of copper oxychloride.

IV. CONCLUSION

Among the tested fungi-toxicants, Propiconazole (15 and 30 ppm) gave 100% mycelial inhibition of the test pathogen being the most effective fungicide followed by other single composition fungicides as well as combination fungicides viz., Fenamidone + Mancozeb 100ppm (88.13%), Mancozeb (82.83%), Metalaxyl + Mancozeb 100ppm (79.72%), Carbendazim + Mancozeb 100ppm (77.48%). The experiment showed variable effect of different fungicides of similar concentration. The effective treatments from the result of this study under in vitro conditions are only indicative and can be used to test for further trial under natural field condition (in pots or fields) for the confirmation of their efficacy against *Bipolaris sorokiniana*.

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Effects of cooking process on the level of heavy metal accumulation in vegetables: Literature review

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Abstract— *People, nowadays, consume vegetables, fruits and herbs contaminating such heavy metals either from polluted air or water sources without awareness on a daily basis. However, some vegetables are regularly consumed after going through the cooking process, while some are eaten freshly. This paper, therefore, gathers information regarding whether cooking methods are able to lower the level of heavy metal in food or not, focusing on vegetables. Finally, It could be concluded that different cooking methods have widely different effects on heavy metal concentration. In other words, some processes potentially minimize certain types of metal, while others may be unable. According to the studies, water is claimed to enhance the reduction of toxic substances concentration through the evaporation, therefore, boiling usually lowers some metal levels, though the change in such levels are not considerable and frequently not that adequate to be healthy. Moreover, there are other ways to eliminate such heavy metals directly in plants, namely flushing, and using DTPA, chemical substances which both are claimed to be potential methods.*

Keywords— ***Cooking methods, heavy metal, vegetables.***

I. INTRODUCTION

There are widespread measurements of heavy metals accumulation in plants. The study areas consider where there is a high probability that plants might absorb large amounts of heavy metals. Hydrophytes, plants living in water, absorb heavy metals from water they live in. Researchers, therefore, usually conduct samples from the river or water resources nearby factories or where people were packed in, which means water is highly utilized and tremendous amounts of wastes are released into the water sources. It is therefore lead to marked accumulation of heavy metals including lead (Pb), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Manganese (Mn), Zinc (Zn), Copper (Cu) and Nickel (Ni) [8] as well as other perilous chemical substances. Similarly, Embryophytes, land plants researchers often conduct samples where there is an avalanche of mining and manufacturing as it causes an enormous accumulation of heavy metals in the vicinity of soil, Embryophytes then absorb excessively such heavy metals from those contaminated soil. In addition to this, using chemical fertilizers or pesticides in plantations also causes the overwhelming heavy metals' accumulation which could lead to fatality in plants.

In recent times, there has been a dramatic increase in the number of globally contaminated vegetables, both home-grown vegetables and herbs [11]. This is due to the fact that the size of manufacture, where the polluted water; possibly contaminated with heavy metal, is normally released into the water sources such as lake and river, has grown substantially year by year and the water is then used for crop. Moreover, the number of passenger cars, buses and other motor vehicles which produce enormous amounts of pollution have also continued to increase exponentially and consequently lead to the rising amounts of heavy metal in the environment. When it comes to current agriculture, due to more and more fertilizer and pesticide being used for accelerating outcomes, soil becomes intensely and continuously contaminated with heavy metal. Organic pesticide is invariably added by several banes such as Copper (Cu), Mercury (Hg) and Arsenic (As) which are residue, so this can also cause contamination in soil and vegetables. It is

undeniable that people regularly eat vegetables such as carrot, onion, lettuce and cabbage in everyday life, thus when vegetables absorb heavy metal from soil [12], people consume them unaware of taking heavy metal into their bodies. Likewise, herbs, popularly used as an alternative treatment and added in many spices, also contain huge amounts of heavy metal. Therefore, related organizations of each country have determined an appropriately standard quantity of heavy metal to limit and make sure that it would not harm people in their countries because some metals are beneficial if intaking proper amounts such as Copper (Cu) and Iron (Fe).

However, when it comes to vegetable consumption, there are several fresh vegetables which people regularly eat, lettuce, cucumber, tomato, red and green oak in salad, for instance, while some vegetables are eaten after they are passed such cooking processes as boiling, steaming, frying, and stir-frying. Whether these cooking processes can lower the contaminants in vegetables and also in meats has sparked heated debates. Several researches have been conducted and has got different results, so this paper will review the effect of different cooking methods on the contents of these metals in certain plants and meats, focusing on vegetables and information was gathered from published papers and the findings from various authors.

II. BACKGROUND

2.1 Heavy metals frequently found in plants and effects on human body

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times [6] greater than that of water, with atomic numbers ranging from 23-92, totaling 72 elements in periods 4-7, such as cadmium, chromium, mercury, lead, etc. Some are useful in industry. Some are beneficial to the body, but some are toxic to the body. [1][2]

2.1.1. Cadmium: Cd

2.1.1.1. Benefits

Cadmium is used in several categories of industry including metal plating process to prevent rust and add luster, used as a lubricant, used in the battery manufacturing process, used as a component of paint, used in the production of plastics, used in the welding process, used as a mixture in fungicides and chemicals used in agriculture, used as a nuclear fission rate regulator in atomic reactors, used in the manufacture of fluorescent lamps, use to mix with other metals as an alloy to increase toughness and corrosion resistance, used in combination with other metals in the jewelry and jewelry industries.

2.1.1.2. Harm to the body

In nature, cadmium is always found in combination with zinc. It has an element with chemical and physical properties similar to zinc, which is a constituent of the enzyme. When entering the body, it can replace the atoms of zinc in enzymes causing toxic effects on the body including digestive system and metabolism failure, inhibition of the formation of red blood cells which then cause anemia, sluggish body, chills and fever, high blood pressure, heart disease and irregular heartbeat, kidney malfunction which leads to kidney failure, 'Itai-Itai disease' which causes pain in the joints and bones, blurred vision, diarrhea, vomiting, liver failure and, even worse, the likelihood of death. Moreover, if the body has cadmium more than 15 ppm, it will cause high proteinuria.

2.1.2. Chromium: Cr

2.1.2.1. Benefits

Chromium is a heavy metal that is widely used in many industries widely including tanning industry as a compound of Cr(OH)SO4), used as an ingredient in the manufacture of stainless steel, used in combination with iron, nickel and other metals to produce corrosion and high heat resistant alloy steels, used as a metal plating agent, metal coating as well as plastic coating to make it shiny and prevent corrosion, such as in the humid industry, automobile parts, etc., used as an ingredient in the production of dyes or pigments such as Chrome oxide green (Cr2O3), Chrome yellow (PbCrO4), Chrome orange (PbCrO4.PbO), as well as a dyeing agent in the textile and dyeing industry, used as a mixture of spray paint, house paint, used as a mixture of fungicides and wood preservative, used to produce dust deterrents and prevent corrosion of diesel engines, used as rust inhibitors such as dichromate compounds, used in the colored glass industry, especially Chromium(III), used in the production of heat-resistant bricks, used as a catalyst for chemical reactions and chemical synthesis, used in medicine such as chromium isotope (Cr-51). Moreover, Chromium (III) is a chromium that can be found in the human body. It plays an important role in the function of the hormone insulin that regulates blood sugar levels.

2.1.2.2. Harm to the body

Chromium toxicity to the human body is often caused by the poisoning of Chromium (VI), which is currently being used a lot in industry as a component of various products that are used by humans causing contamination to the environment and the most likely to enter the human body. Symptoms of chromium poisoning caused by the body's exposure to low to moderate amounts and have accumulated for a long time will gradually begin to appear in various organs of the body including inflammation of

the skin irritation; chronic wounds; slow wound healing; the mucous membranes of various internal organs are irritated and destroyed; kidney, liver and lung malfunction and damage; osteoporosis; cancer in various organs; respiratory failure and risk of sudden death.

2.1.3. Lead: Pb

Lead, when entering the body, has properties similar to calcium, that is, it accumulates in the bones and in the hair.

2.1.3.1. Benefits

Lead is used in various aspects. It is used in the soldering industry, in the petroleum industry and is a mixture of oil and fuel, used to produce batteries, used as a lead compound for mixing paint, used to make ammunition, used as an elemental alloy with copper and iron to enhance turning or cutting properties, and used to control the loudness of the sound of machinery (Thu Chan Nuan, 2007). Lead is, also, a component of important chemicals (Kittiphan Bangyikhan, 2008), such as pesticides.

2.1.3.2. Harm to the body

Intake of lead can cause several severe malfunctions of the body including acute poisoning such as physical exhaustion, dizziness, vomiting and muscle spasms; headache and difficulty sleeping; chronic poisoning which anemia and slim body are accounted. Furthermore, lead has an ability to inhibit the activity of enzymes as well as the process of red blood cell formation causing anemia. It can, in addition, results in bone decay, and creates damages in the nerve cells causing the brain to swell and inhibiting the activity of chemicals in the brain leading to the risk of dementia and neurological symptoms. Moreover, it could lead to kidney damage which then results in renal failure as well as reproductive system malfunction; weak sperm, ovarian atrophy, irregular menstruation and risk of infertility.

2.1.4. Mercury: Hg

2.1.4.1. Benefits

Mercury is an important ingredient in making thermometers. It is, further than that, used in the electrical industry, paper industry, paint industry, and used to synthesize pesticide. In ancient times, mercury was used as a component of syphilis medicine. It also has been used as an important ingredient of red medicine to heal fresh wounds.

2.1.4.2. Harm to the body

Mercury in methyl and ethyl forms is more toxic than metallic mercury or metal compounds. The least toxic mercury is in the form of organic compounds. Mercury has toxic effects on the body in many ways. An example of mercury poisoning is Minamata disease that leaks from an industrial plant in Minamata, Japan, where many people died from drinking water and eating food contaminated with mercury. The toxic effects on the body are abdominal pain, severe diarrhea, gum and salivary glands are destroyed and has a scorched appearance, nervous system malfunction, cause amnesia, blurred vision and may cause blindness.

2.1.5. Manganese: Mn

Manganese is found in water sources, surface and groundwater. If high in content it will turn the water into a cloudy reddish-brown color. This often occurs with iron.

2.1.5.1. Benefits

Manganese is used in wastewater treatment processes or water quality improvement system, used to make alloys to increase the strength of metals, used to produce electric welding rods, used to produce flashlights and batteries, used to make weaving machines, coating containers, paint products, cosmetics, rubber industry, printing machines, metal plating, and pesticides. In agriculture, it is used as a fungicide. Furthermore, manganese is a component of enzymes and bones which human body needs about 3-4 milligrams per day.

2.1.5.2. Harm to the body

If the body receives manganese more than the body's needs will cause poisoning including inflammation of the skin and mucous membranes of the gastrointestinal tract, body weakness and a headache, the nervous system is destroyed, trembling of the forearm and legs, and risk of partial paralysis (Jamlong Pintawong, Anon, Nonthaso and Sathaporn Kavinate, 2011). Moreover, manganese intaking may result in schizophrenia (Schizophrenia), emotionally unstable, difficulty swallowing speechless or hoarse. Tremors similar to Parkinson's disease are also found to occurred in some people.

2.1.6. Zinc: Zn

2.1.6.1. Benefits

Zinc is found existed in human body as a component of various enzymes. It helps to promote development in memory and learning in children, as well as the functioning of the reproductive system. Similarly, it helps a body to achieve fertility according to the appropriate age and stimulates the work of sex hormones. Additionally, it is used in plant growth accelerators or in fertilizers, used in the pharmaceutical industry, used to coat metal to prevent rust, and used as an octane booster in fuel industry.

2.1.6.2. Harm to the body

Zinc is well absorbed in the small intestine. When receiving high amounts of zinc, the body stimulates the

mucous membrane (mucosal epithelium) to synthesize metallothionein. Metallothionein, a protein rich in cysteine, binds to zinc by adhering to the intestinal cell wall and then loosening. However, if the amount of zinc is too high, it will accumulate in the liver and kidney area resulting in the following consequences including: internal organs destroyed, liver and kidney failure, anemia, chromosome abnormalities, risk of cancer, and the deficient in copper of the body due to inhibition of absorption. If zinc is taken more than 2 grams will cause acute poisoning including: diarrhea, vomiting and fever. Obtaining a good soluble zinc salt by ingestion in large quantities will cause nausea, vomiting, dehydration, severe abdominal pain, diarrhea, and if more than 45 g is obtained, more severe toxicity will occurs including: unconsciousness, blood transfusion and low urine output (On-Ing Vejsit, 2008)

2.1.7. Iron: Fe [7]

2.1.7.1. Benefits

Iron is a component of red blood cells and is used in biochemical processes such as digestion processes in the digestive system.

2.1.7.2. Harm to the body

Receiving high amount of iron and accumulation in the body will cause effects on various systems including: a decrease in digestive efficiency, dilation of blood vessels, lower blood pressure, slow blood clotting, decreased liver function, liver deterioration, and causing the activity of enzymes to be inhibited. When receiving less iron or the body is deficient in iron will result red blood cell synthesis to be inhibited, deprivation of oxygen of blood, and causing anemia.

2.1.8. Copper: Cu

2.1.8.1. Benefits

Copper is a metal that is essential to the body. To clarify, it is a part of the formation of hemoglobin and the function of some enzymes which are peroxidase and cytochrome oxidase. Copper also helps in delivering oxygen into cells. In addition, it is a component of many drugs; such as those that induce vomiting, burn wounds, anthelmintic drugs and fungicides. Copper is used in the manufacture of containers, furniture and metal industry, used as an ingredient in metal fabrication, used to produce wires and electronic circuits, used to produce chemical dyes, used in plant growth accelerators or in fertilizers, used as a component of pesticides, used to add into foods such as canned beans and some compotes such as Madan.

2.1.8.2. Harm to the body

If given through inhalation, copper will cause nasal congestion and sores on the roof of the mouth. Likewise, if taken by eating high doses, it will cause nausea, vomiting, abdominal pain, stomach bleeding, thirst, green vomit, dark color foamy urine (may cause fatality in 4 hours), neurotic symptoms, seizures, delirium, paralysis, balance disorder, and jaundice. Copper can cause itching and blistered skin if absorbed through the skin. If it gets into the eyes, it will cause conjunctivitis, swollen eyelids with black eye lesions. Some such have accumulation in tissues, causing a green color at the base of the hair and gums (On Ing Vejsit, 2008). If a human body receives more than 100 milligrams of copper, may has the following symptoms: body weakness, vomiting, anorexia, slim body, red blood cells break down, destruction of the liver, inhibition of the liver function. If the body has copper accumulation in the amount of 25-30 mg / body weight (kg) will cause cirrhosis. In addition, if the copper content in water is more than 0.1 ppm, it can cause toxic effects to aquatic animals as well.

2.1.9. Nickel: Ni

2.1.9.1. Benefits

Nickel is sed as a metal component in electrical appliances and electronic circuits. It is, also, used in combination with other metals to increase strength and luster.

2.1.9.2. Harm to the body

Nickel is an element that the body does not need. However, when it accumulates more higher and higher in the body, it can cause symptoms including: nausea, headache, vomiting and chest pain, weak and slim body, severe pneumonia, abnormally fast pulse, high blood pressure, risk of ruptured blood vessels in the brain which can lead to paralysis, and risk of cancer.

2.2 Plant Categories; considering ability in absorbing heavy metal

According to Baker's and Walker's study [9], some plant species have a physiological ability to adapt themselves surviving in the contaminated soil. As they had observed in their experiments, those plant species are able to grow naturally although they live in an enormously heavy metal contaminated soil. Moreover, each species represented a different ability in accumulation and Baker and Walker categorized plant species into three groups by using the ability to absorb heavy metals and also the organ which dominantly accumulates as a classification criterion.

2.2.1. Metal excluder

Metal excluder; Including plant species which accumulate heavy metals in its roots and no metals were found in other organs. For example, Cyperus articulatus L. is a cadmium excluder.

2.2.2. Metal indicator

Metal indicator; Including plant species which uptake heavy metals by its roots and deliver to other organs, particularly in aboveground parts such as leaves and stems. Due to the concentration of heavy metals in these parts comparable to the level in soil they lived in and directly proportional, these plants have been used as a metal indicator to identify the areas where there was highly heavy metals accumulation. For example, Ludwigia stolonifera (Ghill. & Pers) Raven was classified as cadmium and lead indicator.

2.2.3. Hyperaccumulator

Hyperaccumulator; Including plant species which absorb heavy metals by its roots and deliver to other parts, likewise Metal indicators do, though Hyperaccumulators accumulate substantially more heavy metals in aboveground organs than in soil parts. In addition, many environmentalists value these species and use them to deplete such toxins in soil as they are able to absorb large amounts of heavy metal. Nowadays, there are more than 500 hyperaccumulators including various plant species and families. For example, Leersia hexandra SW. is a lead and nickel hyperaccumulator [10].

2.3 How to measure the level of accumulative heavy metals in plants [13]

Spectrophotometers are a process based on the principle of measuring the absorption and transmittance of monochromatic light to measure biological quantities including chemical substances. The main components are as follows:

2.3.1. Light Source

The light source in the spectrophotometer must be emitted continuously and consistently in the desired wavelength as well as having enough light intensity. There are different types according to the wavelength of light emitted which must be selected to suit the liquid used to measure the absorbance

2.3.2. Cells used to contain the sample solution.

Cell samples are sometimes called cuvettes. The most common form is a simple glass cell. It is available only in the visible range because ordinary glass is absorbed in the UV range.

2.3.3. Monochromator

This component is used to control light by emitting light from a light source which is a monochromatic light and a single wavelength usually using prisms or gratings."

2.3.4. Detector

It measures the intensity of the absorbed radiation by converting the radiation energy into electrical energy. A good signal detector must have high sensitivity. So that, even if the amount of light changes slightly, it can detect the difference signal.

The light source is used to allow light to pass through the sample. A good light source should provide a consistent light intensity and remain constant throughout the wavelength. Currently, the most popular light sources are deuterium arc (190-220 nm), tungsten (350-2500 nm), and xenon lamp (190-800 nm).

Currently, spectrophotometers are being developed. The high efficiency of small sample volumes led to a system called Cuvette-less spectrophotometer.

2.4 How to use a spectrophotometer

2.4.1. Turn on the spectrophotometer. Select a light source and determine the desired wavelength of light. It should be turned on at least 15 minutes before use.

2.4.2. Choose a cuvette to suit the sample. Put the sample in the cuvette. Wipe the sides of the cuvette dry and clean.

2.4.3. Place the cuvette in the slot, close the lid, and read the absorbance from the display. The absorbance of the sample and null values are removed so that the results can be read from the display.

2.4.4. After use, clean the machine. Clean the cuvette and turn it off.

III. LITERATURE REVIEW

3.1 Food processing methods and accumulation of heavy metals [16]

Various studies have indicated heavy metal contamination in foods. Because of the harmful consequences of some of these metals, such as lead, mercury, and cadmium, this has become a more troublesome issue. There are several cooking methods, and these procedures increase the levels of heavy metals in the cooking process. Smoking, grilling, boiling, and frying, among other techniques, are all used in studies. Various amounts of such metals also were observed. On the other hand, there are conflicting data on the impact of various cooking processes on the concentration of these metals in food. While some studies show a drop in concentrations, others have found other results.

For the processing of raw food resources before consuming, a variety of cooking methods are available. Boiling, frying, roasting, barbequing, and grilling are some of the most popular methods.

The amount of metals in cooked fish cooked by grilling and frying was found to be greater than in raw fish. They suggested that the size of the fried fish was one of the causes for this outcome. They claimed that the size of the fish was inversely proportional to the amount of oil consumed and the amount of water lost during the preparation of food. As a result, when small amounts are cooked, more water is lost, resulting in a higher metal concentration.

Because heavy metals in food are soluble in water, they will be reduced during the cooking process. There is the loss of water rising temperature for the drop in metal concentration in the fried sample. Many variables, including the original levels of heavy metals in the cooking procedures might influence the decrease or increase of these components in the flesh.

There are no consistent consequences when it comes to the effects of various cooking processes on the amounts of various heavy metals. Factors from the surroundings during the cooking process have been identified as probable factors of the growth. This shows that the cooking method or heating isn't the most essential part in such experiments. As a result, it is in need of additional controlled studies in this field, with a wider sample. More research is needed on cooking processes in various circumstances (such as time, temperatures, and cooking mediums) in order to decrease the harmful impacts on food.

3.2 Effect of cooking method (soaking, washing, and cooking) on the content of heavy metals in rice [24]

According to Raafat A.,2020, the impact of the method of cooking on the heavy metals content of Iraqi rice available in the local market was examined. The result shows that the process of soaking, washing and cooking rice affected the concentration of the arsenic element to significantly decrease. This is made more solid by the study (Naito et al. 2015) of rice grown in Japan which indicated that the process of soaking, washing and cooking affected the arsenic concentration in it to leveled down about 20 times from initial, from 0.2104 to 0.01032 mg/kg.

Similarly, there is a decline in lead and cadmium concentration in rice after soaking and cooking treatments; being soaked and washed with water before being cooked. Also, a clear decrease in the concentration of lead and cadmium in rice when soaked with salt and acid and then cooked have been found by several studies.

However, there was no big difference in cadmium concentration according to the method of cooking. It was found that cooking without filtering cooking water or with filtering reduced the element concentration significantly, from 0.3922 mg/kg before cooking to 0.0955 mg/kg with no filtering and 0.09102 mg/kg with filtering (Naseri et al., 2014). Additionally, for cooking water, Ziarati and Azizi, 2014 indicated that cooking rice without soaking did not significantly reduce the concentration of cadmium, as it reached 0.3178 mg/kg after cooking compared to its concentration before 0.3276 mg/kg.

3.3 Effects of food processing methods (oil extraction, boiling, and infusing) on migration of heavy metals in oilseeds, noodles and teas [25]

According to Joon-Goo Lee et al., 2019, it was found that heavy metals (lead, cadmium, arsenic, and aluminium) in oilseeds, noodles and teas were reduced by extracting oils, boiling noodles, and infusing teas.

Oil extraction methods including mechanical pressing, solvent and supercritical-fluid extraction were used in the study. The results suggest that heavy metals were transferred from seeds to oils less than 10% by pressing and solvent extractions while the transfer rates of heavy metals were up to 30% in supercritical-fluid extraction. This is due to the fact that supercritical-fluid extraction extracts more heavy metals rather than other extraction processes, and therefore, is more effective in extracting components of seeds rather than solvent and pressing extractions.

Heavy metals were significantly decreased during boiling noodles; including flour noodles and glass noodles, for 3 minutes. Similarly, heavy metals were significantly decreased in tea infusion after infusing tea; including black tea, green tea and Solomon's seal tea, for 2 minutes. In addition, heavy metals contents were increased as the infusion time increased from 2 to 10 and 30 minutes resulted from the fact that the migration of heavy metals into tea infusion is metal dependent. Especially, black tea showed the highest migration rates and green tea had the lowest migration rates. Black tea was made from green tea by oxidation, which is called the fermentation procedure. Heavy metals bio-concentrate to metal chelates in plants and metal chelates would be changed by oxidation in black tea. Therefore, heavy metals in black tea would be easily migrated to infusion.

3.4 Effect of processing methods on heavy metal concentrations in commonly consumed green leafy vegetables available in Sri Lankan Market [14]

According to the effect of processing methods on heavy metal concentrations in commonly consumed green leafy vegetables available in Sri Lankan Market research, certain green leafy vegetables, whose leaves and young stems are edible. These edible parts of each vegetable were passed through 2 cooking methods, including cooking (boiled with coconut milk) and stir-frying. Thereafter, the level of

5 different heavy metals, namely Ni, Cd, Cr, Pb, and Cu are measured and compared with the raw ones.

The results were relatively consistent, with the level of almost all heavy metals in stir-fried vegetables reducing the most, followed closely by cooked ones, though the decrease was fairly minimal.

Moreover, the research indicates that these heavy metals are elaborated through various means, evaporation of water, and solubilization of the element for instance. Also, the metals possibly bind to such macronutrients present in food items as carbohydrate, lipid, and protein.

3.5 Effects of Different Cooking Methods on Heavy Metals Level in Fresh and Smoked Game Meat [15]

However, cooking methods do not only potentially reduce the level of heavy metals in plants but also in meat. Several studies have been conducted and obtained different results. According to Effects of Different Cooking Methods on Heavy Metals Level in Fresh and Smoked Game Meat research, different bush meats are cooked with various processes including, grill, boil, and fry and subsequently examined the level of such heavy metals as Fe, Mn, Cu, Zn, Cd, and Pb. The results were surprisingly inconsistent, especially for Iron (Fe), where boiled and grilled meats were reported to have higher metal levels, when compared to fresh one. When it comes to grilled and smoked meats, the researcher deduced that the increase in Iron concentration can be attributed to an interaction between meat and the metal grid. However, the level of Iron has not been frequently measured, when it comes to vegetables. The study eventually concluded that the different cooking methods had affected different heavy metal levels and the number of toxic substances in a food could be minimized by using the appropriate process.

3.6 Other methods on eliminating heavy metal in plant

3.6.1. Complexing agents; DTPA

The synthetic amino polycarboxylic acids; ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), and diethylenetriaminepentaacetic acid (DTPA) constitute a class of metal-complexing agents used in a range of applications including water treatment, pulp, and paper manufacturing, agrochemicals, photographic chemicals, industrial and domestic detergents, food processing, electroplating, textiles, cosmetics, pharmaceuticals, and medical detoxification [16]. NTA was first synthesized in 1862 and EDTA in 1935 (Egli, 2001). Aminopolycarboxylic acids act as a ‘chelant’, contain several carboxylate groups linked to one or more nitrogen atoms, and are able to form complex metal ions by forming stable heteroatomic rings (known as the ‘chelate effect’). These metal complexes are stable and water-soluble (Fiorucci et al., 2002) and restrict the metal ion from normal chemical interactions [17].

DTPA (Diethylenetriaminepentaacetic acid; C14H23N3O10), one of the commonly used chelant, was suitable for increasing the cadmium removal capacity of plants (i.e. Water hyacinth). Besides, it offers a suitable phytoremediation technique, the use of plants to extract and remove elemental pollutants or lower their bioavailability in soil (Berti and Cunningham, 2000) [18], to help clean contaminated sites [19], or in other words, helps in removal of heavy metals in soil and water where absorption of flora mainly occurs.

3.6.2. Plant flushing

Another effective way to remove heavy metals in plants is “plant flushing” [20]. Watering plants with clear water; without any contaminants (recommended PH of water is around 5.5-6) or using “flushing agent” containing chelating agent [23], specific chemical compounds whose structures permit the attachment of their two or more donor atoms (or sites) to the same metal ion simultaneously, around 1-2 weeks before harvest or depends on growing means [22] , without added any compounds or nutrients. When plants receive an excess amount of fertilizer for long times, salt will be produced and the PH of soil and nutrients will be imbalanced, resulting in ineffective products such as unpleasant chemical taste and smell. Plant flushing, therefore, not only enhances removal of excessive nutrients and compounds, but heavy metals and contaminants will be also removed through the process [21].

IV. CONCLUSION

To summarize, methods of cooking could decrease the level of heavy metal in vegetables, though the decrease has not been significant. However, these heavy metals are found to be evaporated through boiling as well as stir-frying processes, so this can be attributed to the help of water or such soluble as oil. Or else, metals should definitely migrate and accumulate in cooking mediums, frying oil, soup, and cooking stocks, for instance.

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Typology and structural characteristics of the woody population of oasian basins in south-eastern Niger

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Abstract— *The present study aims to characterize the typology of woody stands in oasis basins in the Gouré department: the case of Balla, Kilakina and Tchago. In these oasian basins, 75 survey plots of 50x50m² were carried out in order to collect informations about le floral of the area and its dendrometric characteristics. The woody stand of the basins contains a total of 26 woody species divided into 23 genera and 16 different families. The most represented families are: Mimosaceae (7 species or 26%), Arecaceae (2 species or 7.79%), Asclepiadaceae (2 species or 7.79%), Anacardiaceae (2 species or 7.79%) and Myrtaceae (2 species or 7.79%).The characterization of the woody stand of the oasis basins of Gouré according to the importance value index showed that Hyphaene thebaica (L.) Mart has the highest contribution (852.7 points), and Adansonia digitata L., the least (59.28 points). Three plant groups were identified based on the ascending hierarchical classification from 15% similarity and the dendrometric structure of each group was established. The later defined a positive asymmetric distribution characterizing the dominance of young individuals. Based on the above results, the diversity of the oasian basins is high. Nevertheless, this biodiversity is exposed to severe climatic conditions and anthropogenic pressure, which makes its vulnerability. In this circumstance, our study remains as a tool for better management and exploitation of this woody stand.*

Keywords— Oasian basins, flora, vegetation structure, eastern Niger.

I. INTRODUCTION

In west Africa, the Sahelian domain corresponds to the transition zone between the desert region and the Sudanese region. The first, characterized by hot weather and the later by sufficient rainfall and less vulnerable crops to climatic hazards. Sahel region is an agropastoral zone by excellence which suffer severe degradation for several decades due to worsening climatic conditions and increasing anthropization (Albergel et *al.*, 1986). The conjunction of these natural and anthropogenic phenomena results in deforestation, soil degradation and desertification (Toko and Sinsin, 2008). Its local communities are hence

confronted with a multitude of interactive factors (climate, demography, epidemics, epizootics, locust invasions, unsuitable socio-political, economic and cultural systems, etc.) which considerably limit their adaptive strategies to the environment.

Niger, a Sahelian country, is not an exception. It has indeed been affected by recurrent droughts since the end of the 1960s; the most terrible of which were those of 1972/73 and 1983/84. Nevertheless, these disturbances are becoming more frequent and intense and generate significant impacts. The most visible are the decrease in agricultural and animal production, the degradation of natural resources (water, soil and vegetation) and erosion, thus making food security, environmental management and livelihood precarious. This situation exacerbates the vulnerability of these nigeriens populations.

Several localities are affected by this situation and the department of Gouré, located in the Zinder region, in the east of the Republic of Niger is among the most affected areas by these recurrent droughts , since the end of the 1960s (Ozer et *al.*, 2005). It is patterned with closed depressions commonly referred to as the oasian basins (karimou, 2017). These basins are characterized by three to four inter-dune depressions of varied cross-sections where the vegetation and soil types are organised in concentric aureoles descending gently towards the center with invariably the external aureole consisting of sand dunes, the second aureole of dense palm trees, the third aureole made up of market gardening and finally the central one characterised by more or less natronised bare soil, however, depending on the morphology of the basins, it could be occupied by a pond (PLECO, 2006).

In this area, the active sand dunes are present for nearly 200 km between Gouré and Maine-Soroa, constantly threatening the infrastructures, (such as roads and housing) and these oasian basins which suffer already from general degradation due to wind erosion, silting up, the drop in groundwater levels and the strong pressure on woody resources (Ozer et al., 2005; karimou, 2017).

All these threats to which insufficient rainfall could be added ,repeatedly causes a deficit in agricultural production and the migration of certain species (PLECO, 2010). Despite the role of these basins in the socio-economic life of local populations, these ecosystems do not benefit from any conservation measures and little is known in the scientific literature in Niger. In line with the sustainable exploitation of these resources, it is imperative to carry out more investigations in order to have scientific data, essential tools for the development of sustainable management plans. Hence, the framework for this study, which focuses on the typology and structural characteristics of the woody population of oasian basins.

II. METHODS

Description of study sites

The study was conducted Southeast of the 14th parallel between 09°45'and 11°15' East longitude and the department of Gouré (region of Zinder) which covers an area of 20,517 km² in the Manga oasis system. The area is bounded to the east by the department of Goudoumaria (Diffa region), to the north by the department of Tesker, to the south by the department of Magaria and the Federal Republic of Nigeria and to the west by the department of Mirriah and Tanout (Figure 1).

The department of Gouré is between the isohyets 150 and 400 mm. Its climate is Sahelian type, and is characterized by two distinct seasons: dry and raining seasons.

Three basins were selected in this department namely Balla, Kilakina and Tchago (Figure 1& 2). They are:

- **Balla site**

The Balla basin, falls under the canton of Gouré and extends over a strip of about 10 km. Located 25 km from Gouré in the center area and 2 km from the edge of the RN1 (13°53'50.89"N latitude and 10°24'31.6 " East longitude). This site has three or even four sectors namely the doum belt, the zone exploited under market gardening, the salt zone exploited under market gardening and the strongly salty zone very weakly represented. The village bears the same name as the basin and also has a second basin which is mainly for pastoral purposes and reserved for watering animals, whiles the first is reserved for crops. The village is mainly made up of the Kanouri who have for main activity the exploitation of the basin. However, this basin is also exploited by neighboring villages namely: Ballabré and Allajiri (Issaka, 2018).

- **Kilakina site**

The kilakina site located in the urban commune of Gouré is located 62 km east of the city of Gouré on RN1 (13°43'72.8" North latitude and 10°44'80.5" East longitude). The basin, with intermediate water, is for agro-pastoral purposes. It has a total area of 56 ha, of which 35 are exploitable and 25 are exploited, i.e. 71.4%. It is a large basin which is exploited throughout the year. This site has four very distinct sectors. These are the doum belt, the zone exploited under market gardening and arboriculture, the zone exploited under market gardening and arboriculture which is salty and the naturally natroned bed. According to the population, the village has around more than 1,500 inhabitants and 54 farms (Issaka, 2018).

- **Tchago site**

The Tchago site is located 23 km north-west of the department of Gouré (14°02'30" and 14°02'52" North latitudes and 10°03'39" and 10°04'06" longitudes East). Tchago is one of the villages with a large number of basins in the department of Gouré. There are five (5) basins, the first of which has an area of 1.94 ha, is located southwest of the village at a distance of fifty 50m, the second, the largest of 5 hectares and the most exploited, is also located to the southwest of the village at a distance of 1 km, the third of 0.4 hectares, located to the south of the said village at a distance of 2 km, the fourth of 0.5 hectares, contiguous to the villages of Tchago and N 'Gouro Guidimi, the fifth of 1 hectare is also contiguous to the villages of Dagradi and N'GousroGuidimi and finally a great depression to the West and North of about 20 hectares in which rainfed crops are cultivated. The cultivated plots are fenced by a dead hedge of *Prosopis juliflora* associated with branches of *Phoenix dactylifera*. The cultivated areas in these basins represent only about 1/4 of the total area. (Issaka, 2018). Tchago has two sectors: the doum belt and the zone exploited under arboriculture and market gardening.

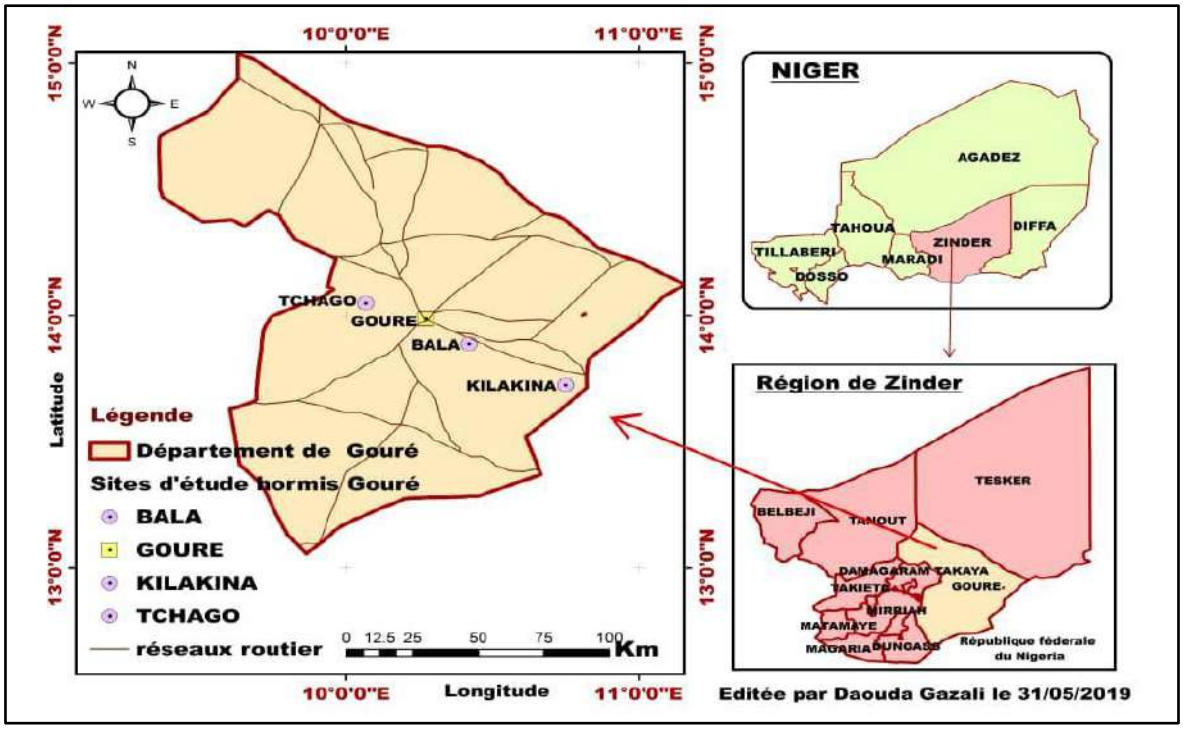

Fig.1: Study sites locations

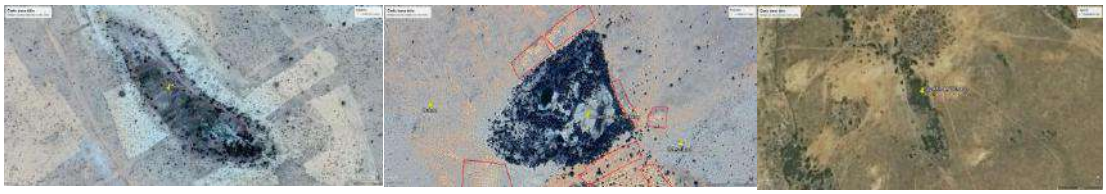

Fig.2: Satellite view of the three sites

Data Collection

In each of the three basins, data was collected using the radiar transect method as defined by Douma (2016). Thus, eight transects were installed from the center of the bassin with a spacing of 45° between the transects. On each of them, four vegetation inventory plots of 2500 m^2 (50mx50m) have been placed at an equidistance of 500 m between plots. In total, 75 vegetation plots were carried out.

In each plot, parameters such as woody density, total height, diameter at 30 cm from the ground and the two crown diameters were measured. The floristic list was established on the basis of the Flore du Sénégal by BERHAUT (1967).

Table 1: Number of transects and surveys carried out in the study area

Village	Balla	Tchago	Kalakina	Total
Ethnic group	Kanouri	Kanouri	Kanouri	
Number of transect	8	8	8	**24**
Number of sampling	26	23	26	**75**

Data processing and analysis

❖ Several indices were estimated. The diversity of the ligneous plants was evaluated by the calculation of :

(1) **The Shannon diversity index, Weaver (1949) (*H*)**formulated as follows (1) :

$$H' = -\sum_{i=1}^{s} \frac{ni}{n} \log_2 \frac{ni}{n}$$

n = total number of species; ni = number of individual of species i.

This index, expressed in bits, is based on information theory. It varies between 0 (zero diversity) and more than 5 bits (very high diversity) (Frontier and Pichod-Viale, 1993).

(2) **The Pielou equitability index (1966) calculated as follows:**

$$E = \frac{H'}{Log2\ S};$$

H'= observed diversity ; $\log_2 S$ = maximum theoric diversity, E= equitability et S = specific richness.

It varies between 0 and 1. It tends towards 0 when there is a phenomenon of dominance and towards 1 when the distribution of individuals between species is regular (no phenomenon of dominance).

(3) The Species Importance Value Index (IVI), developed by Cottam, Curtis (1956), characterizes the place occupied by each species in relation to all species of vegetation. It was calculated to assess the specific preponderance according to the formula (Kouamé, 1998):

IVI = *FREQesp* + *DENSesp* + *DOMesp*,with :

FREQesp, the relative frequency of a species: it is the ratio of its specific frequency (number of plots in which it is present) to the total of the specific frequencies; *DENSesp*, the relative density of a species; it is the ratio of its absolute density to the total of absole densities; and *DOMesp*, the relative dominance of a species; it is the quotient of its basal area (basal area) by the total of the basal areas of the species. and. Specific diversity is defined both in relation to the number of species present (specific richness).

❖ The structural parameters used to characterize the stands are:

(1) the density which expresses the total number of trees per unit area (hectare)

(2) basal area, (en m^2/ha)) was calculated according to the relation:

$$G = \frac{\pi\, d^2}{4}$$

With G in (en m^2/ha) and d expressing the diameter at 1.30 m.

From a threshold of 5 cm, diameter classes of 5 cm amplitude were formed. These classes helped to establish distribution histograms of the diameter.

A test of fit to the theoretical Weibull distribution (Rondeux, 1999) was carried out using the Minitab 16 software. The theoretical Weibull distribution with three parameters (position a, scale or size b and shape c) was used to characterize stand structure, thanks to its flexibility of use and great variability; of the forms of distribution it produces. The parameter a corresponds to the threshold value, that is

to say to the smallest value of diameter (respectively height) retained for the constitution of the histograms. The parameter b is linked to the central value of the distribution of the diameter and height classes. Finally, the parameter c is linked to the observed structure and, depending on its value, leads the Weibull distribution to take several forms.

The overlap gives the average of the dominant abundance class of each species in the stand. It plays an important role in the study of vegetation. It is calculated from the following formula: Rm=∑ Ri/n with Ri the recovery of i^{eme} reading and n the number of reading.

In order to characterized a plant group in the oasis basins, an ascending hierarchical classification of the 75 surveys using the PC-ORD software was done with a dissimilarity rate of 30%.

III. RESULTS

➢ **Floristic richness of the oasian basins**

The woody stand of the basins contains a total of 26 woody species divided into 23 genera and 16 different families. The most represented families are: Mimosaceae (7 species or 26%), Arecaceae (2 species or 7.79%), Asclepiadaceae (2 species or 7.79%), Anacardiaceae (2 species or 7.79%) and Myrtaceae (2 species or 7.79%). These families represent 58.08% in total. The remaining families are poorly represented. These are the Balanitaceae, Bombacaceae, Burseraceae, Caesalpiniaceae, Capparaceae, Lythraceae Euphorbiaceae (Figure 2).

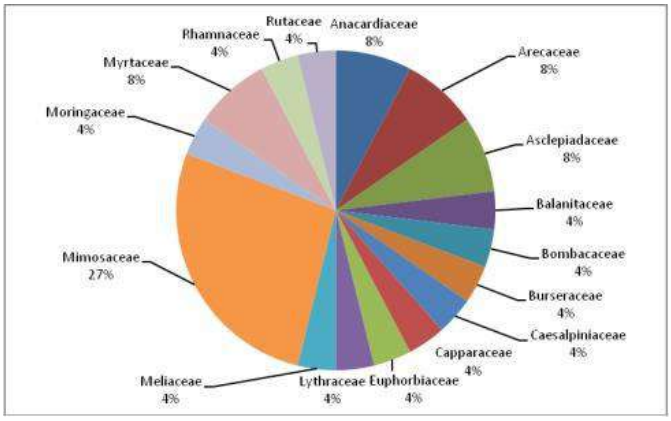

Fig.2: Specific family composition of the oasian basins

➢ **Characterization of the woody stand of the basins**

The characterization of the woody stand of the oasis basins of Gouré according to the importance value index shows that *Hyphaene thebaica* (L.) Mart has the highest contribution (852.7 points), followed by *Leptadenia pyrotechnica* (614.85 points), *Prosopis juliflo*ra (473.2), *Phoenix dactylifera* L. (415.77 points), *Lawsonia inermis* L. (321.96) and *Calotropis procera* (223.28 points) (Table 1). *Hyphaene Thebaica is therefore the species of greatest ecological importance*. and can be used, on its own, to designate the stand.

Table 1: Woody species predominance in the basins

SPECIES	Total Number	Relative Frequency	density (Ind/ha)	St (m²)	Plant cover (m²)	IVI
Hyphaene thebaica (L.) Mart.	737	22,9	35,09	33,86	23,88	852,73
Leptadenia pyrotechnica (Forssk.) Decne	570	17,71	27,14	0	0	614,85
Prosopis juliflora (Sw.) D.C.	415	12,89	19,76	6,4	19,15	473,2
Phoenix dactylifera L.	318	9,88	15,14	42,86	29,89	415,77
Lawsonia inermis L.	298	9,26	14,19	0,02	0,49	321,96
Calotropis procera (Ait.) Ait. F.	207	6,43	9,85	0	0	223,28

Azadirachta indicaA.Juss.	141	4,38	6,71	6,62	27,91	186,62
Acacia raddiana Savi	133	4,13	6,33	1,08	8,49	153,03
Euphorbia balsamifera Aiton	70	2,17	3,33	0	0	75,5
Acacia senegal (L.) Willd.	63	1,95	3	0,31	7,56	75,82
Citrus limon (L.) Burm.f.	37	1,14	1,76	2,44	37,63	79,97
Faidherbia albida Del.	36	1,11	1,71	7,18	35,62	81,62
Adansonia digitata L.	35	1,08	1,67	9,08	12,45	59,28
Others	31	0,93	1,44	3,68	128,73	165,78

➢ **Vegetation groupings**

Three plant groups were identified based on the ascending hierarchical classification from the 75 vegetation plot matrix and 26 species at a rate of 15% similarity (Figure 3). Group I (G1) is represented by 26 records and 21 species The majority of these surveys were carried out at the village of Balla (46.15%), and Kilakina and Tchago recorded respectively 36.46% and 15.38%. The species presenting this group are: *Hyphaene thebaica* (31.46%) followed by *Prosopis juliflora* (19.69%); *Phoenix dactylifera* (15.15%), *Euphorbia balsamifera* (6, 75%); *Lawsonia inermis* (6.27%) and *Azadirachta indica* (4.15%). These species are found mostly on soil characterised by 69.23% clay texture. It also represents a transition zone between the lower slope and the valley floor, therefore a relatively medium slope. At this level, the average species density is 159.38 ± 12.7 plants / ha with an average cover of 31.40%. The basal area of the trees is low 0.90 ± 0.86m² / ha.

Group II(G2)has 33 records for a total of 24 species. 48.48% of the surveys were carried out in the village of Tchago, 30.30% in Balla and 21.21% in Kilakina. The woody flora of this group is characterized by species such as *Leptedania phyrotechnica* (23.61%); *Lawsonia inermis* (17.17%) *Hyphaene thebaica* (13.01%); *Prosopis juliflora* (12.15%); *Phoenix dactylifera* (9.19%) and *Azadirachta indica* (6.85%). These species are found on the sandy-clay texture mid-slope. The tree density in this environment is 155.15 ± 9.93 individual per hectare with an average cover of 36.46%. But these trees give this environment a basal area of 25.67m².

Group III (G3) includes 16 surveys and presents 19 species. These surveys were carried out in the village of Balla (56.25%), Kilakina (43.75%) and Tchago (6.25%). The dominant species in this group are *Hyphaene thebaica* (33.7%), *Leptedania phyrotechnica* (17.12%); *Calotropis procera* (14.77%); *Acacia raddiana* (10; 36%) *Prosopis juliflora* (7.89%); and *Phoenix dactylifera* (5.52%) At this level the slope is relatively low.

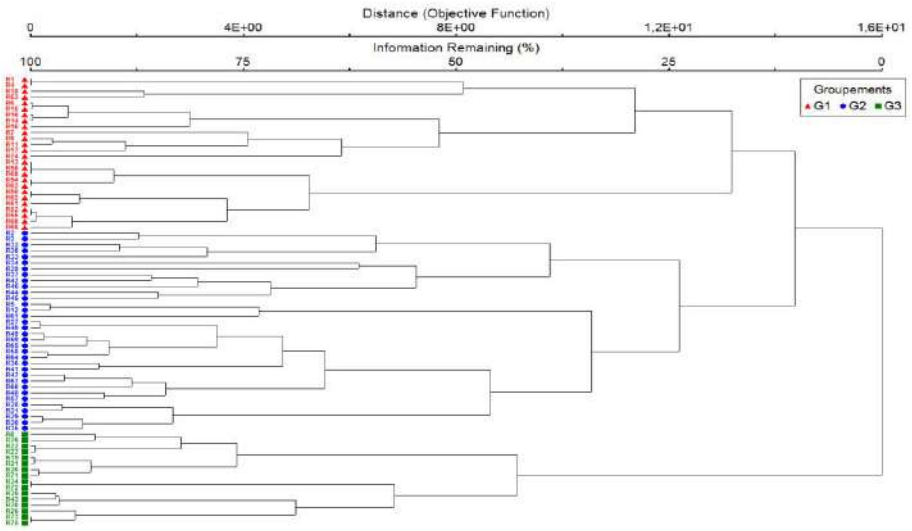

Fig.3: Ascending Hierarchical Classification (CHA) of readings based on a factorial correspondence analysis.

➢ **Diversity and equity of groups**

Table 2 shows the variation of species richness, Shannon diversity index and Pielou equity indices for each group. It appears that group 3 is relatively less diversified in taxa compared to the other two. This is confirmed by the values of the Shannon diversity index (H '= 2.89 and E = 0.68 bits) against respectively (H' = 3.04; E = 0.69) and (H '= 3.32 and E = 0.72) for groups 1 and 2.

Table 2: Species diversity of the different plant groups in oasian basins

Group	S	H’	E
Group 1	21	3,04	0,69
Group 2	24	3,32	0,72
Group 3	19	2,89	0,68

➢ **Dendrometric characteristics of the groups**

Structural characteristics of stands

Table 3 shows the structural dendrometric parameters determined within these three groups. Group I and III have the highest density (159.38 ± 12.17 and 181 ± 15.62 plants / ha) respectively, while the third has the lowest density (155.15 ± 9.93 plants / ha).

Table 3. Structural parameters determined within these three groups

Groups	Density (individuals/ha)	Basal area(m^2/ha)	Plant cover (%)
Group 1	159,38 ± 12,71	0,90 ± 0,86	31,40%
Group 2	155,15 ± 9,93	1,33 ± 1,92	36,46%
Group 3	181 ± 15,62	0,98± 1,28	32,15%

Population structure of the settlement

Distribution by size

Figure 4 below represent the distribution of individuals of the three identified groups.In general, the structure is bell-shaped for all individuals and for the three groups.

The histograms of group I are of a stable type characterized by a large number of individuals of small size classes (young subjects), a small number of large individuals (adult subjects) in the intermediate classes and a regular reduction in the number of individuals. aged upper classes. This type of distribution fits with a theoretical Weibull distribution with a shape parameter c = 1.37 (1 <c<3.6) characteristic of a predominance of young individuals or small diameters.

The diametric structure of the individuals of group II is also characterized by a predominance of individuals of small diameter. This distribution histogram of the observed diameter classes shows a straight asymmetry appearance (1 <c <3.6), characteristic of populations with a relative predominance of young individuals or of small diameters. Meanwhile, the structure of individuals in group III stood out from the others by a high density of individuals in the large intermediate classes (30-40). The analysis of the diameter structure of the present woody plants fits the theoretical Weibull distribution with the shape parameter c = 1.039 (1 <C <3.6).

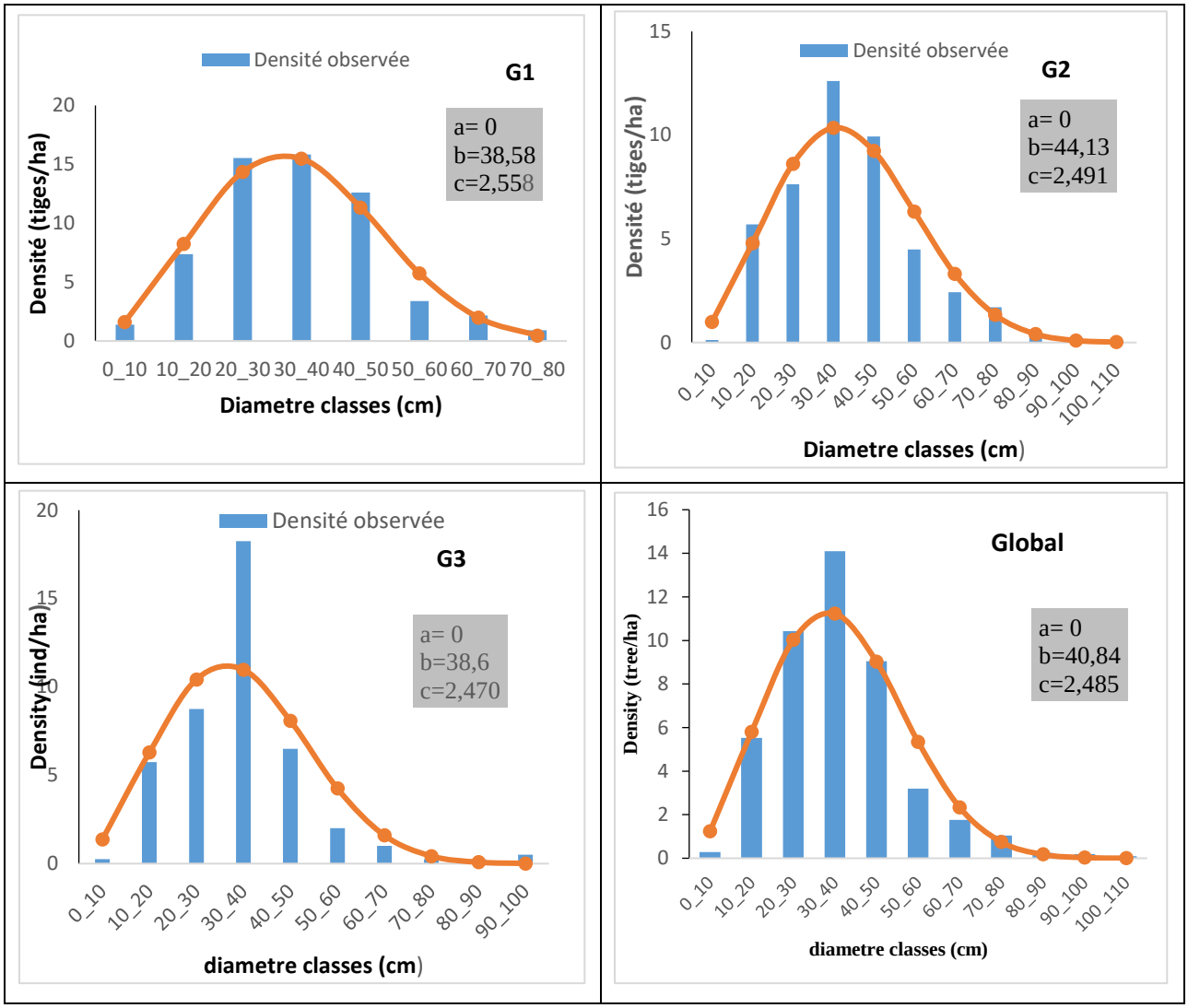

Fig.4: Diameter structure for all woody individuals and for the three plant groups.

IV. DISCUSSION

The woody flora of the Oasian basins of Gouré contains 26 woody species divided into 23 genera and 16 families. This richness is much greater than that obtained by Kaou et al. (2017), on the dunes of Mainé Soroa where they identified 10 woody species divided into 5 families and 8 genera with a dominance of Mimosaceae (37.5%) and Asclepiadaceae (25%). This difference can be linked to the level of organization of the soils in a halo or aureole and to the level of the water table. The basins in the Gouré area seem to be better organized than those in the Mainé area. Indeed, when the soil is well organized, a relatively flat unit is formed, colonized by woody plants dominated by palm date trees and acacias (*A. seyal* and *A. nilotica*) with sporadic doum trees (PAGRN, 2005). This vegetation belt reduces wind speed and in turn weakens erosion, and improves the microclimate for good vegetation establishment (van Aarde et al. 1996).

The best represented families are the Mimosaceae, the presence of which indicates a dry climate (Mbayngone et al., 2008). The most frequent species in these basins are *Hyphaene thebaica, Prosopis juliflora, Leptadenia pyrotechnica and Phoenix dactylifera*. These species are characteristic of this particular ecosystem type. Their importance depends on environmental conditions, in particular the intensity of anthropogenic pressure. These species are arranged in a halo from the outside to the inside of the basin with a high concentration of *Hyphaene thebaica* in the outer belt (PLECO, 2006) and date palms on its inner periphery. This density varies according to the sites, which is itself explained by a variation in the level of the depth of the water table. This has already been observed by (Issa et

al. 2016; and Souley, 2017). However, in terms of floristic richness, the Sorensen index showed great similarity both between the sites and between the concentric halos, which could mean that the three sites share the same environmental conditions. Regarding the diversity of the woody stand, we observe a high diversity in these basins despite disturbances due to human activities of the population including trampling. This means that the operation does not affect the diversity of the pits. According to Forman and Godron (1986) and Burel and Baudry (1999), up to a certain threshold, disturbances increase the diversity of an ecosystem. These authors indicate that the disturbances are intense of the peripherals generally tend towards a diversification while in the internal zones with weak disturbance tend towards a tend towards a homogenization of the medium (Barot et al., 1999; Henkel, 2003). They explained this state of affairs by a change in facies induced by the edge effect which promotes both the heterogeneous development of certain species such as heliophilic species, ruderal species and that of species adapted to various disturbances (trampling, water stress and human activities) (Tomimatsu and Ohara, 2004; Verheyen and Hermy, 2004; Harper et al., 2005).

Considering the villages, there is a variation in diversity which may be due to the variation in the level of the shallower water table and to the way in which the woody stand is managed by the local populations. This shows that the Balla site is the least diversified of the three sites. This low diversity seems to be due to the resurgence of bush fires which have had an impact on certain species (Tidjani,2016). Overall, it is at the level of the second halo that the lowest density is recorded. On the other hand, the recovery rate is very high. This means that the stand is made up of large trees with a giant habit. The soil appears to be fertile and *Hyphaen tebaica* is the dominant species, these 80% surrounded pits are surrounded by Arecaceae with a specific contribution between 60-92.6%. Analysis of the structure of the woody stand shows a distribution characterized by the predominance of young individuals or of small diameters. Individuals between the 30 to 40cm class are best represented. This type of distribution was described by (Volle, 1985) in an environment with little or no grazing where the relatively low population densities corresponded to a recent installation or to aging. Environmental factors play a significant role in the discrimination of plant groups (Ababou et al., 2009).

The three individualized plant groups explain the heterogeneity of the stand. These groupings, although distinct, are 80% similar. The valuesof the diversity indices show that the species therefore share the ecological niche in an equitable manner. Group I which brings together the 21 species distributed in 26 predominantly *Hyphaene thebaica*. It is made up of readings from the natron zone and those from the market gardening zone which corresponds respectively to the third and fourth halo of the basins. This group represents the transition zone between the lower slope and the valley floor. This transition zone is also characterized by a relatively medium slope and a 69.23% clay texture. Factors, soil types and the intensity of anthropogenic pressure seem to explain the aggregation of species in this grouping. The average density of the species is 159.38 ± 12.7 vines / ha with an average crown cover of 31.40% and a basal cover of 0.90 ± 0.86m² / ha.

Group II corresponds to the vegetation of the transition zone between the second and third halo of the basins, ie between the zone of market garden crops and the strip of palm groves. The woody flora of this group is predominantly Leptedania phyrotechnica..C is the sandy-clay texture mid-slope which most often represents a meadow established under the edge effect. The tree density is 155.15 ± 9.93 vines / ha which provides an aerial cover of 36.46%. Trees seem to be better adapted to this environment. The basal area of 25.67m².

Group III, which includes months of surveys and less species. These surveys were carried out in the village of Balla (56.25%), Kilakina (43.75%) and Tchago (6.25%). It corresponds to the vegetation that overlaps between the second and first sandy texture halo. This is the transition zone between the palm grove and the dune front. The dominant species in this group are *hyphaene thebaica* (33.7%), *Leptedania phyrotechnica* (17.12%); *Calotropis procera* (14.77%); *Acacia raddiana* (10; 36%) *Prosopis juliflora* (7.89%); and *Phoenix dactylifera* (5.52%) At this level the slope is relatively low.

Analysis of the woody stand structure of these oasis basins

shows an asymmetric distribution of individuals characteristic of monospecific stands and a dominance of individuals with small diameters. This result is consistent with that of Aboubacar, et al., 2016) in the Dallol Bosso. For the height structure, in the study area and for the different groupings, the distribution also has the same shape as that of the diameter which is a positive asymmetric distribution with a predominance of young individuals, which is said to be disturbances (natural and anthropogenic) recurrent.

V. CONCLUSION

The present study contributes to the existing information on the woody population of the oasisian basins of Gouré. The flora of this stand is rich in 26 species divided into 23 genera and 16 families. The dominant family is Arecaceae (33%) followed by Asclepiadaceae with 24% Mimosaceae 21%, Lytraceae 9% and finally Meliaceae 4%. The average tree density is estimated at 132.87 vines / ha. It is a relatively covered, diverse stand. Analysis of the demographic structures of the trees in these present basins shows a positive asymmetric distribution characterizing a monospecific population. Floristic compositions vary from one site to another; the hierarchical classification has made it possible to distinguish three groups of vegetation: (1) Group I, which groups together the species in the transition zone between the lower slope and the valley floor. The soil type factors and the intensity of anthropogenic pressure seem to explain the grouping of species. (2) Group II corresponds to the vegetation of the transition zone between the second and third halo of the basins. It is the mid-slope with a sandy-clay texture that most often represents a meadow installed under the edge effect. (3) Group III which includes fewer surveys and species.

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Willingness to pay for Pesticide-Safe Vegetables in Nepal

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Abstract— *Demand for pesticide-safe vegetables in affordable price is increasing because of consumers' consciousness for their health. The objective of this study was to assess the consumers' willingness to pay for pesticide-safe vegetables in Nepal. A sample of 720 respondents, surveyed at four major markets in Nepal, analysed by Choice Base Conjoint method. The result showed that price was prioritized in majority of consumers in Pokhara, Chitwan and Butwal; and production method was prioritized in majority of consumers in Kathmandu. There was significantly negative preference to price in all markets and in all consumer segments. Almost all consumer segments in all markets had significantly positive preference for labelled products. Products produced under conventional systems were disliked by consumers, irrespective of market. Majority of consumers were willing to pay for switching to higher quality food-safety levels from low quality food safety levels. Therefore, the study concludes that there is a strong potential for establishing spacility market for pesticide-safe vegetables in Nepal.*

Keywords— ***WTP; Choice Base Conjoint; Pesticide-free, Traditional and Organic vegetables; Labelling.***

I. INTRODUCTION

There is increasing trend of demand for various types of pesticide-free/pesticide-safe agricultural and food products in recent decade (Aswathy & Thomas, 2019; Edenbrandt, 2018) amid increased consumer awareness on health, environmental safety, harmful impact of pesticides and may be the impact of various 'buy local' movements (Ndlovu *et al.*, 2016). Reciprocating to the changes in consumer preference, various production typologies such as - organic farming, agro-ecological farming, low input agriculture, good agriculture practice (GAP), biological farming, permaculture – have been proposed as technological solution to produce pesticide-safe agricultural commodities. There is also an increased attention to revive the traditional farming system due to various environmental and economic benefits (Singh and Singh, 2017). All these global patterns about change of consumer preference and search for alternative production methods are pertinent for Nepal.

The traditional farming system, which is still present in high hills and mountains, tends to be organic or chemical-free by default due to century long production methods (Gauchan *et al.*, 2020; Bhatta and Doppler, 2011). However, the pesticide use is growing very sharply in the country. Pesticide use of Nepal is 0.35 kg/ha, which is only 13% of worlds average of 2.69 kg/ha (FAOSTAT, 2021). Rapidly growing commercial and semi-commercial fresh vegetable enterprises use highest amount of pesticides and have also stirred discussion about pesticide residue among urban consumers. There is increased media attention on pesticide residue on fresh vegetables which often puts vegetable growing farmers as culprit. Due to this, there is strong push for growers to adopt alternative production methods, like GAP, organic agriculture, integrated pest

management (IPM), or return to traditional agriculture practices for supporting food safety issues.

Insects and pests cause about 35 percent crop loss in Nepal which compels application of insecticides and pesticides for crop protection (PQPMC, 2019). In addition, the current agricultural markets do not have any structure to differentiate different levels of pesticide-safe products to reap incentive for farmers to adopt alternative production methods. There are few organic outlets in few cities who claim to sell organic products but not only they are excessively expensive but their authenticity, product quality and traceability are uncertain (Singh & Maharjan, 2015). Therefore, pesticide toxicity has been mentioned as a classic case of market failure since neither the negative externality of the pesticide is accounted nor the positive externalities of alternative methods are appreciated (Becker, 2017).

Market is an important pull factor for promotion of alternative production technologies. No effort for promotion of pesticide-safe vegetables would be sustainable without building incentive mechanisms within the market system. In Nepal, the demand for organic vegetables (and food products in general) is rapidly growing. Studies have also indicated that the health-conscious consumers in urban areas are interested to pay premium price for organic or pesticide-free vegetables (Khanal, 2020; Bhatta *et al.*, 2010). It is also evident in few specialized organic market outlets who have been running the speciality market charging premium price to consumers.

One general limitation of this system is that this system gives consumers only binary choice from two ends of spectrum. Consumers can choose highly expensive organic products from specialty market or pesticide-laden products from conventional markets. There are relatively safer, mid-way alternatives such as: pesticide-free (not organic product because farmers may use chemical fertilizer, which is possible under pest control through IPM or GAP systems), traditional products (which are similar to organic product without organic certification), none of these mid-way alternatives are made available for consumers to choose. Very little is understood about how consumers perceive about these mid-way safer-than-conventional and cheaper-than-organic products which could provide a balanced compromise for food safety and affordability to urban consumers. Understanding consumer attitudes towards and preferences for these production methods, as well as determining consumers' willingness to pay (WTP) to these alternatives would, therefore, be of immense value to producers, marketers, and policy makers in the country.

In this context, this study was conducted to understand the market segmentation and consumers' WTP for different types of pesticide-safe vegetables produced under pesticide-free condition, traditional system and pure organic system in markets of Nepal. The specific objectives of this study were to: 1) assess consumers' preference for different attributes of pesticide-safe vegetables for different segments of market in Nepal by using choice-based conjoint technique, 2) quantify consumers' WTP for switching from conventional to pesticide-free, traditional, and organic farming products.

II. MATERIALS AND METHODS

Study Area

The primary data were collected through consumer survey in December 2019 from vegetable markets of four biggest urban centres of Nepal, i.e. - Kathmandu (Kalimati), Chitwan (Bharatpur), Butwal and Pokhara, through structured questionarrie.

Sampling

A systematic random sampling technique was applied at each market centre. Sampling was done among the consumers present at the vegetable market gates at the peak time of vegetable purchase. Systematically, every 4th consumer passing from the gate was interviewed to observe the randomness in the sampling. If any sampled respondent did not answer, next person coming out from market gate was interviewed.

All together 720 consumers were surveyed for this study which accounted 180 from each market. The sample size of 180 was decided because this size was enough to ensure the validity of the Choice Base Conjoint (CBC) design with the intended levels of attributes.

The vegetables such as tomato, potato, cauliflower, and radish were considered for the study as these are most common vegetables traded in Nepalese market. However, very little or no difference was observed in respondents' responses by type of vegetable. Therefore, only tomato was retained for final survey as it has highest share in market transection and is available in all these market in all seasons.

Analytical methods

CBC model was chosen because it can analyse the data having large number of attributes of a good and it involves one attribute in monitory cost, and WTP can be calculated on estimated preference. It is possible to construct the preference over all attributes of each consumer.

For objective 1, to assess consumers' preference for different attributes of pesticide-safe vegetable for different

consumer segments, CBC analysis was conducted. CBC was developed since 1960 for stated preference studies. The strategy of this method involves making inferences about the Part Worth of attribute levels from respondents stated choices (Raghavarao *et al*., 2010). Surveys asks respondents to make choice among alternatives based on levels of their attributes. Each attribute consists of different levels which is associated with different prices. Thus, CBC survey asks respondents to indicate their preferences for combinations of different levels of two or more attributes. The attributes and their levels for the CBC questionnaire with consumers for this study were as follows (Table 1):

Table 1: Attributes and levels for CBC analysis

Attributes	Levels	
1. Production methods	1.1)	Conventional (use of both pesticide and chemical fertilizer)
	1.2)	Pesticide-free (no use of pesticide but may have used chemical fertilizer)
	1.3)	Traditional (no use of pesticide and no use of chemical fertilizer)
	1.4)	Organic (not allowed to pesticide use, not allowed to chemical fertilizer use)
2. Labelling/certification	2.1)	Without labelling/certification
	2.2)	With labelling/certification
3. Price	3.1)	Regular market price
	3.2)	Maximum 15% higher than market price
	3.3)	Maximum 30% higher than market price
	3.4)	Maximum 45% higher than market price

The first attribute was vegetable production method, particularly related to how the insect/pest control has been done in field. This attribute had four levels starting from conventional (which uses pesticide-based pest control), pesticide-free (where pesticide use is not allowed but use of chemical fertilizers permitted), traditional production systems (no use of chemical fertilizer and no use of pesticide) and organic farming system (where both pesticide and chemical fertilizers are not permitted). Different levels of the first attribute provided the alternative, mid-way safer vegetable production methods which the study intended to assess WTP by consumers. The second attribute was labelling/certification which had binary levels – without or with labelling. Third attribute was price which was specified with four levels of premium price for greater levels of earlier two attribute combinations, i.e. no premium, 15% premium, 30% premium and 45% premium. Different levels of premium price were determined through focus group discussion and verified in pre-testing.

The questionnaire for the WTP was framed as the CBC. Ten different versions of the CBC questionnaire were generated by Sawtooth Software by randomizing the combinations of the choice options. Each version had 12 different choice sets where each choice set had 3 different combinations of different levels of the attributes.

Example question for CBC for latent class analysis

Attributes	Option 1	Option 21	Option 36
Pest control methods	Existing method	Traditional	Organic
Labeling	No labeling	No labeling	Labeling
Price	Market price	Price 15% higher than market price	Price 45% higher than market price
Which option you like?			

Robustness of CBC design

The levels within first attributes have Standard Error (SE) of 0.026, whereas the second attribute had SE of 0.021 and third attribute had SE of 0.032. Higher SE for attribute with higher number of levels is observed since each level appears fewer times in the design when the number of levels increases. SE of none of the main effects were larger than 0.05. In CBC design, SE of less than 0.05 is accepted for main effects as thumb rule for the robustness of the design, minimum sample sizes and minimum acceptable precision (Orme, 2005). This design with 180 respondents with 10 version and 12 tasks from each version meet the higher standard for this particular attribute list and set of effects.

The final analysis was carried out using the latent class model as often done in market segmentation studies. In the latent class model, the consumers were classified to different segments by the model based on the similarity and differences regarding their response to the product attributes and their levels. The Consistent Akaike Info Criterion (CAIC) criteria identified two group models as the best fitted model, but the numbers of segments were too low for drawing out meaningful conclusion. Therefore, the model with three segments, which has next lowest CAIC, were selected for the interpretation in all sites. The latent class model of three groups grouped the consumers in three distinct segments according to preferences of consumers which was useful to make meaningful interpretation for consumer segmentation. The sign of 'Part Worth Utility (PWU)' values were read as the consumer preference where the positive (+) PWU indicating 'like' and negative (-) PWU indicating 'dislike' to the attribute level. The statistical significance of the PWU were analysed for P=0.01 and P=0.05 whereas PWU not significant at 95% confidence level were considered non-significant preference or indifference.

For objective 2, consumers' WTP for switching from conventional to pesticide-free, traditional, and organic farming products were quantified by using the equation 1 in MS Excel spread sheet. This was calculated by using the method described by Orme (2001). The formula for calculation of WTP for switching from one level to other level was as follows:

$$CS = -\frac{1}{\beta m}(V' - V);$$

where: CS *is willingness to pay*, βm *is parameter estimate for price*, V' *is the utility of desired level and* V *is utility of initial level*

III. RESULTS AND DISCUSSIONS

Consumers' preference for different attributes of pesticide-safe vegetables for different segments

Different consumer segments had clear and distinct characteristics regarding their preference and importance for different attributes of the vegetables. Figure 1 shows the relative importance of the different attributes for different consumer segments separated by the four markets.

For the largest consumer segment in Kathmandu market, production method was the most important attribute carrying about 40% weight followed by price (32.8% weight) and labelling (27.2% weight) (Figure 1). For the second segment, however, the price was the most important attribute (55.1% weight) whereas production method was second important (30.4% weight) and price was the least important attribute (14.6% weight). For the third consumer segment, production method carried even higher importance (71.0% weight); price was second important attribute carrying 21.3% weight whereas this group gave very little importance (7.7% weight) on the labelling.

In Pokhara market, for the largest consumer segment, price was the most important attribute carrying about 64.1% weight followed by production method and labelling. For the second segment, the price was the most important attribute (42.5% weight) whereas production method was second important (38.1% weight) and labelling was the least important attribute (19.4% weight). For the third consumer segment, production method carried even higher importance (50.3% weight), price was second important attribute carrying 25.6% weight whereas this group gave very little importance (24.1% weight) on the price.

For the largest consumer segment of Chitwan market, price was the most important attribute carrying about 53.5% weight followed by production method and labelling. For the second segment, the production method was the most important attribute (51.7% weight) whereas price was second important (25.6% weight) and labelling was the least important attribute (22.7% weight). For the third consumer segment, production method carried even higher importance (61.2% weight), price was second important attribute carrying 21.9% weight whereas this group gave very little importance (17.0% weight) on the price.

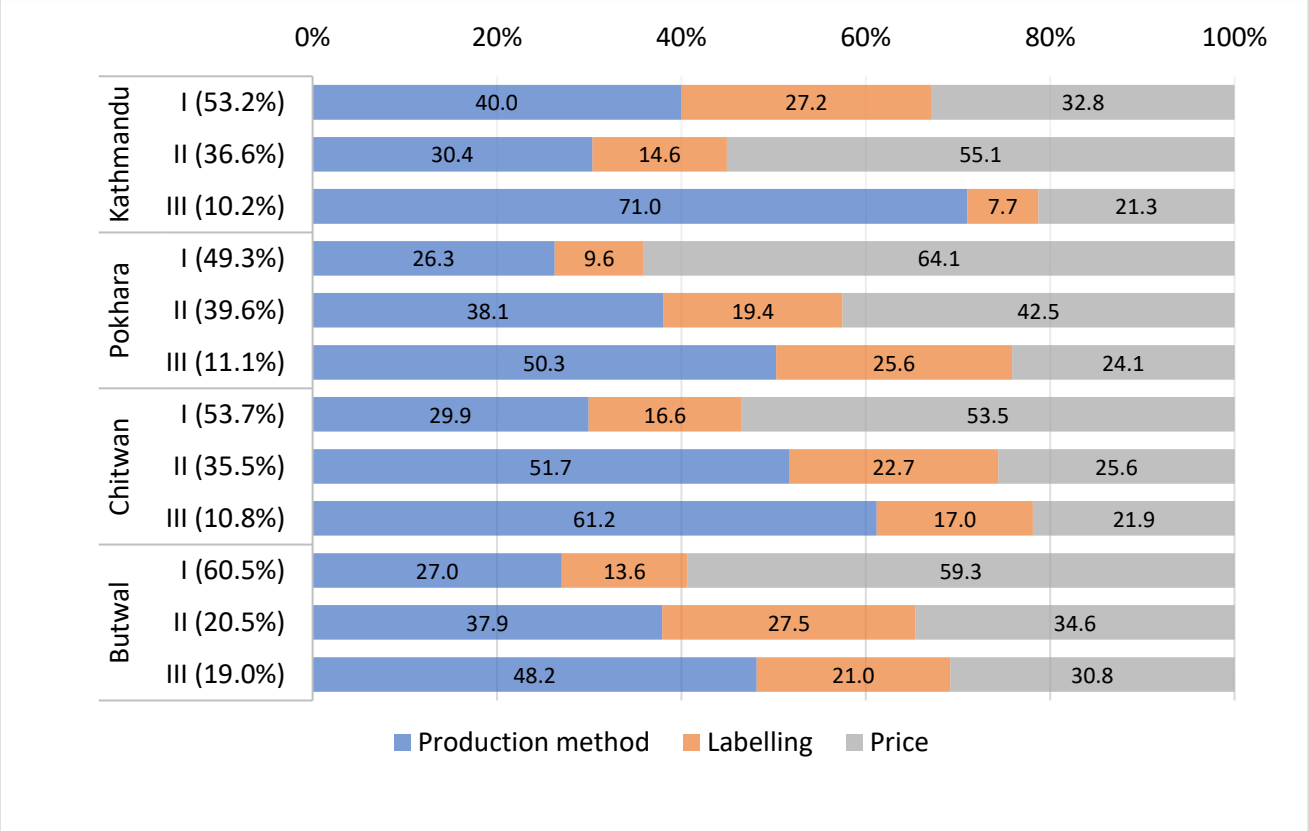

Figure 1: Relative importance of attributes by consumer segments by markets (importance are in percentage) (source: consumer survey)

Largest consumer segment of Butwal market, price was the most important attribute carrying about 59.3% weight followed by production method and labelling. For the second segment, the production method was the most important attribute (37.9% weight) whereas price was second important (34.6% weight) and labelling was the least important attribute (27.5% weight). For the third consumer segment, production method carried even higher importance (48.2% weight), price was second important attribute carrying 30.8% weight, whereas this group gave very little importance (21.0% weight) on the labelling.

Thus, price was the most prioritized attribute for majority of consumers in Pokhara (78.9%), Chitwan (53.7%) and Butwal (60.5%) and sizable 36.6% of consumers in Kathmandu. There are dearth of studies relating premium price to traditional or pesticide-free production in Nepal however in case of organic products, 'high price' has been always reported as one critical constraint for promoting organic vegetables (Bhatta *et al.*, 2009; Bardhan *et al.*, 2019; Sharma *et al.*, 2016). This suggests that there is need for producing safe food without much expected premium price. This is possible by promoting safer mid-way alternatives to vegetable grown under pesticide-laden conventional system as well as expensive organic system.

One strategy could be promoting products from traditional system which covers 96%, 80% and 41% agriculture lands in Mountain, Hill and Terai region of Nepal, respectively, which produces pesticide-safe food but are marketed as conventional products (PDD, 2014; Gurung *et al.*, 2016; Palikhey *et al.*, 2017). Another strategy could be promoting pesticide-free products allowing supplemental application of production limiting soil nutrients. Lower crop yield under organic system has also been reported in Nepal (Dahal and Dhakal, 2016; Singh *et al.*, 2015). Deficiency of nitrogen is one of the main factors limiting productivity under organic farming (Askegaard *et al.*, 2011). Therefore, allowing supplemental nitrogen fertilizer but restricting pesticide can be mid-way compromise to produce affordable and pesticide-safe vegetables. This study also verified that consumers are willing to pay some premium price for these mid-way alternatives to organic products. Production method was the most important attribute for 63.4% consumers in Kathmandu, 50.7% consumers in Pokhara, 46.3% consumers in Chitwan and 39.5% consumers in Butwal, indicating there is still potential of premium price for products grown under those production methods. Consumers in Kathmandu were found to be prioritizing production method over price possibly due to relatively higher income and greater awareness

about pesticide-safety issues in Kathmandu. Adhikari *et al.* (2012) also reported educated and economically better consumers in Kathmandu preferred product quality over price.

Consumers' preference for different levels of attributes

The consumer segments identified by the selected three-group latent class models are presented in Table 2. Firstly, the result showed significantly negative preference to price attribute in all markets and all consumer segments, which indicated confirmation to the negatively sloped demand curve/law of demand, thereby indicating robust construct validity of the models. Similarly, almost all consumer segments in all markets had significantly positive preference for products with label which is understandable as labels provide the information and assurance about product quality. These findings are aligned to previous findings suggesting consumers were willing to pay higher for products with organic certification and labelling in Nepal (Kattel *et al.*, 2020; Bhattarai, 2019; Aryal *et al.*, 2009) and globally (Padilla *et al.*, 2007; McCluskey & Loureiro, 2003).

The results showed that in Kathmandu, all three segments of the consumers had significantly negative preference for vegetables grown under conventional system (applying both chemical fertilizers and pesticides). The largest consumer segment in Kathmandu constituted 53.2% consumers and it was characterised by significant positive preference for pesticide-free, traditional, and organic products. These consumers had positive preference for labelled products. The second segment constituted 36.6% consumer who were also similar to first segment except that these consumers were indifferent to pesticide-free products unlike the first segment who were positive toward pesticide-free. The third segment, which constituted a small 10.2% consumers, but had unique preference. These consumers had positive preference toward organic products while being in different to any other alternatives.

All consumer segments in Pokhara had significant negative preference for vegetables coming from conventional system. Differences were observed in preferences regarding the production methods. The largest consumer segment (49.3%) in Pokhara had significantly positive preference for organic products. This segment was indifferent to pesticide-free products but had significant positive preference to traditional products. The second largest segment in Pokhara constituted 39.6% consumers, who had significant negative preference for pesticide-safe products but had positive preference to traditional and organic products. The third segment of consumer constituted 11.1% of people, who had positive preference for products grown under pesticide-free methods, traditional methods and organic methods.

Like in earlier markets, all three consumer segments in Chitwan had significant negative preference for the vegetables produced under conventional system. All segments had significant positive preference to vegetables grown under traditional and organic systems as well as products with labels. The difference in the preference was observed in preference toward pesticide-free production methods. The largest consumer segment in Chitwan with 53.7% of the consumers in the segment were indifferent to pesticide-free products. The second segment was composed of 35.5% consumers who had strong positive preference for pesticide-free products. The third segment constituted 10.8% of consumers, who had significant negative preference to pesticide-free production methods.

In Butwal market, all consumer segments had significantly negative preference to vegetables produced under conventional system applying both pesticide and fertilizers. The difference of consumer preference was once again due to differential preference for production methods. In Butwal market, all consumer segments had strong significant positive preference to traditional and organic products. However, the largest consumer segment in Butwal, which constituted 60.5% of consumer, were indifferent to the products grown under pesticide-free methods. The second segment constituting 20.5% of consumers had significant positive preference to pesticide-free products. The third segment composed of 19.0% of the consumers had negative preference for products grown pesticide-free products.

The largest consumer segments in Pokhara, Chitwan and Butwal market (constituting about 49-61% consumers) had similar characterisation – negative preference to conventional products; positive preference to vegetable grown under traditional system and organic systems; and positive preference to labelled products. There was mixed preference for pesticide-safe products as larget consumer section in Kathmandu market also had positive preference to such products whereas in other market where consumers did not had significant preference on this possibly due to this mid-way compromise not being well understood to them. The result also indicated that there was a small but sizable segment of consumers (10-19% depending on market) who had positive preference for organic and traditional products except in Kathmandu. This consumer segment could be key for establishing speciality markets for organic and traditional products.

Products produced under conventional systems were disliked by consumers, irrespective of market, indicating an increased knowledge about the food safety issues among urban consumers in Nepal. It was evident that

majority of the consumers preferred safer production alternatives – either pesticide-free or traditional or organic products which showed a great scope for promotion of these products. Despite such strong consumer preference toward pesticide-free, traditional and organic products, the lack of sizable market transection of vegetables grown under these systems is a contradiction. This contradiction is mainly due to mismatch between consumer's WTP and affordability. Consumers who dislike conventional products may still consume those products due to several individual factors like value and risk taking attributes as well as external constraints like unavailability of alterative product, high transection cost, low quality assurance and low affordability (Eide and Toft, 2013; Tandon *et al.*, 2021; Singh and Verma, 2017).

Another striking finding across most of the market that there were sizable numbers (53.2% in Kathmandu, 11.1% in Pokhara, 35.5% in Chitwan, 20.5% in Butwal) of consumers who preferred organic products also preferring pesticide-free products indicating consumers were more worried about pesticide toxicity than fertilizer. This suggested toward the possibility of promoting pesticide-safe production methods without falling in potential production hiccup for converting to organic. For all alternative production methods, labelling was important. Therefore, innovative certification and labelling without adding much to the cost, such as participatory guarantee systems (PGS) or geographical labelling, would be useful for meeting labelling requirements.

Consumers' WTP to switch from inferior to superior levels of each attributes

Table 3 shows maximum percentage of additional price consumers were willing to pay for switching to greater level of food-safety categories from poor level of food safety category. Since most of the current consumers were consuming vegetables with both pesticide and fertilizer residue or produce without label, no reverse switching was relevant for this study.

The analysis of WTP showed that the largest consumer segment in Kathmandu (53.2%) had willing to pay 41.8% for pesticide-free products, 47.3% higher for traditional products and 54.7% higher for complete organic products. Those consumers were also willing to pay 37.2% higher for labelled products compared to non-labelled products. About 36.6% consumers in Kathmandu were willing to pay additional 14.3%, 17.0% and 24.8% for pesticide-free, traditional and organic products, respectively, and 11.9% higher for labelled products. Remaining 10.2% consumers were willing to pay 51.2% higher to organic products compared to conventional products and 16.4% higher to labelled product compared to non-labelled products. In Pokhara, the largest consumer segment (49.30%) had WTP 10.4% higher for pesticide-free, 12.1% higher for traditional and 18.5% higher for organic products. Those consumers were also willing to pay 6.8% higher for labelled products. Similarly, 39.6% consumers from second segment were willing to pay 20.6%, 31.6% and 40.2% higher for pesticide-free, traditional and organic products, respectively. Remaining 11.10% consumers were willing to pay 75.8%, 83.2% and 93.5% higher for pesticide-free, traditional and organic products, respectively. Further, the results indicated that the largest consumer segment in Chitwan (53.70%) had WTP 15.5% higher for pesticide-free products, 20.6% higher for traditional and 25.3% for organic vegetables. Those consumers were also willing to pay 14% higher for labelled products. The second largest segment (composed of 35.50% consumers) was willing to pay 75.7%, 79.6% and 90.8% higher for pesticide-free, traditional and organic produce, respectively. Remaining 10.80% consumers from third segment in Chitwan were willing to pay 40%, 105.9% and 125.3% higher for pesticide-free, traditional and organic products, respectively. The largest consumer segment in Butwal (60.50%) was willing to pay 11.7% higher for pesticide-free products, 14.9% higher for traditional products and 20.5% for organic products. Those consumers were also willing to pay 10.3% higher for labelled products compared to non-labelled products. Other 20.50% consumers in Butwal were willing to pay 35.5%, 45.8% and 49.2% higher for pesticide-free, traditional and organic products whereas remaining 19.0% consumers from third segment were willing to pay 31.8%, 50.6% and 69.8% higher for pesticide-free, traditional and organic products, respectively.

It is apparent that sizable proportion of consumers were willing to pay premium price to shift from low food-safety levels to higher food-safety levels in all markets. It is understandable that consumers want to switch to higher quality of food. Few previous findings have also suggested that urban consumers were willing to pay premium price for safe food, particularly in context of organic food. For instance, Bhattarai (2019) reported that WTP on an average of 25% higher for organic products. Atreya *et al.* (2012) reported that individuals were willing to pay 53-79% more than the existing pesticide price to protect their health and environment. Similarly, Rai and Adhikari (2016) reported that consumers are willing to pay up to 31% additional prices for organic vegetables. Aryal *et al.* (2009) found that 58% of the consumers in Nepal were willing to pay 6-20% price premium, whereas 13% consumers were willing to pay up to 50% premium. This study also confirmed the findings from like Balogh *et al.* (2016) who reported that traditional food products can

command a substantial premium price if effective quality certification and authentic product composition is maintained. There is no previous study about consumer WTP for pesticide-free products in Nepal, but the findings are aligned with studies indicating positive WTP price premium for pesticide-free products (Khan *et al.*, 2018; Hayati *et al.*, 2017) or pesticide-safe products (Cobbinah *et al.*, 2018; Vidogbéna *et al.*, 2015).

Despite that, caution is warranted in interpreting these results. It is likely that consumers who expressed strong WTP to organic, pesticide-free, or traditional products in hypothetical market may not actually pay premium price in real market. Using a meta-analysis of 80 worldwide studies, Li and Kallas (2021) suggested that WTP estimate derived from hypothetical market scenario like choice experiment was higher than real transection analysis due to hypothetical bias. Regardless of this, the result indicates toward strong potential for specialty market and opportunity to promote safer mid-way alternative production methods.

IV. CONCLUSION AND RECOMMENDATION

Different typologies of safe-food production systems are available in the continuum between conventional, traditional, green-revolution paradigm and pure organic system which may produce pesticide-safe food in affordable price. Among them, traditional production system which is by-default organic but lacks certification, and pesticide-free system where pesticides are not allowed but synthetic fertilizer is allowed, can be mid-way safer compromise for poor, food insecure countries, such as Nepal. However, these alternatives are seldom recognized and made authentically available to consumers. This study explored consumers' perceptions about these mid-way alternatives along with organic system and found that sizable segment of consumers has positive preference to these alternatives. Our study also validated that consumers are willing to pay highest premium price to pure organic products; however, there are large groups of consumers who do not prefer to pay high premium price for pure organic products. Thus, it seems that there is strong market demand for pesticide-free and traditional products which need to be harnessed for improving agriculture marketing as well as food-safety agenda in Nepal. Alternative certification and labelling practices which would not put high price hike for safe-food like organic certification does could be optimal solution to develop speciality market and realize the price premium for products coming from relatively safer food production systems. These alternative production systems need to be streamlined in policy about agriculture marketing and food-safety to supply safer food for consumers.

Table 2: Part Worth Utility of consumer preference by market and consumer segments (source: consumer survey)

	Kathmandu			Pokhara			Chitwan			Butwal		
No of segments	Seg1	Seg2	Seg3	Seg1	Seg2	Seg3	Seg1	Seg2	Seg3	Seg1	Seg2	Seg3
Consumer segment size	53.2%	36.6%	10.2%	49.3%	39.6%	11.1%	53.7%	35.5%	10.8%	60.5%	20.5%	19.0%
Production method												
Conventional	-4.17**	-2.85**	-13.14ns	-2.53**	-2.68**	-8.27**	-2.55**	-7.75**	-5.15**	-2.24**	-5.74**	-2.70**
Pesticide-free	0.68**	0.21ns	1.26ns	0.17ns	-0.29**	1.66*	0.13ns	1.79**	-2.12**	-0.04ns	0.50**	-0.44**
Traditional	1.31**	0.54**	5.11ns	0.40*	0.99**	2.63**	0.83**	2.28**	2.90**	0.60**	2.32**	0.89**
Organic	2.17**	2.10**	6.76*	1.96**	1.98**	3.98**	1.60**	3.69**	4.37**	1.67**	2.92**	2.25**
Labelling												
without label	-2.16**	-1.19**	-1.08**	-0.82**	-1.19**	-3.11**	-1.15**	-2.51**	-1.32**	-0.99**	-3.14**	-1.08**
with label	2.16**	1.19**	1.08**	0.82**	1.19**	3.11**	1.15**	2.51**	1.32**	0.99**	3.14**	1.08**
price	-0.12**	-0.20**	-0.13**	-0.24**	-0.12**	-0.13**	-0.16**	-0.13**	-0.08**	-0.19**	-0.18**	-0.07**

Note: ** and * indicate the significant at P=0.01 and P=0.05 respectively, ns indicate value not significant at 95% confidence level

Table 3: Consumers' Willingness to Pay to switch from lower food-safety attributes to higher food-safety categories (source: consumer survey)

Switching		Kathmandu			Pokhara			Chitwan			Butwal		
From	To	Seg 1 (53.2 %)	Seg 2 (36.6 %)	Seg 3 (10.2 %)	Seg 1 (49.3 %)	Seg 2 (39.6 %)	Seg 3 (11.1 %)	Seg 1 (53.7 %)	Seg 2 (35.5 %)	Seg 3 (10.8 %)	Seg 1 (60.5 %)	Seg 2 (20.5 %)	Seg 3 (19.0 %)
Conventional	Pesticide-free	41.8	14.3	NR	10.4	20.6	75.8	15.5	75.7	40.0	11.7	35.5	31.8
	Traditional	47.3	17.0	NR	12.1	31.6	83.2	20.6	79.6	105.9	14.9	45.8	50.6
	Organic	54.7	24.8	51.2	18.5	40.2	93.5	25.3	90.8	125.3	20.5	49.2	69.8
Pesticide-free	Traditional	5.4	2.7	NR	1.6	11.1	7.4	5.0	3.9	66.0	3.2	10.3	18.8
	Organic	12.8	10.5	51.2	8.1	19.6	17.7	9.7	15.1	85.4	8.8	13.8	38.0
Traditional	Organic	7.4	7.8	51.2	6.4	8.5	10.3	4.7	11.2	19.4	5.6	3.4	19.2
Without label	With label	37.2	11.9	16.4	6.8	20.5	47.5	14.0	39.9	34.8	10.3	35.7	30.5

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Effect of Concentration of *Sargassum polycystum* and Fermentation Time on thickness and Yield of Nata de Sargassum

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Abstract— *Sargassum polycystum is one type of brown seaweeds that is abundant in Indonesian waters, including in Bone Bay, South Sulawesi. S. polycystum is an underutilized species and considered by coastal communities as trash that polluting the sea. Nevertheless, it contains carbohydrates which are essential in the nata making process. This research aimed to determine the concentration of S. polycystum and the fermentation time that produce the best thickness and yield of nata de sargassum. Th research was conducted at the laboratory of Fisheries High School (SUPM) Bone, South Sulawesi. Sample of S.polycyctum was collected from Tanjung Palette's water, Bone Bay. A completely randomized factorial design was used with 2 factors,namely concentration of S.polycystum and fermentation time, ang each factor consisted of four levels. The concentrations used were 2, 3, 4 and 5% (w/v) and the fermentation time were 7, 10, 13 and 16 days. Each treatment was carried out in three replicates. Results showed that the concentration of S. polycystum and fermentation time imparted a significant effect ($p<0.05$) on the thickness and yield of the nata de Sargassum. The thickness (5.6 mm) nata produced was at a concentration of 3% and a fermentation time of 16 days. While the largest yield (32.2%) was produced at a concentration of 5% with a fermentation time of 16 days.*

Keywords—***Brown algae, nata de sargassum, underutilized, Acetobacter xylinum, carbohydrates.***

I. INTRODUCTION

Sargassum polycystum is one of the abundant brown seaweeds in Indonesian waters, including Bone Bay, but so far is still underutilized. Although many studies on its contents and benefits, the existence of *S. polycystum* tended to be ignored and considered as trash that pollutes the ocean[1],[2], Dried *S. polycystum* contains 17.69% water, 24.51% ash, 0.50% fat, 3.65% protein, 53.66% carbohydrates, 3.81% crude fibre, and minerals such as magnesium (Mg) 89.9 mg/g, iron (Fe) 0.50 mg/g,potassium (K) 26.9 mg/g, sodium (Na) 22.23 mg/g andcalcium (Ca)18.06 mg/g [3]. According to [4],[5],materials containing high carbohydrate are good source of nata production. With over 50% carbohydrate content, *S. polycystum* may therefore be a potential material for the production of nata de seaweed.

Nata de seaweed is an alternative product processed from seaweeds that is well known and favoured by many communities, especially Japanese. Nata was first produced in the Philippines[6] from coconut water, and the product is worldwide known as *nata de coco*. Nata is a layer of extracellular polysaccharide obtained through fermentation using *Acetobacter xylinum*. Nata has a chewy texture, white gel-like appearance and floats on the surface of aqueous solution containing sugar and acid [7],[8]. Nata is essentially a mass of cellulose formed through polymerization of glucose by *A. xylinum* during fermentation process.

The key factors influencing the success of nata making are substrate concentration and fermentation time.

[9]studied the production of nata de seaweed using *Kappaphycusalvarezii* and reported that the optimum concentration of *K.alvarezii* was 2% with a fermentation period of 16 days. The longer time of fermentation the thicker the nata, but too long fermentation time affects the quality of the nata[4],[5].

The common indicator used in evaluating the success of the nata making is by measuring the thickness and yield of the nata produced. Both the thickness and the yield are indicators of how effective and efficient are the conversion of sugar (glucose) into cellulose (nata). Therefore, this paper describes characteristic of the thickness and yield of the nata de Sargassum produced at different concentration and fermentation time.It may be worth to note that this is the first study to use brown seaweed *Sargassum* as a substrate for the production of nata de seaweed.

II. MATERIALS AND METHODS

2.1. Materials and Equipment

Materials used to make nata de Sargassumwere *S. polycystum*obtained from the waters of Tanjung Palette, Bone Bay South Sulawesi, distilled water, vinegar, sugar, *Acetobacter xylinum*, ammonium sulphate, magnesium sulphate.Equipment used were plastic jar, measuring cup, stainless sieve, stove, stainless steel pan, wooden stirrer, stainless steel knive, blender, balance, newsprint, rubberbands.

2.2 Procedures of nata de Sargassum making

2.2.1.Equipmentand *S. polycystum*preparation

Equipment used, except balance and rubber bands, must beclean and sterile[10],[11].All the equipment were washed with soap, thoroughly rinsed under running tap water, dried, and then sprayed with 70% ethanol solution. *S. polycystum* was also thoroughly cleaned and washed to remove mud and other undesired materials attached to the thallus, and if necessary soaked in fresh water. Upon drained, the seaweed sample was then chopped to reduce its size to 1-2 cm long.

2.2.2. Preparation of *A. xylinum*starter

The pre-chopped *S. polycystum* was homogenized in the distilled water using a commercial house blenderat a ratio of 1:50 (w/v). The homogenized sample was then filtered through a stainless steel sieve to separate the solid and the filtrate. Furthermore, the filtrate was boiled in a boiling pan, and then sugar(10%, w/v), vinegar (1%, v/v),ammonium sulphate(0.5%, w/v), magnesium sulphate(0.5%, w/v) of the filtrate volume were added. After boiling, the solution (substrate)waspoured into a bottle, close tightly and let the solution to stand at room temperature for about 8 hours until cool.Then, 100 ml of *A. xylinum* stock was added into 1000 ml of substrate. The substrate was allowed toferment until cellulose layer was formed (about 6 days). This fermented substrate, also known as starter, was then used in the natade Sargassum making process.

2.2.3. Preparation of nata de Sargassum substrate

Fresh *S. polycystum* was washed thoroughly until the fishy smell disappeared.After draining the seaweed was homogenized in distilled water using a commercial homogenizer with the ratio of water:seaweedof 1,000:20, 1,000:30, 1,000:40, and1,000:50. Then the homogenized seaweed samples were filtered over a stainless sieve andthe filtrates were poured into separate boiling pans. Further, 10% (w/v) ofsucrose, 0.5% (v/v) of vinegar, 0.5% (w/v) of ammonium sulphate and 0.5% (w/v) of magnesium sulphate, all by volume of the filtrate, were added into each boiling pan.The mixtures were stirredevenly and then boiled.

2.2.4. Fermentation process of nata de Sargassum

Separately, as much as 500 ml of the boiled filtrates (substrate)of each treatment ratio were immediately transferred into a pre-assigned sterilized fermentation containers, covered with newsprint, and then tied with rubber bands. Allow the substrate to cool and let to stand for approximately 8 hours. Then, by opening the newsprint coverslightly, 50 ml of *A. xylinum starter solution* were poured into each of the fermentation containers without stirring. The newsprint cover was then immediately reclosed to prevent contamination. Process was done at room temperature (27-28^{0}C) for 7, 10, 13 and 16 days.

2.2.5. Harvesting of nata de Sargassum

At the end of each of the fermentation period, the nata formed was harvested, cleaned by removing its top layer (the epidermis), washed, and then cut into desired shape. The nata was then squeezed using a calico cloth until it resembles a sheet of paper. To get rid of the sour smell, then at Awa soaked in fresh water for 3 days.

2.3 Parameter measurement

2.3.1. Determination of the Nata thickness

The thickness of the nata was measured using a caliper and the thickness value was expressed asthe average of five measurements.

2.3.2. Determination of thenata de Sargassum yield

After soaking in the fresh water, the nata was allowed to drain and measured for its weight. The yield was then calculated using aformula as follow:

$$\text{Yield} = \frac{\text{Weight of the nata (g)}}{\text{Weight of ingredients (g)}} \text{ X } 100\%$$

2.4. Experimental design

The experiment was carried out by employing a completely randomized factorial design with two treatments factors, namely the concentration of the *S. polycystum* and the fermenting time. The concentration used was 2, 3, 4 and 5%, whereas the fermenting time was 7, 10, 13 and 16 days. Al treatments were repeated three times.

2.5. Data Analysis

Data of the thickness and yield of the nata de Sargassum were analysed using two-way ANOVA at 95% level of confidence. Since analysis of variance (ANOVA)indicated the presence of significant difference, the analysis was proceeded with a Tukey-test to determine which treatment has the significant difference.

III. RESULTAND DISCUSSION

3.1 Nata Thickness

The thickness of the nata de Sargassum produced in this study ranged from3.2 mmto 5.6 mm, being thinnest for 3% (w/v) concentration of 7 days fermentation and thickest for the 3% (w/v) concentration with a fermentation time of 16 days (Table 1).According to Indonesian National Standard(SNI) 01-4317-1996[12], nata that meets the quality requirements must have a thickness of about 1-1.5 cm, thus the nata de Sargassum resulted in this study, werestill far below the standard thickness by the SNI.

Table-1.The thickness of the nata de Sargassum produced at differentS. polycystum substrate concentration and fermentation time.

Consentration of *S.policystum* (%)	Thickness (mm)			
	7 days	10 days	13 days	16 days
2	3.46 ± 0.04^{ax}	4.53 ± 0.12^{xy}	4.86 ± 0.09^{x}	5.06 ± 0.09^{x}
3	3.2 ± 0.16^{ax}	4.7 ± 0.08^{y}	4.7 ± 0.08^{x}	5.6 ± 0.16^{y}
4	3.26 ± 0.09^{ax}	4.66 ± 0.09^{y}	4.76 ± 0.12^{x}	5.16 ± 0.16^{x}
5	3.73 ± 0.24^{ax}	3.9 ± 0.65^{x}	4.8 ± 0^{x}	5.2 ± 0.14^{xy}

Valuesfollowed by the samesuperscriptin the same row(a,b,…)or column (x,y…) show no differenceat 95% probability level($p>0.05$).

Results of ANOVA indicated that the concentration of *S. polycystum* and fermentation time exerted significant effects on the thickness of the nata de Sargassum. The Tuckey test showed that the3% (w/v) concentration with afermentation time of 16 days produced a significantly thicker nata as compared to the other treatments, except with that of the 5% concentration of the same fermentation time. Regardless of the absence of significant difference between the 3 and 5% concentration for the 16 days of fermentation, the tendency of the higher thickness of the nata for the 3% concentration may indicate that 3% of *S. polycystum* is the best and optimum concentration for the production of the nata de Sargassum.

The smallest thickness value of the nata obtained at a concentration of 3% of*S. Polycystum* and 7 days of fermentation time was presumably due to an inadequate fermentation duration, so thatthe capacity of *A. xylinum* bacteria to form the nata layer has not reached its plateau. Meanwhile, the thickest Nata produced at 3% concentration of *S. polycystum*ata fermentation time of 16 days indicated that *A. xylynum* has reached its maximum capacity in converting carbohydrates to cellulose. Based on the above trends, it is clear that the conversion of carbohydrates(glucose)by *A. xylinum* bacteria in the fermentation substrate into celluloseis a function of fermentation duration, thus the thickness of the nata is, up to a certain time period, a fermentation time-dependent.[4],[13],[14]reported that the longer the fermentation time the thicker the nata will be.It was also noted in this study that fermentation time was the most influencing factor in the synthesis of cellulose, and therefore on the thickness of the nata de Sargassum.

The addition of carbohydrates as a carbon source must be in an appropriate amount. Excessive carbohydrates will cause the work of *A. Xylinum* bacteria become not optimal due to part of carbohydratesis converted into acid, thusdecreasing the pH of the fermentation medium[15]. Excessive carbohydrates may also cause brown colour due to Maillard reaction. On the other hand,too little carbohydrate added will affect the thickness of the nata layer produced because of the lack of carbon source forthe *A. xylinum* to form the nata layer [16].

3.2. Yield

The yield of nata de Sargassum at various concentrations of *S. polycystum* and fermentation time ranged from 27.9 to 32.2% (Table 2). The shortest fermentation time produced the lowest yield, regardless of the concentration of the substrate. The result of Anova showed that the concentration of *S. polycystum* did not impose any effect on the yield of the nata de Sargassum while the fermentation time did. Further, the Tukey test showed that the fermentation time of 7 days produced significantly lower yield of the nata de Sargassum as compared to the other fermentation times, while the fermentation times of 10, 13 and 16 days produced a similar yield of the nata de Sargassum.

Table-2. Yield of nata de Sargassum at various concentrations of S. polycystum and fermentation time.

Concentration of S. polycystum(%)	Yield (%)			
	Day 7	10 Day	13 Day	16 Day
2	$28.53 \pm 0,35^{ax}$	$30.48 \pm 0,27^{bx}$	$30.87 \pm 0,48^{bx}$	$31.58 \pm 0,12^{bx}$
3	$27.94 \pm 0,32^{ax}$	$30.73 \pm 0,26^{bx}$	$30.61 \pm 0,22^{bx}$	$32.12 \pm 0,15^{bx}$
4	$28.05 \pm 0,12^{ax}$	$30.51 \pm 0,27^{bx}$	$31.01 \pm 0,30^{bx}$	$31.77 \pm 0,16^{bx}$
5	$28.88 \pm 0,77^{ax}$	$29.33 \pm 1,24^{abx}$	$30.78 \pm 0,22^{bx}$	$32.24 \pm 0,42^{bx}$

Values followed by the same superscript in the same row (a,b,..) or column (x,y,.) show no difference at 95% confidence level ($p>0.05$)

Raw materials play an important role in the process of formation of cellulose/nata[17]. Materials containing high carbohydrates can be used as ingredients for making nata. Besides high content of carbohydrate, *S. polycystum*also provides sufficient macronutrients and micronutrients for *A.xylinum* bacteria to grow and develop, thus may increase the yield of the resulting nata. In addition to the raw materials, the length of fermentation timeis also a crucial determining factor to the yield of the nata. Table 2 above shows thatthe fermentation time of longer than 7 days significantly ($p<0.05$)increases the yield of the nata *de Sargassum*.[18] stated that during fermentation process there is a breakdown of sugar into simpler components namely glucose and fructose, and the formation of cellulose-forming carbohydrates. Therefore, longer fermentation time provide a continuous accumulation of glucose which is then converted cellulose to form the nata.

However, the similar yield of the nata obtained for all substrate concentration at any of the same fermentation time may raise a question of whether the bacterial count of *A. xylinum* in the starter solution is sufficient to convert most, if not all, the carbohydrates (sugar) in the fermentation substrate. Logically, if most or all sugar has been converted to cellulose, then a higher substrate concentration should produce a higher yield of the nata as well.So, there is a possibility that the similar yield obtained from different substrate concentration is due to insufficient concentration of *A. xylinum* used in the process. The same is applicable to the similar thickness of the nata de Sargassum as well. Therefore, further researchers are needed to determine the best or optimum conditions for the production of good characteristics of the nata de Sargassum.

IV. Conclusion

This study is the first to use*S. polycystum*as araw material to produce nataand proves that this type of brown seaweed can be used to produce nata *de Sargassum*. Both the thickness and the yield of the nata de Sargassum were fermentation time-dependent; longer fermentation time produces thickerand higher yield of the nata *de Sargassum.* Therefore, there is no urgency to use the concentration*S. polycystum* substrate of above 3% in the production of the nata de Sargassum. However, since the concentration of the bacterial starter was not varied in this study, it is still unknown whether different or higher concentration of *A.xylinum* bacterial starter imposes any effect on the characteristics of the nata de Sargassum; a subject commands a further study.

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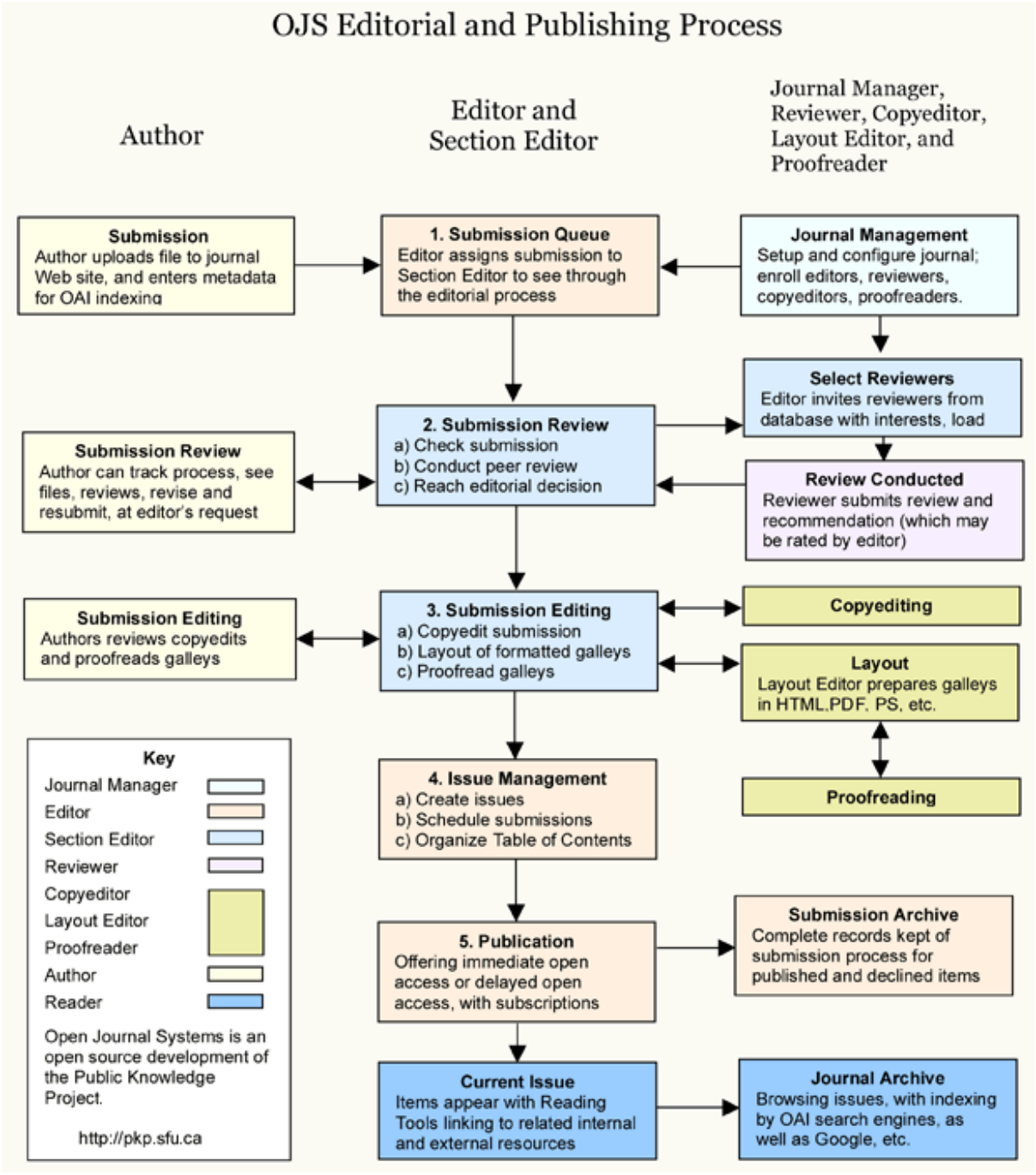

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