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FOREWORD

I am pleased to put into the hands of readers Volume-5; Issue-4: Jul-Aug 2020 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: Sept, 2020

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Analysis of the Development of the Export Seaweed Processing Industry in South Sulawesi

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***Abstract*—** *This research was conducted in the seaweed industries in South Sulawesi, especially in the Makassar industrial area (KIMA). The purpose of this study is to develop a strategy for developing seaweed commodity processing industries to increase added value. Analysis of the data used is using SWOT analysis. With SWOT analysis, it can produce several alternative strategies in making good decisions to be carried out. So that it can determine the development strategy of the seaweed industry. The results showed an IFAS score of 2.79 and a score for the EFAS results of 2.85. When converted into IE Matrix diagram, it is located in quadrant V, where the results of the company enter into the phase of Growth and Stability. The right strategy for companies in quadrant V is SO strategy, using a strategy that utilizes existing opportunities by utilizing the strengths they possess, namely by intensification and extensification of cultivated land, diversification of seaweed processed products and development of fishery products that have high added value including the biotechnology-based industry.*

Keywords*— *Seaweed, Industrial Development Strategy, SWOT Analysis, IFAS-EFAS Matrix.

I. INTRODUCTION

Seaweed (seaweed) is a marine plant belonging to the multicellular algae (algae) thallophyta division. Unlike perfect plants in general, seaweed has no roots, stems and leaves. Seaweed lives on the ocean floor that can be penetrated by sunlight so it has a variety of colors which are then used to classify seaweed. In general, edible seaweed is a type of blue algae (cyanophyceae), green algae (chlorophyceae), red algae (rodophyceae) and brown algae (phaeophyceae) (Atmadja, 2012). However, the term seaweed is more often used for red algae and brown algae. Brown algae, which is a source of alginate, live mostly in temperate regions.

Seaweed is one of the international trade commodities that have been exported to more than 35 countries. Seaweed cultivation potential in Indonesia is found in provinces such as Aceh, North Sumatra, DKI Jakarta, West Java, Central Java, East Java, Bali, NTT, NTB, North Sulawesi, Central Sulawesi, South Sulawesi, Maluku and Papua (Ministry of Maritime Affairs and Fisheries , 2012). Seaweed as a commodity is also potential to be developed because seaweed cultivation production techniques are relatively easy and inexpensive with very low risk of crop failure, high productivity, and harvests can be done every 45-60 days or about 4 times a harvest a year. Seaweed farming can also absorb a large workforce and create large and broad economic multiplier effects. Seaweed is generally traded in the form of: dried seaweed, products that can be directly consumed, and hydrocolloid products.

South Sulawesi is located on the southern peninsula of the island of Sulawesi. The province with its thousand cities in Makassar is administratively, in the north bordering West Sulawesi and Central Sulawesi Provinces. South Sulawesi Province is located at 0 ° 12 '- 8 ° South Latitude and 116 ° 48' - 122 ° 36 'East Longitude. Its area is 45,764.53 km². The province is bordered by Central Sulawesi and West Sulawesi to the north, Bone Bay and Southeast Sulawesi to the east, the Makassar Strait to the west and the Flores Sea to the south. Geographically it is located in a very strategic position, because it is at the crossroads of passenger traffic, trade in goods and services from the western region of Indonesia (KBI) to the eastern region of Indonesia (KTI).

With this potential, the amount of production is very large but the absorption of seaweed in the country is still relatively small because most seaweed processing industries in the country are also still limited to the base product level and have not yet developed to the level of end products and product formulations (blended products).

This resulted in several losses including inefficiency of the seaweed processing industry due to lack of raw materials, loss of potential added value, employment and potential tax revenue. Therefore, seaweed exports in the form of raw materials need to be regulated to ensure the availability of raw materials for the domestic seaweed processing industry that produces end products and formulation products.

Other problems in the seaweed industry in South Sulawesi are the low quality of seaweed produced by farmers, the lack of product diversification, global market requirements, competition between producers, cost efficiency, technology support, waste management (Setyawan, 2018). Based on the foregoing, this research is aimed at determining strategies in the development of the export seaweed processing industry in South Sulawesi.

The purpose of this study is to identify internal factors that are strengths and weaknesses in the development of the export seaweed processing industry in South Sulawesi, identify external factors that are opportunities and threats in the development of the export seaweed processing industry in South Sulawesi. This study also aims to establish alternative development strategies that are appropriate for the export seaweed processing industry in South Sulawesi.

II. METHOD

Research activities on the analysis of the development of the export seaweed processing industry are carried out in several seaweed industries in South Sulawesi, particularly in the Makassar industrial area (KIMA). The research location is determined purposively with the consideration that the Makassar industrial area (KIMA) is an industrial center in eastern Indonesia, where there are industrial and warehousing complexes, KIMA houses companies that manage and export seaweed. This research was conducted for 3 months.

Data collection

Based on the research objectives, the data collected in this study consisted of two sources, namely primary data and secondary data. Primary data is a method of collecting data that is observational through direct observation in the field using several data collection techniques, such as through documentation, filling in questionnaires, and in-depth interviews (Indept Interview) with related parties in accordance with the desired data needs. Secondary data used in this research is to use literature studies or literature studies that are relevant or related to the formulation of the problem and retrieve data that can be obtained from various sources, either through national agencies or through collecting documents that can support this research.

SWOT Analysis

SWOT analysis is a qualitative analysis to identify various factors systematically and formulate an activity strategy. This analysis is based on logic that maximizes strengths and opportunities, but simultaneously minimizes weaknesses and threats (Rangkuti, 2016).

The steps taken in the SWOT analysis are as follows: (a) Identification of strengths/weaknesses and opportunities/ threats and (b) The analysis stage through mapping the factors identified in the form of the SWOT matrix.

Strategic factors in the development of the export seaweed processing industry in South Sulawesi are arranged in a matrix that can clearly illustrate how external opportunities and threats faced can be adjusted to their strengths and weaknesses. The resulting strategy consists of several alternative strategies. To determine the priority of the strategy that must be chosen, a weighting sum is derived from the interrelationship between the elements of strengths, weaknesses, opportunities, and threats contained in the alternative strategies in the following manner :

a. The SO (Strength and Opportunity) strategy is to add value to the elements of strength and opportunity that are interrelated with the strategy.

b. The WO (Weaknesses and Opportunity) strategy is to add up the value of weaknesses and opportunities that are interrelated with the strategy.

c. The ST Strategy (Strength and Threats) is to add the value of the strength and threat elements that are interrelated with the strategy.

d. The WT (Weaknesses and Threats) strategy is to add the values of weaknesses and threats that are interrelated with the strategy.

Table 1. SWOT Matrix

IFAS / EFAS	Strenght Internal strength factor	Weaknesses Internal weakness factor
Opportunity External opportunity factors	Strategi SO Use your strength to take advantage of opportunities	Strategi WO Overcome weaknesses by taking advantage of opportunities
Ancaman (Threats) External threat factor	Strategi ST Use force to avoid threats	Strategi WT Minimize weaknesses and avoid threats

Source: Rangkuti, 2016.

Next do a QSPM analysis. This analysis is an analytical tool that enables strategists to evaluate various alternative strategies objectively, based on important external and internal success factors identified earlier (David, 2011), so as to optimize the results obtained (Zulkarnaen & Sutopo, 2013). QSPM uses input analysis from the EFAS Matrix, IFAS Matrix and SWOT Matrix to objectively determine the strategy to be carried out among alternative strategies.

III. RESULTS AND DISCUSSION

Internal Factor Analysis

Based on the analysis that has been carried out, some strengths and weaknesses are obtained in the development of the export seaweed processing industry in South Sulawesi. Strengths include : (1) Great seaweed resource potential, (2) Large domestic market with per capita consumption of fishery products and by-products, (3) Government support and regional policies, (4) A large number of the fishing industry. Weaknesses include : (1) Exports of processed seaweed products are still low, (2) Limited supply of raw materials, (3) Seaweed processing technology has not been integrated yet, (4) HR in the seaweed processing industry is still not ready, (5) Infrastructure to support the development of the seaweed processing industry is still limited, (6) The production capacity of the seaweed processing industry is not optimal.

External Factor Analysis

Based on the analysis, several opportunities and threats were obtained in the development of the export seaweed processing industry in South Sulawesi. Opportunities include : (1) The increasing world consumption needs of fishery products (the size of export opportunities), (2) Opportunities for diversification of marine products, (3) Opportunities for the development of non-food seafood products with high added value, (4) A large quantity of human resources scattered in various centers of marine products. Threats include : (1) Food safety issues, (2) Requirements and standardization of products that refer to international standards, are still difficult to adopt and implement, (3) Export requirements are getting stricter, (4) Very tight competition in obtaining raw materials.

Internal Matrix Analysis

A weight score of 2.79 indicates that the industry is in a strong position. This means that this industry is relatively stronger in utilizing its strengths and is able to overcome its weaknesses. The main strength of this industry is the potential of large marine resources with a score of 0.56. In second place is a large domestic market with a score of 0.41. In the third position is government support with a score of 0.33. In the fourth position is the regional policy with a score of 0.30 and in the fifth position is the large number of the fishing industry with a score of 0.25. More details can be seen in Table 2.

Table 2. Results of Internal Factor Analysis

Factor	Weight	Rating	Score
Strength			
1. Large potential of marine resources	0.14	4	0.56
2. Large domestic market	0.11	3.75	0.41
3. Government Support	0.12	2.75	0.33
4. A large number of seaweed industries	0.09	2.75	0.25
5. Regional Policy	0.11	2.75	0.30
Total Strength Score			**1.85**
Weaknesses			
6. Exports of processed seaweed products are still low	0.07	2	0.14
7. Limited raw material supply	0.07	2.25	0.16
8. Seaweed processing technology has not yet been integrated	0.07	2	0.14
9. Human resources in the processing industry are still not ready	0.07	2	0.14
10. Infrastructure is still limited	0.08	2.5	0.20
11. The production capacity of the processing industry is not yet optimal	0.08	2	0.16
Total Weaknesses Score			**0.94**
Total	**1.00**		**2.79**

Source: Primary Data After Processing, 2018

This industry has internal environmental conditions in the form of strengths and weaknesses. The main weakness in the development of the seaweed processing industry in South Sulawesi is that infrastructure is still limited with a score of 0.20. In the second and third positions are limited supply of raw materials and production capacity of the processing industry which is not optimal with a score of

0.16. While in the third, fourth and fifth positions are exports of marine products is still low, not yet integrated seaweed processing technology and human resources in the processing industry which are still not ready with a score of 0.14 respectively.

External Matrix Analysis

Based on the external matrix, the total weighting score is 2.85. This means that the development of the export seaweed processing industry in South Sulawesi is able to respond to external factors by utilizing existing opportunities to overcome threats. The main opportunity in the development of the seaweed processing industry in South Sulawesi is the increasing world consumption needs of seaweed products with a score of 0.53. The second opportunity is the development of the seaweed industry with high added value with a score of 0.49. The third opportunity is the diversification of seaweed products with a score of 0.46 and the last opportunity is the quantity of human resources that are numerous and scattered in various centers of marine products with a score of 0.45. more details can be seen in Table 3.

Table 3. Results of External Factor Analysis

Oppertunities			
Factor	**Weight**	**Rating**	**Score**
1. The world consumption needs of seaweed products are increasing	0.14	3.75	0.53
2. Opportunities for diversification of marine products	0.13	3.5	0.46
3. Development of seaweed industry with high added value	0.13	3.75	0.49
4. The quantity of human resources is widely spread in various centers of marine products	0.12	3.75	0.45
Total Strength Score			**1.92**
Threats			
5. about food safety	0.08	1.75	0.14
6. The requirements and standardization of seaweed, which refers to international standards, are still difficult to adopt and implement	0.09	2	0.18
7. Export requirements are increasingly stringent	0.08	2	0.16
8. Application of integrated technology in competitor countries	0.07	1.25	0.09
9. Very tight competition in getting raw materials	0.08	2.5	0.20
10. Local products are less competitive	0.09	2.25	0.20
Total Weaknesses Score			**0.94**
Total	**1.00**		**2.85**

Source: Primary Data after Processing, 2018

The development of the export seaweed processing industry in South Sulawesi has external environmental conditions in the form of opportunities and threats. The main threat they have is very tight competition in obtaining raw materials and less competitive local products with a score of 0.20. The second threat is the seaweed standardization requirements that refer to international standards, are still difficult to adopt and apply with a score of 0.18. The third threat is the increasingly stringent export requirements with a score of 0.16 and the last threat is the application of integrated technology in competitor countries with a score of 0.09.

Then an alternative strategy is determined based on a SWOT analysis. From the SWOT matrix, 4 main strategies can be arranged, namely S-O, W-O, S-T, and W-T. Each of these strategies has its own characteristics and should be implemented, then implemented together and support each other (Tjoe and Sarjono, 2010)

- Strategy S-O (Strengths - Opportunities). This category contains various alternative strategies that take advantage of opportunities by utilizing their strengths.
- Strategy W-O (Weaknesses – Opportunities). Categories that take advantage of external opportunities to overcome weaknesses.
- Strategy S-T (Strengths–Threats). An alternative category of strategies that utilize power to overcome threats.
- Strategy W-T (Weaknesses–Threats). An alternative category of strategy is the solution to the assessment of weaknesses and threats faced, or efforts to avoid threats to overcome weaknesses.

Alternative strategies are shown in table 4. Based on the analysis of the SWOT diagram in table 3 and the values in the EFAS and IFAS tables, that strength is greater than weaknesses and opportunities are greater than threats then the company is in quadrant 1 with an aggressive strategy that matches the SO strategy in the SWOT matrix.

SWOT matrix and analysis as one of the strategic planning methods used to evaluate strengths, weaknesses, opportunities and threats in fisheries industrialization strategies to support regional economic development. Table 4 outlines the strengths, opportunities, weaknesses, and threats of the fisheries industrialization conditions, resulting in 13 strategies as follows :

1. S1: Strategy 1, Improving the regional economy through increasing the population of the seaweed processing industry
2. S2: Strategy 2, Initiation of the development of seaweed processing industries that have high added value including biotechnology-based industries
3. S3: Strategy 3, Increasing the role of central and regional governments in maintaining food safety of processed seaweed products
4. S4: Strategy 4, Strengthening supply chains, partnerships and market expansion
5. S5: Strategy 5, Adjusting the potential of fisheries resource areas to downstream industries that have high added value
6. S6: Strategy 6, Provision of appropriate equipment and technology

Table 4. Alternative Strategies

SWOT ELEMENTS	STRENGTHS	WEAKNESS
	1. Large potential marine resources, including aquaculture and seaweed 2. Large domestic market 3. Government Support 4. A large number of seaweed industries 5. Regional Policy	1. Exports of processed seaweed products are still low 2. Limited supply of raw materials, especially the lean season 3. Seaweed processing technology has not yet been integrated 4. Human resources in the seaweed processing industry is still not ready 5. Infrastructure to support the development of the seaweed processing industry is still limited 6. Seaweed processing industry production capacity is not optimal
OPPORTUNITY	**S-O**	**W-O**
1. Increasing world consumption needs of seaweed products (large export opportunities) 2. Opportunities for diversification of marine products 3. Opportunities for the development of the seaweed industry with high added value 4. Quantity Human resources are abundant and scattered in various centers of marine products	• Intensification and extensification of cultivated land • Diversification of processed seaweed products • Initiation of the development of seaweed industry that has high added value, including biotechnology-based industries	• Improve cultivation technique skills to increase production • Provision of appropriate equipment and technology • Application of Cultivation Technology • Increasing the establishment of seaweed industry which has added value and diversification of high products. • Improvement of processing feasibility certification (SKP)

THREAT	S-T	W-T
1. Issues regarding food safety 2. The requirements and standardization of fishery products that refer to international standards, are still difficult to adopt and implement 3. Export requirements are increasingly stringent 4. Application of integrated technology in competitor countries 5. Very tight competition in getting raw materials 6. Local products are less competitive than China, Vietnam and Thailand	• Increased role of central and local governments in maintaining food safety of processed products • Optimizing existing production capacity • Improving the quality of processed seaweed products • Strengthening the Supply Chain, Partnerships and Market Expansion	• Improving facilities and infrastructure to support seaweed industry activities • Improving quality assurance, food safety, and improving sanitation in industry • Acceleration of the Development of Fisheries Industry Growth Center with high added value at the locus of choice

Source: Primary Data After Processing, 2018

7. S7: Strategy 7, Increasing the supply of raw materials through the utilization of fisheries potential in areas with high LQ values
8. S8: Strategy 8, Application of cultivation technology
9. S9: Strategy 9, Increase the establishment of the seaweed industry which has added value and high product diversification.
10. S10: Strategy 10, Improvement of processing feasibility certification (SKP)
11. S11: Strategy 11, Strengthening infrastructure in areas that have high potential but utilization is still low
12. S12: Strategy 12, Improving quality assurance, food safety, and improving sanitation in industry
13. S13: Strategy 13, Acceleration of the development of seaweed industry growth centers with high added value at the locus of choice (optimization of installed capacity of the fishing industry)

IV. CONCLUSION AND RECOMMENDATION

Conclusion

The conclusions from the results of research on the analysis of the development of the export seaweed processing industry in South Sulawesi are as follows:

1. Internal factors in the development of the export seaweed processing industry in South Sulawesi as strengths include : (1) Large seaweed resource potential, (2) Large domestic market with per capita consumption of fishery products and by-products, (3) Government support and regional policies, (4) The number of the fishing industry is large, while the weaknesses include : (1) Exports of processed seaweed products are still low, (2) Limited supply of raw materials, (3) Seaweed processing technology has not been integrated yet, (4) Human resources in the seaweed processing industry are still not ready, (5) Infrastructure to support the development of the seaweed processing industry is still limited, (6) The production capacity of the seaweed processing industry is not optimal.
2. External factors in the development of the export seaweed processing industry in South Sulawesi as opportunities include : (1) The increasing world consumption needs of fishery products (the size of export opportunities), (2) Opportunities for diversification of marine products, (3) Opportunities for the development of non-food seafood products with high added value, (4) A large quantity of human resources scattered in various centers of marine products, while the threats they have include : (1) Food safety issues, (2) Requirements and standardization of products

that refer to international standards, are still difficult to adopt and implement, (3) Export requirements are getting stricter, (4) Very tight competition in obtaining raw materials.

3. Based on the results of research from the internal side, the analysis of the development of the export seaweed processing industry in South Sulawesi has greater strengths than its weaknesses, while from the external side the opportunities are greater than threats, so the appropriate strategy is the SO strategy, namely using strategies that take advantage of opportunities there is by utilizing the power possessed. What can be done is by way of intensification and extensification of cultivated land, diversification of processed seaweed products and initiation of the development of seaweed industry that has high added value including biotechnology-based industries

Recommendation

By looking at various problems in the production and processing of seaweed in the country, it is necessary for government policies that encourage the growth and development of seaweed cultivation and seaweed processing industry. Seaweed farmers are advised to be able to produce high quality seaweed periodically by implementing the latest innovations. Related to efforts to improve the quality of Indonesian seaweed, a policy that can be carried out is to apply sea water quality standards for the cultivation and supply of quality seeds. In addition, training can also be done to increase knowledge, cultivation abilities that lead to increased seaweed productivity.

REFERENCES

[1] Atmadja, W., S. (2012). Apa Rumput Laut itu sebenarnya? Diunduh pada tanggal 12 Juli 2018 dari http://www.coremap.or.id/print/article.php?id=264.

[2] David, F. 2011. Strategic Management, 14th Edition. New Jersey: Prentice Hall.

[3] Kementrian Kelautan dan Perikanan. 2012. DKP Dorong Rumput Laut Sebagai Sumber Pangan dan Energi. Siaran Pers. Jakarta : Kementrian Kelautan dan Perikanan.

[4] Rangkuti, Freddy (2016). Analisis SWOT: Teknik Membedah Kasus Bisnis Cara Perhitungan Bobot, Rating, dan OCAI Cet. Ke-22. Jakarta: PT Gramedia Pustaka Utama.

[5] Setyawan, E.Y. 2018. FGD Struktur dan Kinerja Pemasaran Komoditas Rumput Laut di Sulawesi Selatan. Diunduh pada 31 Juli 2018 pukul 13:28 dari https://kkp.go.id/brsdm/sosek/artikel/7294-fgd-struktur-dan-kinerja-pemasaran-komoditas-rumput-laut-di-sulawesi-selatan

[6] Tjoe, T.F. dan Sarjono, H., (2010), Strategi Bisnis Pada PT. CTL dengan Pendekatan Metode TOWS, Binus Business Review, 1(2), 434-447

[7] Zulkarnaen, H. O. dan Sutopo. 2013. Analisis Strategi Pemasaran pada Usaha Kecil Menengah (UKM) Makanan Ringan (Studi Penelitian UKM Snack Barokahdi Solo). Journal of Managmenet 2 (3): 1-13. Universitas Diponegoro, Semarang.

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Hydroponic— The Future of Farming

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Abstract— *Hydroponics is the modern solution for modern problems. Due to extensive traditional agriculture and various man made activities like deforestation. Fertile and arable land is decreasing day by day. While transporting the food we eat, so much of fossil fuels goes into transportation. The volume of water which is used in conventional farming is shocking. For the irrigation in these conventional farming, large volume of water is needed and water is one of the most crucial resource to be saved. Loads of chemical fertilizers and pesticides that are used in conventional farming flows into the water bodies and make them polluted. To tackle these problems hydroponics can be used as the effective method. In hydroponics plants are grown in a room without the use of any soil. Plants do not require soil for their growth all they need is water and various micro and macro nutrients that are present in soil. In hydroponics water and these nutrients are provided to the plants directly. With the effect of which plants do not have to spend their energy in the expansion of their root system for the search of water and minerals. The use this energy for the production of better food products. Hydroponics have numerous advantages over soil base farming. In this paper all the advantages, history, types and basic components of hydroponics has been explained.*

Keywords— Advantages, Drip system, DWC (Deep Water Culture), Ebb and Flow System, Grow Tray, History, Hydroponic farming, NFT (Nutrient Film Technique), Reservoir, RO (Reverse Osmosis), Wick System.

I. INTRODUCTION

In traditional agriculture, plants are grown in soil. But, plants do not need soil to grow, they need the nutrients that are present in the soil for their growth. Soil contains various micronutrients and macro-nutrients that are essential for the growth of plants. When water is added to the soil, it carries nutrients along with it to the plants. On traditional soil based farming and planting, plants have to spend lots of energy for the development of their huge root system as, roots has to search deep inside for water and minerals. In short, plants need water and minerals for their growth either from the soil or without soil. In soilless farming suggests hydroponic these micronutrients and macronutrients are delivered to the plants directly. The word hydroponics are delivered from two Greek words that are 'hydro' meaning water and 'ponics' meaning labour. In hydroponic farming various micro and macro nutrients that are required by plants are being directly given to them. These nutrients are premix in the water. By this method plants get everything they need at the right proportion and the right time so that plants can utilise it more efficiently. In soilless farming as plants need not to waste their energy for the growth of the complex root system the utilise that energy in food production and show its full genetic potential.

Hydroponics is a system of agriculture that utilizes nutrient rich water rather than using soil. This type of soil less farming have several advantages over traditional methods of farming. In traditional farming loads of chemical fertilizers, pesticides, Fungicides and herbicides are used. Lots of water is required for irrigation purpose. These things somehow disturb the ecological balance and deteriorate the environment. Hydroponics does not use any chemical based fertilizers and pesticides, but also reduces the use of water that helps with water conservation.

II. COMPONENTS OF HYDROPONICS

- **Temperature controller**

Plants require a specific range of temperature for their optimal growth. Temperature control of both, the environment in which hydroponic is done and the temperature of the nutrient solution is required. The best water temperature for hydroponic should be between 65° Fahrenheit and 80° Fahrenheit. The best grow room temperature during vegetative stage is between 70° to 78° Fahrenheit, when the lights are on during the daytime, and it should not be more than 10° to 15° degree cooler during the night time, with the relative humidity of 45 to 55%.

The setup, which helps in the temperature control of the environment in which the hydroponic is performed have things like

1. Exhaust fan
2. Pad cooling system
3. Foggers
4. Sensor
5. Shade Cloth

1. Exhaust fan-

Exhaust fan facilitates proper ventilation inside the plantation room. They are mostly present on the roof of the grow room. These help in removal of hot air and oxygen rich air from the room. This helps in the proper circulation of air inside the grow room. Removal of hot and oxygen rich air facilitates the entry of Co2 rich air inside the room. Ventilation balances the humidity percentage and helps in heat management inside the room. It also helps in pest control, because stagnant and humid air has negative impact on the surface of growing room it encourages the entry and accelerate the reproduction of pest which damages the plants. Due to the stagnant and humid air the growth of fungi, mold and mildew also increase. Ventilation is very important for the optimal growth and protection of plants grown in hydroponics.

2. Pad cooling system-

It is also known as evaporative pad and fan system. It is the most common way of cooling down the grow room temperature. They are placed on the walls of grow room. Cooling pad has their separate water tank that is fitted outside the grow room. The water is performed by the pump or motor on the cooling pads which wet them completely. This water is then evaporated by using the heat present inside the grow room leaving the room cool. And this evaporated heat is dissipated outside the grow room through exhaust fans.

Cooling pads are made up of three types:-

- Wood
- Cellulose
- Plastic

Wood fibre is produced during shaving Aspen trees. They provide a cushion like structure with better air and water balance. It allows more air to travel through the air, which makes the air inside the room cooler. The would absorb just one part of the water and starts cooling it. They are less expensive, but have a short life. On the other hand cellulose pads are made with cellulose paper moulded with the honeycomb construction. As it is a thick material, it holds more water in it. This type of pad is used in the dry area where the water can the evaporated rapidly. Cellulose pads are more costly than wood. Plastic parts are not used commonly because they are not effective as compared to other type of pad material. Plastic is not a good absorbent of water. They are thinner and more cost effective, but they can only be used for shorter term.

3. Fogger-

Fogger adds water in the atmosphere by creating fog. They are fitted on the roof of the grow room. When the temperature is raised above the certain set point the fogger start creating fog which add tiny droplets in the environment, decreasing the temperature and maintaining the humidity of the grow room.

4. Sensor-

Sensors present in the grow room works on fuzzy logic. They record the room temperature and command temperature controllers accordingly in order to maintain room temperature. The sensors are connected with temperature controllers. Sensors are also present in temperature controllers which controls the temperature of nutrient rich water.

5. Shade cloth

Shade cloth is used to provide comfortable and environment friendly temperature for the plants. It provides protection from various pests and insects. Shade cloth also provides a greenhouse effect to the plants that are grown hydroponically. Shade cloth is made up of loosely woven polyester or even aluminium. They are of various densities degrees of shade ranging between 5% to 95%. There are two types of shade clothes-

a. Knitted shade cloth- knitted shade cloth is made up of light weighted polyethylene.
b. Woven shade cloth- woven shade cloth is made up of 100% polypropylene.

Type of Shade cloth is used according to the environment. Shade cloth also protects plants from harmful UV radiations of the sun.

- **Water coolant**

During hydroponics farming, one of the major problems that is faced is increased temperature of water. As the water keeps on circulating in a loop due to this water gets heated up. This increase in temperature of water decreases the amount of dissolved oxygen in it and make the water oxygen deficient. So it is very important to keep the water temperature maintained.

To solve the problem of increased water temperature, a water coolant system should be connected to the water tank to maintain the desired temperature of the water.

For small scale hydroponics, a diy method can be used for lowering the water temperature.

Procedure

1. Take 2 litre water bottle
2. Fill it with water and add one tablespoon of common salt in it.
3. Keep the bottle in the freezer and convert it into ice.
4. Once, whole water gets converted into ice. Immerse frozen water bottle to the water tank.

Salt is added to the water before freezing at because salt increases the melting point of ice.

- **Water tank**

In hydroponic farming water tank is considered to be the heart of the system. Tank holds what plants need to live: nutrient rich water. All Hydroponic tanks are almost of same design. They hold water and nutrients that are to be delivered to plants. These water tanks are fitted with RO system so that any hardness that is present in water can be removed because this hardness can affect the growth of plants. Different type of hydroponic systems has slightly different tanks.

1. **Ebb and flow**

In this type of hydroponic system tank need not to be placed just below the Growing tray. Water is pumped using a water pump, into the growing tray till the water reaches to a certain level after the certain level is achieved water starts draining away from the growing tray. Many systems have a drainage line connected back to the water reservoir, i.e. nutrient-rich water flows in a loop system.

2. **NFT**

In this system tank can be placed away from the growing tray. In, nutrient film technique water reaches to the tray passing from the nutrient reservoir at a constant speed. Trays are placed slightly in slanted angle, therefore pump is needed to move water up to the growing tray.

3. **DWC**

In deep water culture hydroponic system, the plant's roots are suspended directly into the water reservoir, due to which water tank is placed just below the growing tray.

4. **Drip**

In drip hydroponic system, water tank is placed just below the growing tray. Tubes carry water to plants from the water reservoir, each plant receives water through the hole place in tubes. The tank is placed just below the growing tray as it helps to conserve any runoff water.

5. **Wick**

In wick hydroponic system, the tank is placed just below the growing tray as the growing tray has holes from which absorbent cord passes and connect from the tank. They absorb the nutrient solution from the tank and provided to plants in the growing tray.

- **Nutrient tank**

In hydroponic farming, a separate nutrient tank is required. This nutrient tank is attached to the water tank, it contains various nutrients that are required by plants for their growth. Both water reservoir and nutrient tanks are connected to an automatic machine which keeps on adding nutrients in the water tank as per the requirement. In nutrient and the TDS of the solution is measured by using TDS metre and pH buy pH metre. Maintenance of both TDS and pH is necessary for the optimal growth of plants.

Different types of plant required different TDS for example, nutrient solutions for cannabis require TDS of around 500 to 600 ppm for young clones and seedlings, whereas vegetative plants and flowering plants requires TDS from 800 to 900 ppm and 1000 to 1100 ppm respectively. TDS should be around 400 to 500 PPM when flushing the nutrient solution from the growing tray. The TDS keeps on decreasing as plants absorb nutrients from the solution. TDS helps track when you have to add fertilizers and nutrients or when you have to completely change your reservoir.

How pH of the nutrient solution is maintained?

The pH plays an important role in the growth of plants so it is very important to keep check on the pH of the nutrient solution and if the pH is fluctuating from the set range then it has to be maintained. The idle pH requirement for hydroponics rangers between 5.5 to 6.5 that is slightly alkaline. Hydroponic farming is performed under specialised personal so these things are easy to manage. There are two ways by which the pH of the nutrient solution can be balanced-

1. Phosphoric acid
2. Potassium hydroxide (Caustic Potash)

1. **<u>Phosphoric acid</u>**

Phosphoric acid has a pH 2.14, it means that it is highly acidic in nature. When the pH of of the nutrient solution becomes basic in nature and ranges between 8 to 9 then this method can be used to maintain the required pH of the nutrient solution.

Procedure-

i. 1 litre hot water (60°-70°C) and 1 gram phosphoric acid.
ii. Mix both of them into a beaker.
iii. Slowly, Add this diluted phosphoric acid into the nutrient water.
iv. Left it for some time till the pH gets adjusted.
v. Check the pH of nutrient water after 35 to 40 minutes using a pH metre.
vi. Again add the leftover diluted phosphoric acid into the nutrient water
vii. Check the pH using pH metre
viii. Keep on repeating disturb unless the pH of nutrient water ranges between 5.5 to 6.5.

In the place of phosphoric acid, citric acid and white vinegar can also be used for the same.

2. **Potassium hydroxide (Caustic potash)**

Potassium hydroxide has the pH 10.98 that means it is alkaline in nature. When the pH of nutrient solution becomes acidic (around pH 4) potassium hydroxide can be added to maintain the pH level.

Procedure

i. Take 1 litre of lukewarm water and 1 gram of potassium hydroxide
ii. Mix both of them in a beaker
iii. Slowly add diluted potassium hydroxide mixture into the nutrient solution.
iv. Leave it for some time. (35-40 min)
v. After sometime measure the pH of nutrient solution using pH metre.
vi. Keep on repeating the step unless the pH of the nutrient solution reaches 5.5 to 6.5.

Note- do not mix potassium hydroxide and phosphoric acid together.

- **Seed growing medium**

Hydroponics is a soilless plantation method in which the plants are provided with all the essential micronutrients and macronutrients for their growth. These nutrients are provided to them with water because plants need only nutrients and water for their growth and survival.

Soil is not used in hydroponics therefore seed need to be germinated before transplanting them into hydroponic system. There are various seed growing medium in which seeds are germinated and grown to form a baby plant. Then this plant is transferred to the hydroponic system for the further growth.

Growing medium that is used commonly are-

a. Rockwool
b. Cocopeat

    a. **Rockwool-** We can use Rockwool for germinating seeds because Rockwool have high growing ratio as compared to other medium. Rockwool is a rock based mineral fibre that is made up of basalt rock and recycled slag. Basalt is an igneous rock and slag is the byproduct of steel and copper industry. These minerals are melted and spun together to form fibre. It is used because it is light weighted and more convenient. Rock wool has high germination ratio and a special feature due to which insect attack ratio is very low.
    b. **Cocopeat-** Cocopeat is also used for seed germination for hydroponics because it has high water holding capacity. It is made up of coconut husk. Due to its high water holding capacity, it is preferred for the germination of those which require constant and high moisture around it to germinate.

- **Thermometer**

Thermometer is used to measure or for mapping the temperature of the plants. Increase in temperature of plants helps us to know about stress. We have to check the temperature of individual plants regularly, if any, stress is found in a particular plant then that individual plant is removed from the grow tray and is planted in a suitable environment to reduce its stress level. When stress is recorded in a particular plant then that individual plant is removed from the grow tray, not the whole tray is removed from the system.

III. TYPES OF HYDROPONIC SYSTEM

There are six separate types of hydroponic system

a. Wick system
b. Water culture
c. Ebb and flow
d. Drip
e. N.F.T. (Nutrient film Technology)
f. Aeroponic system

- **Wick system**

Wick system is the most basic form of hydroponics and is the most easiest to set up. They are passive, it means that they have no moving parts. Wick system is comparatively cheaper than other methods of hydroponics. It uses various wicks that are in contact with the roots of plants. One side of the wick is emerged in in the nutrient rich water where as other end surrounds the plant's root. The movement of nutrient rich water from the reservoir to the grow tray takes place by the capillary action.

This type of hydroponic system is only efficient with plants like rosemary which does not require a lot of water, but it does not work well with plants which require the large amount of water such as the bell peppers and tomatoes.

Four main components of wick system are explained below-

1. **The Grow Tray -** The grow tray in wick system does not require net pots to hold the growing medium. The seedlings are directly transplanted into the growing medium that fully covers the grow tray. The growing medium which does not drain the nutrient medium to fast and efficiently utilise the capillary action of the wicks are preferred like vermiculite and soilless mixes.
2. **The Reservoir**- The reservoir contains a nutrient rich solution. It is placed just below the grow tray and supplies nutrients to the plants.
3. **The Aeration System-** The aeration system consists of an airstone and a pump. The air stone is a porous stone which helps in the formation of bubbles when the air is pumped in it through the air pump. These bubbles ensure the supply of oxygen to the nutrient solution and nutrient solution does not get stagnant.
4. **The Wick**- the grow tray is connected to the reservoir with the help of two or more wicks. The easiest wick to use is cotton wick, but it has to be changed time to time as it got rot easily, so instead of the cotton wick nylon wick is also used because it does not rot easily. Nutrient solution travels from the reservoir to the grow tray through these wicks by capillary action. These wicks are inserted through the holes that are present in the grow tray and are immersed in nutrient solution present in the reservoir. Number of wicks that are to be used depends on the type of plants grown in the grow tray.

- **Water culture**

Water culture hydroponic system is another simple type of hydroponic system as it does not involve any complex setup. It is a very inexpensive type of system. Things that are required to build a water culture are-

a. Reservoir
b. Aeration system
c. Baskets, pots or cups
d. Some type of growing media
e. Grow tray

The Reservoir contains the nutrient rich solution that is placed just below the grow tray. Grow tray have holes with baskets or net cups, plants are placed in these baskets or cups. The grow tray is placed in such a way so that the plant's roots are fully immersed in the nutrient solution directly into the reservoir.

The aeration system ensures the proper amount of oxygen in the nutrient solution so that the roots can get oxygen rich nutrient solution. Airstone or soaker hose is placed on the floor of nutrient solution and it connects with the air pump that is present outside the nutrient solution tank with the help of an air line. Air pump supplies oxygen to the airstone which creates bubbles in the nutrient solution. Aeration is important as it provides oxygen to the nutrient solution and so to the roots, and does not allow the nutrient rich solution to become stagnant.

Deep water culture is just the variation of water culture system, the whole procedure is same the only difference between deep water culture and water culture is that in deep water culture water depth in the system is deeper than 8 to 10 inches whereas in water culture the depth is less than 8 inches.

- **Ebb and flow system-**

Ebb and flow system is also known as flood and drain hydroponic system. It has the intermediate difficulty level with relatively low cost to set up. Basic components of of the ebb and flow system are-

a. Plant tray
b. Reservoir
c. Submersible pump with timer

a. Plant tray- plant tray is also called flood tray. It is large and shallow. It has perforated pots filled with growing medium such as perlite in which seedlings are transplanted. The pot in which the plants are grown should be placed in such a way that they get fully emerged when the tray is flooded with nutrient solution. Water is pumped from the reservoir to the flood tray till roots get completely immersed in nutrient solution after sometime water get drained back to the reservoir, allowing the roots to dry and oxygenated before the nutrient rich solution again floods the tray.

b. Reservoir- reservoir is placed just below the flood tray. It is connected with the flood tray by two tubes- fill tube and a drain tube. The fill tube is attached to the immersible pump that is placed in the nutrient solution in the reservoir, it controls the flow of water up to the flood tray. This immersible pump is attached to the timer to regulate the nutrient rich solution flow to the flood tray. The drain pipe is connected between the flood tray and the reservoir, the flooded solution drains back to the reservoir by the action of gravity from flood tray.

- **Drip System-**

In drip hydroponic system, the nutrient rich solution is provided to plant by the process of drip irrigation. This type of hydroponic system is very water efficient as it does not flood the nutrient rich solution to the grow tray, but provide the nutrient rich solution to the base of each plant individually by slow drip.

In drip system, a pump is placed in the nutrient solution reservoir, it is accompanied by a timer which controls the irrigation schedule. The pump helps to flow the nutrient rich solution to the grow tray. The pipe that carries the nutrient rich solution to the plants have various drip emitters that deliver nutrient solution to the roots of each plant.

There are two types of drip system in hydroponics-

a. Recovery system
b. Non recovery system

a. Recovery systems

Recovery system is the system in which the nutrient solution is recovered and recycled again and again through the system.

Recovery system is basically used in vertical gardens in which the nutrient solution flows from top to bottom and drains back into the nutrient tank. The same nutrient solution is recirculated to the plant. This saves and recycle nutrients, the only thing that should be monitored carefully is pH and TDS of the nutrient solution. One needs to adjust the pH level and replace the nutrient solution with the fresh batch periodically.

b. Non recovery system

In non recovery system the nutrient solution is not recycled but is drained out of the system. It sounds counterintuitive, but this system is more resource efficient than recovery system. It is the most preferred technique for commercial hydroponic system. In non recovery system the drip cycle needs to be very precise, so that each plant receives the right amount of nutrient solution that is required by it. It is highly accurate with cycle time and irrigation time. Each drip emitter provides just the right amount of nutrient solution to the individual plant so that the growing medium around each plant can be moistened, and plant utilises water and nutrients completely and nothing goes unused.

As the nutrient solution is not being recycled then there is no need to monitor pH and TDS again and again, simply one have to refill the reservoir with fresh nutrient solution.

- **Nutrient Film Technique (NFT)**

Nutrient film technique is similar to that of the ebb and flow system as it has same components but they have a different configuration.

NFT is the active system of hydroponics because it have moving parts in its working. NFT is similar to that of ebb and flow system the only difference between both of them is that in the ebb and flow system, there is of a flood and drain mechanism whereas in NFT nutrient solution flows through the grow tray continuously.

Generally, in other hydroponic systems plants are grown in net pots filled with growing media, places in grow tray. Whereas, in NFT system a dense mat is used on which plants are grown. The roots of the plants grow into the mat in the channel. Pump is used to deliver water from the reservoir to the grow tray. The grow tray is placed at an angle due to which excess nutrient solution drains back to the reservoir using drain tube. The roots of the plants hang from the mat and touches the nutrient flowing below them. The roots and not fully emerged in the following nutrient solution, but get water without being entirely immersed. Due to this shallow flow of nutrient solution roots can have access to oxygen in the air directly. Plants like strawberries, which have a short root system and are light weighted, I prefer for this hydroponic system.

- **Aeroponic system**

In aeroponic system, plants receive nutrients in the form of mist. In this system root are held in a soilless growing medium like Coco coir on the grow tray, on these roots nutrient rich solution is being sprayed by using specially designed misting devices. These misting devices are connected with the pump that flows nutrient rich solution from the reservoir to the misting devices. These pumps and devices are connected to a timer which insures spraying of nutrient rich solution periodically.

IV. ADVANTAGES OF HYDROPONICS

As in hydroponics there is no soil needed for the cultivation of crops, therefore plants in crops can be grown in places where the land is limited or is heavily contaminated. Hydroponics has been considered the 'farming of the future' by NASA, as crops can be grown in space where there is no soil for astronauts.

In hydroponics we can grow the number of plants in the small area. Traditional agriculture is done in soil and each plant it has to be planted at certain distances, so that they do not compete with each other for nutrients and water. Whereas in hydroponic each plant can be grown close to each other because they do not have to compete for nutrients and water as both are provided to them sufficiently. So we can plant, number of plants in the small area.

In hydroponic plantation the environment that provides for the growth of plants is manually controlled, whereas, traditional agriculture is completely relied on the natural environment and climate. Due to this the chances of crop failure are reduced. Various calamities like flood, drought, etc. Does not hamper the production. This increases the production and maximize the business profit.

As the climate is controlled artificially, seasonal fruits and vegetables can also be produced throughout the year. Without depending upon the seasons we can enjoy our favourite fruits and vegetables throughout the year.

In traditional agriculture irrigation requires large amounts of water. As the population increases water consumption is also increasing which is causing water scarcity all over the world. Plants grown in hydroponics uses 10% of water compared to the plants grown traditionally. In hydroponic water moves in a loop that means same water is recirculated. Water loss only occurs into form under hydroponic - evaporation and leaks from the system. In an efficient hydroponic system these leakages are minimized or stopped completely. Hydroponic require less amount of water and there is no water runoff or water wastage which also helps with water conservation.

In hydroponic, we have 100% control of the nutrients at plant needs. Before planting, plant growers can check about the requirements of particular crop and then can provide the plants with those specific nutrients in specific amount.

Since soil is not being used, so there are no chances of weeds. So there is no need of chemical fertilizers, weedicides, and pesticides. This helps in growing cleaner and healthier food.

V. HISTORY

Although hydroponic today involves tables, parts, pumps and high-tech lights for indoor gardens. It is nothing new. It is a technique that was used by the ancient Mayans and Babylonians.

In 600 BC, hydroponic principles were used in the creation of hanging gardens of Babylon. These gardens were built along the Euphrates River in Babylon. Since the climatic condition of Babylon was dry and rarely saw the rain, people believe that the ancient, people believe that the ancient Babylonian use a chain pull system for watering the plants. In this method the water was drawn from the river and cold on the top of the garden by using chain pull, water dropped to the steps or landing of the garden.

In 900- 1000 AD, there were floating gardens in the Aztecs. To fed their enormous population, the Aztecs indigenously built chinampas, or floating gardens, to convert the marshy wetlands of lake Texcoco into arable farmland. Each garden was 300 feet long by 30 feet wide. To make a garden, sticks were weaved together to form a giant raft, these rafts work covered with thick soil that was taken from the bottom of the lake. The layer of soil was of around 3 feet thick. These rectangular gardens were anchored to the lake by planting willow trees at each corner. Each garden was lined on all sides by canal to allow canoes so that work can easily reach each of the gardens. This network of gardens extended to 22,000 acres across the surface of the lake.

In late 1200s Marco Polo, the explorer noted in his writing that he saw floating gardens in China.

Around 1600 AD, Belgian Jan Van Helmont indicated with his experiment that plants can obtain nutrients from water, but somehow he failed to to explain that plants also need carbon dioxide and oxygen from air.

In 1699, John Woodward, a British scientist study the growth of plants using water culture. He found that plants can grow best in water with nutrients rather than in distilled water.

In 1930s, William Gerickle, a Berkeley scientist, demonstrate the benefits of soil less gardening. He was also credited for giving this a name 'Hydroponic'

In 1938, Two Berkeley scientists named Dennis Hoagland and Daniel Arnon, did an experiment and published "The Water Culture Method For Growing Plants Without Soil", this text is considered to be the most important text regarding hydroponics. They concluded in their research that the quality of crops grown in hydroponics I know better than the crops grown in quality soil. They developed three nutrient solutions that are still used today.

VI. CONCLUSION

Hydroponic farming has numerous advantages. Hydroponic is also considered as the future of farming. Products that are produced by hydroponics are full of nutrients as compared to the products that are produced by conventional farming. There are various types of hydroponic farming, which are adopted according to the plants that are grown. Hydroponics provides a nutrient solution directly to the roots of plants, plants don't have to spend their energy in the search of nutrient and water. Due to this, plants show their best genetic output. Fruits and vegetables that are produced in hydroponics high nutritive value and free from various chemical fertilizers and pesticides.

REFERENCES

[1] https://www.agritecture.com/blog/2019/3/7/soilless-agriculture-an-in-depth-overview
[2] https://www.greenandvibrant.com/advantages-disadvantages-of-hydroponics
[3] https://www.greenandvibrant.com/history-of-hydroponics
[4] http://www.soilless.org/hydroponics/soilless-farming-hydroponic-crop-cultivation/
[5] https://fourstringfarm.com/2014/04/01/the-floating-gardens-of-aztecs/
[6] https://hydrobuilder.com/learn/why-hydroponic-water-temperature-matters/
[7] https://www.greenandvibrant.com/grow-room-ventilation#:~:text=A%20grow%20room%20ventilation%20is,and%20the%20indoor%20grow%20area.&text=And%20circulation%20fans%20are%20used,intend%20to%20start%20with%20Hydroponics.
[8] https://www.evapoler.com/different-types-cooling-pad-materials/#:~:text=Cellulose%2DThese%20pads%20are%20made,because%20of%20the%20thick%20material.
[9] https://www.thespruce.com/hydroponic-gardens-wick-system-1939222#:~:text=Wick%20Systems%20are%20the%20most,incredibly%20easy%20to%20set%20up.&text=The%20difference%20is%20that%20a,submerged%20in%20the%20reservoir%20itself.
[10] https://thehydroponicsplanet.com/hydroponic-tanks-everything-you-need-to-know/
[11] https://hightimes.com/health/science/understanding-hydro-tds-measurements/
[12] https://generalhydroponics.com/ph-dynamics#:~:text=The%20ideal%20pH%20range%20for,is%20between%205.5%20and%206.5.
[13] http://www.homehydrosystems.com/hydroponic-systems/water-culture_systems.html
[14] https://www.thespruce.com/hydroponic-gardens-ebb-and-flow-systems-1939219
[15] https://smartgardenguide.com/drip-system-hydroponics/
[16] https://www.greenandvibrant.com/nutrient-film-technique
[17] https://www.greenandvibrant.com/seed-starting-hydroponics#:~:text=Like%20mature%20plants%2C%20seeds%20also,can%20hold%20air%20and%20water.
[18] https://www.cmac.com.au/blog/choosing-right-shade-cloth-for-your-plants
[19] https://modernfarmer.com/2018/07/how-does-aeroponics-work/

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Biosugar Production from *Kappaphycus alvarezii* by Hydrolysis Method using Fungi *Trichoderma harzianum*

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Abstract— *Kappaphycus alvarezii is one of the natural resources containing high carbohydrate, and it has potential as an alternative raw material for sugar industry. This study aims to analyze the optimum conditions of the fungi Trichoderma harzianum in degradating polysaccharides from K. alvarezii into sugar. The seaweed was collected in Takalar Sea, South Sulawesi, Indonesia. The fungi was previously activated at 30°C for three days in 2% of seaweed substrate. The efficiency of the T. harzianum in producing biosugar was evaluated under various concentrations of inoculum (5%, 10%, 15% and 20%) and hydrolysis time (0 h, 24 h, 48 h, and 72 h). The result implied that K. alvarezii contain 55,58% of carbohydrate which was dominated by starch 35.83% and cellulose 12.21%. Furthermore, the highest content of sugar indicated by 12,27 g/L of reducing sugar was obtained at the combination of 20% of the fungi and 24h incubation. In conclusion, the fungi T. harzianum has ability to convert carbohydrate of K.alvarezii to sugar through hydrolysis process.*

Keywords— ***seaweed; K. alvarezii; biosugar; T. harzianum; hydrolysis.***

I. INTRODUCTION

Sugar is one of the basic human needs consumed by all levels of society both for household and industrial needs. Indonesian sugar needs in 2018 reached 6.6 million tons while the production of sugar based sugarcane only 2.17 million tons (Ministry of Industry, 2018). This lead to a significant increase in sugar import. Nowadays, based on data from Statistical Central Agency in 2018, Indonesia became the largest sugar importer in the world, reaching 4.6 million tons (Katadata.co.id, 2018).

Sugarcane as the main raw material of sugar industry continues declining due to limited availability of cultivation land (IDN TIMES, 2019). Various raw alternatives of the sugar industry have been reported for instances cassava, corn, and sago containing high level of carbohydrates (Rahmawati *et al*., 2017; Mahyati *et al*., 2017). However, the availability of raw materials is limited because of its main function as staple foods. Therefore, it is necessary to search for other alternatives as raw materials which are rich carbohydrate based natural resources in abundant availability e.g. seaweeds.

Indonesia is the largest producer of seaweeds with a total production of 9,9 million tons in 2019 (kkp.go.id, 2020). This country has contributed almost 40% of the total world seaweed production (FAO, 2018). In general, seaweeds are widely used in food, engineering, and medicinal industries (S. W. Kim *et al*., 2015; Parenrengi & Sulaeman, 2007). South Sulawesi is one of the seaweed producing center, especially the red seaweeds *Kappaphycus alvarezii* and *Gracilaria sp* with a total production of 2,4 million tons in 2013 and increasing to 3,6 million tons in 2017 (Department of Marine and Fisheries South Sulawesi, 2018). The abundance of seaweed *K. alvarezii* (65% of the total production) becomes a potential alternative as raw material for sugar industry.

The constituent wall of seaweed cells consisted of agar, caragenan, cellulose, manan, and xylen can be converted into monosaccharides through various methods such as acid hydrolysis, enzymatic hydrolysis, hydrothermal, and fermentation (Meinita *et al*., 2015; Parenrengi & Sulaeman, 2007). Several related studies have been reported, the hydrolysis of carbohydrates using sulfuric acid besides yielding high sugar it also has side toxic compounds (Carvalho *et al*., 2013). Decomposition of carbohydrates with hydrothermal methods showed less results due to high temperature causing damage of carbohydrate structure (D. H. Kim *et al*., 2014). The enzymatic hydrolysis produced high oligosaccharide, environmental friendly, low side effects of toxic compounds but took relatively long time with high cost (Vanegas. 2015).

Acceleration of hydrolysis of seaweed carbohydrate using microbe which has enzyme activity is an effective alternative method. The potential microbe used in degradation of carbohydrates through the hydrolysis method is the fungi *Tricoderma harzianum* because its cellulotic properties (Lee *et al*., 2017; Jamil *et al*., 2009). The enzyme produced by *T. harzianum* is about 25% more efficient than *Trichoderma reesei* (Souza *et al*., 2018). In the present study, we investigated biosugar production from the seaweed *K. alvarezii* using the fungi *Tricoderma harzianum*.

II. MATERIALS AND METHODS

2.1 Seaweed Materials

The sample *K. alvarezii* was collected from the Brackish Aquaculture Fisheries Hall, Takalar. It was washed with freshwater to remove attached mud and salts, then dried for two days. The dried *seaweed* was grinded using the hummer mill to obtain powder with size of 40 mesh.

2.2 Culture of *Trichoderma Harzianum*

The fungi *T. harzianum* was subcultured in a petri dish containing a PDA (Potato Dextrose Agar) medium and incubated at 30°C for 48 h. The fungi culture was stored in a refrigerator before its further use.

2.3 Activation process of *Trichoderma Harzianum*

The Fungi *T. harzianum* was activated in three stages. (1) A small amount of sub cultured fungi was picked up and inoculated into 5 mL of seaweed media then shake incubated at 30°C for 24 h. (2) 1 mL of the inoculum was diluted in 9 mL of seaweed media then incubated at the same condition of the previous stage. (3) 5 mL of the inoculum was activated in 45 mL of the seaweed media.

2.4 Hydrolysis Process

The hydrolysis process use seaweed that has been through the pretreatment process. The seaweed flour is dissolved in aquades and the heating process for 120 min with a warming temperature of ± 100°C and then cooled to a temperature of ± 40°C. Then autoclave about ± 90 min. Selected parameters for optimization in experimental hydrolysis using inoculum *T. harzianum* with varying concentrations (5%, 10%, 15% and 20%) and the duration hydrolysis (0 h, 24 h, 48 h and 72 h). After the reaction, the supernatant were separated from liquid by centrifugation at a speed of 9.000 RPM for 10 min. All preparation were performed at the Marine Microbiology Laboratory, Faculty of Marine Science and Fisheries, Hasanuddin University. The supernatant was collected to measured from the sugar level by the phenol sulfate acid.

2.5 Data Analysis

Data was analyzed by randomized design completed with factorial patterns. Total sugar was analyzed by two ways ANOVA to determine the influence of inoculum concentration and duration of hydrolysis on sugar production. If there was any influence, then proceed with the Tuckey test at a confidence level of 95% ($\alpha = 0.05$) to identify the optimum combination indicated by the highest amount of sugar.

III. RESULTS AND DISCUSSION

3.1 Analysis of carbohydrate content of seaweed *K. alvarezii*

Carbohydrate content of the seaweed *K. alvarezii* was evaluated and obtained that it was about 55,58% dominated by starch and cellulose which were 35,83% and 12,21%, respectively. Carbohydrates are also called polysaccharides which are combination of monosaccharides linked by the glycoside bonding, for instance polysaccharides are cellulose, glycogen, and starch.

Starch and cellulose are the main carbohydrates of *K. alvarezii*, other components are hemicellulose, pectin and lignin. Starch resulted in photosynthesis pathway of plants is deposited as energy for growing. It consists of amylose and amylopectin in different composition.

The seaweed *K. alvarezii* possesses starch higher than other seaweeds. This is supported by previous studies that *Caulerpa lentillifera* and *Ulva Ohnoi* contained starch of 29.82% and 21.4% (Tapotubun, 2018; Prabhu *et al*., 2019).

On the other hand, cellulose is the main polysaccharides of seaweed. Cellulose is one of the most abundant biopolymers in nature and the major component of plant cell wall (Börjesson & Westman, 2015). It associates with other polysaccharides such as hemiselulose or lignin (Holtzapple *et al*., 2003). Thallus of seaweed also has cell wall consisting of cellulose (Linder & Teeri, 1996). This study showed that *K. alvarezii* contained cellulose of 12.21%. It is supported by previous research on the red algae *Gracilaria* reported by Sari *et al*. (2013)

showing cellulose content of 20,17%. In addition, Milala *et al*. (2005) described cellulose content in plants reaching 40-50% of plant mass. High polysaccharides on the seaweed *K. alvarezii* can be converted into mono sugar by hydrolysis method.

3.2 Analysis of the effect of inoculum concentration on total sugar

Pretreated seaweed was hydrolyzed using activated inoculum *T. harzianum* with various concentrations of 5%, 10%, 15% and 20%, and hydrolysis time 0 h, 24 h, 48 h and 72 h. Samples were analyzed to determine the total sugar produced. Based on Two Way ANOVA and Tuckey test showed that the total sugar significant difference from each treatment ($p<0,05$). Figure 1. shows total sugar produced by every single treatment combination.

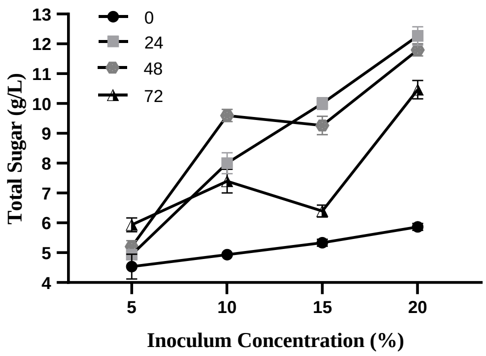

Fig.1: Total sugar from various inoculum concentration.

The result showed that generally, conversion of polysaccharide to total sugar increases due to increased inoculum concentration and hydrolysis time. The tendency is shown by the treatment of hydrolysis time 48 and 72 which increased to 10% inoculum concentration then decreased at a concentration of 15% and increased at the next concentration. Hydrolysis for 24 h showed an increase in sugar production with increasing inoculum concentration. The interesting from the results of this study is the control at the 0 h also shows the increase in total sugar with increasing inoculum concentration. Total sugar detected is sugar produced during the activation process of *T. harzianum*. Thus, the calculation of the total sugar produced must be corrected with the sugar content at the 0 h for all concentrations. The addition of 20% inoculum resulted in relatively equal total sugar after hydrolysis for 24 and 48 hours.

The hydrolysis process of carbohydrates into total sugars is carried out using the fungi *T.harzianum* with several parameters of concentration and duration of hydrolysis. Hydrolysis is a chemical reaction that splits a molecule into two parts by adding water molecules with the aim of converting polysaccharides into monosakarida. Cellulose is the main element of seaweed cell walls that can be degraded into monosugar by the hydrolysis process. Under normal conditions, only a few reactions occur during the hydrolysis process, therefore it is necessary to have a catalyst that can accelerate reactions such as the addition of acids, alkali or enzymes. Enzymes can work 10^8 to 10^{11} times faster than the rate of reaction without a catalyst (Poedjadi, 2006).

T.harzianum produces cellulase enzymes that can degrade cellulotic substrates into sugar (Jamil *et al*., 2009). The use of microbes in hydrolysis provides the advantage of a fast process and does not produce toxic compounds. Souza *et al*., 2018 stated that β-glucosidase produced by *T. harzianum* strains was higher than *T. reesei* by using lactose as a carbon source.

The results indicated that the inoculum concentration and duration of hydrolysis have the significant effect on reducing sugars and there were significant interactions between the two treatments. The highest reducing sugar was 12,2g/L obtained from an inoculum concentration of 20% and a hydrolysis duration of 24 h. Moreover, the lowest reducing sugar is at 5% inoculum concentration treatment and 0 h hydrolysis time. Figure 1. shows the tendency to total sugar levels increases with increasing inoculum concentration. This research indicated that the activity of *T. harzianum* in hydrolyzing cellulose to sugar increases with increasing inoculum concentration. However, high cellulose content in seaweed is a source of carbon needed to obtain energy for the growth of *T. harzianum* in producing cellulase enzymes and degrading cellulose into monosugars. Ul-Haq *et al*., 2005 stated *Trichoderma sp*. is a producer of cellulases and crude enzymes. *T. harzianum* is a potential fungus in producing cellulase enzymes that can hydrolyze chain β-glucosidase (cellobiase) that break down cellobiose to produce glucose (Wang *et al*., 2020). The fungi also produces higher levels of cellulolytic activity and β-glucosidase activity compared to *T. reesei* (Souza *et al*., 2018).

The carbohydrates contained in K. Alvarezii are applicative media for the *Trichoderma* growth. High cellulose and starch elements in the substance can be potential source of nutrients for fungal growth. The 20 percent concentration of inoculum seaweed into the media can produce high cellulase enzymes for the degradation of carbohydrates into monosaccharides. Brijwani *et al*. (2010) stated that *T. Harzianum* has ability to produce enzymes by the growth medium consisting of nitrogen and carbon. The results of the research of Yong Syuan *et al*. (2018) shows rice straw as substrate of *T. Harzianum* potentially produce cellulase and xylanase. Higher substrate concentrations indicate the number of molecular substrates involved with enzyme activity. Morever, low substrate concentrations mean fewer substrate molecules that can be attached to the enzyme causing reduced enzyme activity.

3.3 Analysis of the effect of hydrolysis time on total sugar

The hydrolysis time affects the total sugar produced. This is related to the ability of the fungi *T.harzianum* in degrading carbohydrates into monosugar. Based on the Tuckey test, it showed the significant difference between treatments (P <0.05).

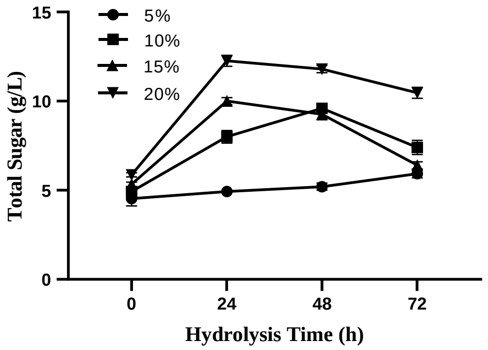

Fig.2: Total sugar from various hydrolysis time

The hydrolysis time affects the total sugar produced. This is related to the activity of *T. harzianum* which able to produce cellulase enzymes to degrade carbohydrates. The treatments with concentrations of 15 and 20% showing a significant increase in the incubation time of 24 h and decreasing in the following hours (Figure 2). This shows that the cellulase enzyme produced by *T. harzianum* is only able to work for a maximum of 24 h. In the treatment of 48 h and 72 h the enzyme activity decreases as indicated by a decrease in sugar levels.

To increase reducing sugar levels from 0 h to 24 h shows the activity of *T.harzianum* in producing cellulose enzymes to degrade carbohydrates from *K. alvarezii* seaweed. Treatment with an inoculum concentration of 20% is an excellent source of nutrition for *T. harzianum* to produce enzymes. At the beginning of the hydrolysis process, there are many sources of nutrients for *T. harzianum* available. After 24 h, growth nutrients for *T. harzianum* are reduced because they have been used in producing enzymes to degrade seaweed carbohydrates. This causes the concentration of the enzyme to become unbalanced, causing the enzyme performance to also decrease.

Treatment with an inoculum of 20% concentration after 24 h showed the amount of reducing sugar produced from the hydrolysis process decreased. This is caused by the accumulation of the final product of the hydrolysis process which inhibits the performance of the cellulase enzyme (Binod *et al*., 2019). Glucose and ethanol are the end products that can inhibit cellulase performance (Chen & Jin, 2006). At constant seaweed substrate concentrations, the rate of enzymatic reaction increases with increasing enzyme concentration. This indicated that the more enzymes to a certain extent, the more substrates are converted because the higher the enzyme activity. In addition, enzymes are specific to the substrate, excessive concentrations will also affect the rate of the enzymatic reaction.

IV. CONCLUSIONS

This research has provided information about the potential of seaweed which used as raw material for producing sugar. Previous studies have shown in sugar production from seaweed uses several methods. However, this study is the first method using *T. harzianum* in degrading polysaccharides into monosugar. The finding of this research can demonstrated the ability of *T.harzianum* to convert carbohydrate from the *K. alvarezii* to biosugar production through the hydrolysis process.

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REFERENCES

[1] [FAO] 2018. The Global Status of Seaweed Production, Trade and Utilization Vol. 124. Roma.
[2] [Departement of Marine and Fisheries South Sulawesi] 2018. Seaweed production data of South Sulawesi Province.
[3] Binod, P., Sindhu, R., Janu, K. U., & Pandey, A. (2019). Hydrolysis of cellulosic and hemicellulosic biomass. *Biomass, Biofuels, Biochemicals: Biofuels: Alternative Feedstocks and Conversion Processes for the Production of Liquid and Gaseous Biofuels*, 447–460. https://doi.org/10.1016/B978-0-12-816856-1.00019-1
[4] Börjesson, M., & Westman, G. (2015). Crystalline Nanocellulose — Preparation, Modification, and Properties. *Cellulose - Fundamental Aspects and Current Trends*. https://doi.org/10.5772/61899
[5] Brijwani, K., Oberoi, H. S., & Vadlani, P. V. (2010). Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. *Process Biochemistry*, *45*(1), 120–128. https://doi.org/10.1016/j.procbio.2009.08.015

[6] Carvalho, A. F. A., Neto, P. de O., da Silva, D. F., & Pastore, G. M. (2013). Xylo-oligosaccharides from lignocellulosic materials: Chemical structure, health benefits and production by chemical and enzymatic hydrolysis. *Food Research International*, *51*(1), 75–85. https://doi.org/10.1016/j.foodres.2012.11.021

[7] Chen, H., & Jin, S. (2006). Effect of ethanol and yeast on cellulase activity and hydrolysis of crystalline cellulose. *Enzyme and Microbial Technology*, *39*(7), 1430–1432. https://doi.org/10.1016/j.enzmictec.2006.03.027

[8] IDN TIMES. (2019). [*Sugarcane Farmers' Productivity Decreases, Ministry of Agriculture Targets 3 Million Tons of Sugar*]. https://www.idntimes.com/business/economy/indianamalia/produktivitas-petani-tebu-menurun-kementan-targetkan-3-juta-ton-gula/4

[9] Jamil, A., Ahmed, S., & Al, E. T. (2009). Production and Purification of Cellulose- Degrading Enzymes From a Filamentous Fungus. *Pak. J. Bot.*, *41*(3), 1411–1419.

[10] katadata.co.id. (2018). [*Indonesia's Largest Sugar Importers in the World 2017/2018*]. *Im*, 1–7. https://databoks.katadata.co.id/datapublish/2019/01/09/indonesia-importir-gula-terbesar-dunia-20172018

[11] Kim, D. H., Lee, S. B., & Jeong, G. T. (2014). Production of reducing sugar from Enteromorpha intestinalis by hydrothermal and enzymatic hydrolysis. *Bioresource Technology*, *161*, 348–353. https://doi.org/10.1016/j.biortech.2014.03.078

[12] Kim, S. W., Hong, C. H., Jeon, S. W., & Shin, H. J. (2015). High-yield production of biosugars from Gracilaria verrucosa by acid and enzymatic hydrolysis processes. *Bioresource Technology*, *196*, 634–641. https://doi.org/10.1016/j.biortech.2015.08.016

[13] kkp.go.id. (2020). *Siaran pers*. [To Boost Export Value, KKP Targets 10.99 Million Tons of Seaweed Production in 2020]. [Internet]; cited 29 Januari 2020; Available from: https://doi.org/10.1111/1540-5885.1110069

[14] Lee, H., Min, Y., Mok, Y., Lee, J., Seok, J., Young, K., & Kim, J. (2017). Industrial Crops & Products Utilization of agricultural residues for enhancement of cellulolytic enzyme production and enzymatic sacchari fi cation by Trichoderma harzianum. *Industrial Crops & Products*, *109*(April), 185–191. https://doi.org/10.1016/j.indcrop.2017.08.042

[15] Linder, M., & Teeri, T. T. (1996). The cellulose-binding domain of the major cellobiohydrolase of Trichoderma reesei exhibits true reversibility and a high exchange rate on crystalline cellulose. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(22), 12251–12255. https://doi.org/10.1073/pnas.93.22.12251

[16] Meinita, M. D. N., Marhaeni, B., Winanto, T., Setyaningsih, D., & Hong, Y. K. (2015). Catalytic efficiency of sulfuric and hydrochloric acids for the hydrolysis of Gelidium latifolium (Gelidiales, Rhodophyta) in bioethanol production. *Journal of Industrial and Engineering Chemistry*, *27*, 108–114. https://doi.org/10.1016/j.jiec.2014.12.024

[17] Milala, M., Shugaba, A., Gidado, A., Ene, A., & Wafar, J. (2005). Studies on the Use of Agricultural Wastes for Cellulase Enzyme Production by Aspegillus niger. *Research Journal of Agriculture and Biological Sciences*, *1*(4), 325–328.

[18] Ministry of Industry. (2018). [*Sugar Industry Boosted.* 18 Maret 2019. [Internet] cited : 10 Oktober 2020]; Available from: https://kemenperin.go.id/artikel/20447/Industri-Gula-Digenjot

[19] Parenrengi, A., & Sulaeman. (2007). [Get to know Seaweed, *Kappaphycus alvarezii*]. In *Media Akuakultur* (Vol. 2, Issue 1, pp. 142–146).

[20] Prabhu, M., Chemodanov, A., Gottlieb, R., Kazir, M., Nahor, O., Gozin, M., Israel, A., Livney, Y. D., & Golberg, A. (2019). Starch from the sea: The green macroalga Ulva ohnoi as a potential source for sustainable starch production in the marine biorefinery. *Algal Research*, *37*(November 2018), 215–227. https://doi.org/10.1016/j.algal.2018.11.007

[21] Sari, R. N., Sugiyono, S., & Assadad, L. (2013). [Optimization of Hydrolysis and Fermentation Process Time in Bioethanol Production from Industrial Agar (*Gracilaria sp*.) waste]. *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*, *8*(2), 133. https://doi.org/10.15578/jpbkp.v8i2.57

[22] Souza, M. F. de, Silva, A. S. A. da, & Bon, E. P. S. (2018). A novel Trichoderma harzianum strain from the Amazon Forest with high cellulolytic capacity. *Biocatalysis and Agricultural Biotechnology*, *14*(October 2017), 183–188. https://doi.org/10.1016/j.bcab.2018.03.008

[23] Tapotubun, A. M. (2018). [Chemical Composition of Seaweed (Caulerpa lentillifera) from Maluku Kei Waters with Different Drying Methods]. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *21*(1), 13. https://doi.org/10.17844/jphpi.v21i1.21257

[24] Ul-Haq, I., Javed, M. M., Khan, T. S., & Siddiq, Z. (2005). Cotton Saccharifying Activity of Cellulases Produced by Co-culture of Aspergillus niger and Trichoderma viride. *Journal of Agriculture and Biological Sciences*, *1*(3), 241–245.

[25] Wang, H., Zhai, L., & Geng, A. (2020). Enhanced cellulase and reducing sugar production by a new mutant strain Trichoderma harzianum EUA20. *Journal of Bioscience and Bioengineering*, *129*(2), 242–249. https://doi.org/10.1016/j.jbiosc.2019.08.016

[26] Yong Syuan, K., Ong Gaik Ai, L., & Kim Suan, T. (2018). Evaluation of cellulase and xylanase production from Trichoderma harzianum using acid-treated rice straw as solid substrate. *Materials Today: Proceedings*, *5*(10), 22109–22117. https://doi.org/10.1016/j.matpr.2018.07.077

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Effects of Climate Change Adaptation Strategies on Technical Efficiency of Poultry Production in Benue State, Nigeria

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Abstract— *This study determined the effects of climate change adaptation strategies on technical efficiency of poultry production in Benue State, Nigeria. The population of the study consist of all poultry farmers in Benue State. A sample size of 198 was selected using multistage sampling technique. Data for the study were collected from primary source using a structured questionnaire. Data were analyzed using descriptive statistics such as frequencies distribution, percentages and mean as well as inferential statistics such as Stochastic frontier production function. Farmers' perceived intensity of climate change as very high temperature, excessive rainfall, drought, irregular relative humidity, excessive sunshine and long period of harmattan with almost all variables exceeding the cut-off mean of 2.0. Majority of the respondents used adaptation strategies to cope with climate change. The result of the stochastic frontier analysis showing the climate change adaptation strategies influencing inefficiency of poultry output showed that, raising of broods and sell, tree planting around poultry house, more water served were found to reduce farmers' economic inefficiency of poultry output at 10% level of significance. The study hence, revealed that climate change adaptation strategies had significant relationship with economic inefficiency of poultry production in the study area. Therefore, the study recommended the need to improve on farmers' access to information on climate change and appropriate adaptation strategies as well as providing credit facilities to help improve farmers' capacity to adapt to climate change.*

Keywords— ***Climate change, Adaptation strategies, Technical efficiency and Poultry production.***

I. INTRODUCTION

Agriculture and climate are mutually dependent. Agriculture both contributes to climate change and is affected by climate change. Agricultural activities including indirect effects through deforestation and other forms of land conversion account for about one third of total global warming potential from greenhouse gas (GHG) emissions today (IPCC, 2001a). As a result of large-scale activities, inadequate management and improper implementation, agriculture is a significant contributor to land and water degradation, and in particular a major emitter of greenhouse gases (Intergovernmental Panel on Climate Change IPCC, 2007a).The issue of climate change has become more threatening not only to the sustainable development of socio-economic and agricultural activities of any nation but to the totality of human existence (Adejuwon 2004).

Climate change, refers to a change in the state of the climate that can be identified (e.g using statistical test) by changes in the variability of its properties, and that persist for an extended period, typically decades or longer (IPCC,2007b). Climate change causes variations in weather conditions which in turn has impact on agricultural productivity. Increase in temperature causes excessive vaporization leading to poor agricultural productivity.

One of the most urgent problems of the second millennium is the changing climate of the universe. Unfortunately, a lot of countries including Nigeria are already living with the results of this global problem. Climate change, which is largely a result of burning fossil fuels, is already affecting the Earth's temperature, precipitation, and hydrological cycles. Continued changes in the frequency and intensity of precipitation, heat waves, and other extreme events have impact on agricultural production. Incidences of food crisis arises from a combination of factors, reduced productivity arising from lower yield is suspected to be exacerbated by climate change and related events (Nnaji, 2001; Onyenechere and Igbozurike, 2008).Climate change is a serious environmental threat to farmers and it worsens poverty because of its impact on agricultural productivity. Almost all sectors of agriculture depends on whether and climate whose variability have meant that rural farmers who implement their regular annual farm business plans, encounter total failure due to climate change effects. (Ozor, Madukwe, Enete, Amaechina, Onokala, Eboh, Ujah and Garforth, (2010). However, livestock production is likely to be adversely affected by climate change (Thornton, 2010). Thus, having a resultant effect on poultry production.

Poultry production is the process of raising domesticated birds for the purpose of producing meat and eggs for food. Poultry are birds that include fowl, turkey, duck, goose, ostrich, guinea fowl, etc which render not only economic services but contribute significantly to human food as a primary supplier of meat, egg, raw materials to industries (feathers, waste products), good source of animal protein, source of income and employment to people compared to other domestic animals (Demeke, 2004).The effects of climate change on poultry production have called for the need to adopt adaptation strategies to cope with its harmful effects.

Adaptation is the adjustments which moderates harm or exploit beneficial opportunities in response to actual or expected climate stimuli or their effects (IPCC, 2007b). Livestock genetic diversity and climate change adaptation, reveals that adaptation strategies address not only the tolerance of livestock to heat, but also their ability to survive, grow and reproduce in conditions of poor nutrition, parasites and diseases(Hoffmann, 2008). Efficiency in itself is concerned with relative performance of the processes used in transforming a set of inputs into output. The concept of efficiency has been interpreted in many forms or ways. These are technical (or physical) and allocative (or price) efficiency. Technical efficiency is the ratio of total output to total input. Chavanapoonphol *et al.* (2005) described technical efficiency of an individual farmer as the ratio of observed output to its corresponding stochastic frontier output, given the levels of the inputs used by the farmer.

Though a few studies have been conducted to assess the impact of climate change on poultry production in some states in Nigeria, there is a dearth of literature on this impact in Benue state, Nigeria. The knowledge of farmers on effects of climate change adaptation strategies will enable farmers to overcome the threats climate change poses on agricultural productivity.To address this gap, this study was designed to determine the effects of climate change adaptation strategies on technical efficiency of poultry production in Benue state, Nigeria.

The specific objectives are to:

1. assess the perception of poultry farmers on intensity of climate change;
2. identify adaptation strategies of poultry farmers to climate change in the study area;
3. determine the effect of climate change adaptation strategies on technical efficiency of poultry production.

II. METHODOLOGY

The study area for this research is Benue state. Benue State is one of the 36 states in Nigeria. The State derives its name from River Benue and lies in the middle belt region of Nigeria. Its geographical coordinates are longitude 7º 47' 0"and 10º 0' 0"East and Latitude 6º 25' 0" and 8º 08' 0" North (NPC 2006). Benue State has the total area of about 30955 km^2 and is divided into 23 Local Government Areas with the Headquarter in Makurdi.

A multistage sampling technique was used to select a sample size of 198 respondent for the study. In the first stage population of the study was stratified into two agricultural zones based on the existing zones in Benue state. The second stage involved a purposive selection of two local government area from each agricultural zones due to high level of poultry production in these area. Thirdly, three communities each were randomly selected from each local government area making a total of twelve communities. Fourthly, a sampling frame was developed for each of communities using a proportion of 30% (0.3) across board. A total sample size of 198 respondent was selected (table 1). But only190 copies of the questionnaires were returned from respondents and analyzed for the study.

The instrument of data collection used was a set of structured questionnaire that covered all the information to analyze the research objectives. The type of reliability test that was used is the test retest method. This was carried out among 20 respondents. Pearson product moment correlation was use to obtain a correlation coefficient (r)of 0.99, which implies that the instrument was reliable.

Model specification

Descriptive statistics was used to analyze objective I and II whileStochastic Frontier Production Function was used to analyze objective III

Stochastic Frontier Production Function

Stochastic frontier production function was used to estimate the Maximum Likelihood Estimates of parameters in Cobb-Douglas stochastic production function for the effects of climate change adaptation strategies on the technical efficiency of poultry production.

The model is specified as:

$lnY = \beta_0 + \beta_1 lnX_1 + \beta_2 lnX_2 + \beta_3 lnX_3 + \beta_4 lnX_4 + \beta_5 lnX_5 + \beta_6 lnX_6 + \beta_7 lnX_7 + \beta_8 lnX_8 + \beta_9 lnX_9 + (v_i - u_i)$

$v_i \sim N(0,\sigma^2 v)$

Where:

β = parameters estimates. Σ is the sign of summation.

Y = the value of poultry output in kilograms;

X1=the total labour used in poultry production in man days;

X2=the total farm size (poultry house) used for poultry production in metres;

X3= the total cost of feed used for poultry production in naira;

X_4= the total capital used for poultry production in naira;

X5= the total cost of drugs and vaccine used for poultry production in naira;

X_6= total cost of litter used for poultry production in naira;

X_7=total cost of equipment used for poultry production in naira;

X_8= total cost of stock (chicks) in naira;

Vi= are random variables which are assumed to be independent of *Ui*, identical and normally

distributed with zero mean and constant variance.

Ui= which are non-negative random variables which are assumed to account for technical

inefficiency in production and are often assumed to be independent of *Vi* such that *U* is

the non-negative truncated (at zero) *U* of half normal distribution with |N (0, σ2v)|.

The inefficiency of production, *Ui* is modeled in terms of the climate change adaptation strategies that are assumed to affect the efficiency of poultry production by poultry farmers.

The Technical Inefficiency Effects Model:

The technical inefficiency effect, μ_i is defined as:

$\mu i = \delta_0 + \delta_1 I_1 + \delta_2 I_2 + \delta_3 I_3 + \delta_4 I_4 + \delta_5 I_5 + \delta_6 I_6$

μ_i= inefficiency effect,

I_1 = Keeping of resistant breeds

I_2 = Prompt and extra vaccination of birds

I_3 = Raising of brood and sell

I_4 = Tree planting around poultry house

I_5 = more space per bird

I_6 = more water served

I_7 = Better hygiene

$\delta_0$andδ_i = coefficients (unknown parameters to be estimated along with the variance parametersδ^2 and γ. The varience of the random errors, δ_v^2 and that ofthe technical inefficiency effectsδ_μ^2 and the overall variance of the model are related;

δ^2=$\delta_v^2 + \delta_\mu^2$.The δ^2indicates the goodness of fit and the correction of the distributional form assumed for the composite error term.

The ratio $\gamma = \delta_u^2 / \delta_v^2$ measures the total variation of output from the frontier which can be attributed to technical inefficiency. The estimates of the parameters of the stochastic frontier production function and the inefficiency model will be obtained simultaneously using the program frontier version 4.1

The technical efficiency is defined in terms of the ratio of observed output (Y_i) to the corresponding frontier output (Y_i) conditioned on the level of input used by the farmers. Hence the technical efficiency (TE_i) of the poultry farmers will be expressed as:

$TE_i = y_i / y_i^*$ =f (X_i,B) exp (V_i-μ_i)/f(Xi;β) exp V=exp (-μi)

Where

Y_i = Observed output

Y_i^* = Frontier output

TE_i = Ranges between 1 and 0

III. RESULTS AND DISCUSSION

Perception of Poultry Farmers on Intensity of Climate Change

Table 1 showed the distribution of the average scores of respondents on perception of poultry farmers on intensity of climate change in the study area. Majority of the farmers in the study area perceived intensity of climate change as very high temperature (Mean=3.3), excessive rainfall (Mean=2.2), drought (Mean=2.04), irregular relative humidity (Mean=2.1, excessive sunshine (Mean=3.0), wind effect (Mean=1.8), and long period of harmattan; with each variable exceeding the cut-off mean score of 2.0 except wind effect which had a mean of 1.8. This implies that the respondents have perceived evidence that climate has changed and therefore will be willing to adopt adaptation strategies related to climate change in other to reduce its effects on poultry production. This agrees with the findings of Chah, Odo, Asadu and Enwelu (2013) that excessive sunshine (90.0%), excessive rainfall (80.0%), short period of hamattan (75.5%) and increased incidence of drought (66.7%) were seen by respondents as evidence of climate change in Enugu state.

This corroborates with the findings of Adesiji etal (2013) that 78.4% of the respondents all agreed that temperature fluctuate and 98.8% observed increased sunshine intensity in Ondo state. This is also similar to the opinion of Gueye, (2003) who reported that climate changes in form of drought, temperature variability, too much sunshine and windstorm have negative effects on agricultural productivity especially on poultry production, corroborating with Yahaya, (2009) who stated that the unusual weather change which brings about rain in different parts of Nigeria in January is an indication of serious negative effects of climate change.

Table 1: Perception of Poultry Farmers on Intensity of Climate Change

Variables	Non applicable	Very high	Mean	Std. Deviation
High temperature	0	4	3.31*	0.77
Excessive rainfall	0	4	2.19*	0.82
Drought	0	4	2.04*	1.08
Irregular Relative Humidity	0	4	2.13*	0.92
Excessive sunshine	0	4	3.01*	0.85
Wind effect	0	4	1.78	0.94
Long harmattan	0	4	2.14*	0.94

Source: Field Survey, 2018

Climate Change Adaptation Strategies used by Poultry Farmers in the Study Area

Findings in table 2 showed that 97.4% of the respondents agreed they were aware of climate change with 85.8% of them having less than 7 years of awareness. This is to say that, majority of the poultry farmers in the study area have noticed variation in climatic elements. Hence they perceived climate change in various ways.

Majority of the respondents (75.8%) used better hygiene as climate change adaptation strategy since dirty environment can aid breeding of disease causal organisms. This implies that farmers used better hygiene to reduce the growth and spread of infection caused by fluctuations in climatic factors. More space per bird was used by 73.7% of the respondents as a coping strategy. This implies that farmers ensure there is enough space in the poultry house for birds to freely move about to enhance ventilation and prevent heat. This agrees with the findings of Alade and Ademola (2013) that giving more spacing per average bird will prevent generation of heat from birds. 72.1% of the respondents served more water as a coping strategy to climate change. This is because birds tend to drink more water when the temperature is high to conserve heat.

Prompt and extra vaccination of birds was used by 68.4% of the respondents to build the immune system of birds to resist and reduce the effect of diseases on birds. Keeping of resistant breeds was used by 63.2% of respondent as a coping strategy to withstand the effect of climate change to an extent. About 59.5% of respondents used regular practice of routine management as a coping strategy to climate change to ensure that birds are well attended to and also to reduce the effect of climate change on birds. About 57.9% of respondents installed cooling equipment to reduce the heat in their poultry houses. Tree planting around poultry house was used by 55.8% of respondents to provide ventilation in their poultry houses and greatly reduce heat wave caused by high temperature and excessive sunshine. About 55.3% of respondents used keeping of early maturing birds as a coping strategy to climate change.

Table 2: Adaptation Strategies of Poultry Farmers to Climate Change in the Study Area (n = 190)

Variables	**Frequencies**	**Percentage**
Climate change awareness		
Yes	185	97.4
No	5	2.6
Years of climate change		
Awareness		
≤ 6	163	85.8
7 – 12	21	11.1
13 – 18	3	1.6
≥ 19	3	1.6
Adaptation strategy		
Keeping of resistant and improved breeds	120	63.2
Installing cooling equipment	110	57.9
Keeping of early maturing birds	105	55.3
Extension management services	17	8.9
Keeping birds varieties	44	23.3
Prompt and extra vaccination of birds	130	68.4
Raising of broods and sell	42	22.1
Tree planting around poultry house	106	55.8
More space per bird	140	73.7
More water served	137	72.1
Better hygiene	144	75.8
Regular practice of routine management	113	59.5
Others	43	22.6

*Multiple responses

Source: Field Survey, 2018

Maximum Likelihood Estimates of Parameters in the Stochastic Frontier Analysis for Effects of Climate Change Adaptation Strategies on Technical Efficiency of Poultry Production.

The analysis of the data for the technical efficiency estimates was achieved through the Maximum Likelihood Estimation (MLE) which involved the estimation of stochastic frontier model with inefficiency effects. The maximum likelihood estimates of parameters in stochastic frontier function are presented in table 3 below. The elasticity parameters are contained in the upper segment of the table while the determinants of inefficiency are also contained in the lower segment of the table. The sigma square (1.23) is significant at 5% level which implied that the stochastic frontier production model was the model that best fit the data. The significance of the estimates of gamma () (0.93) at 1% showed that the inefficiency effects jointly estimated with the production frontier function were not simply random errors. This implied that climate change adaptation strategies as well as farm and farmer specific characteristics had significant influence on the efficiency of poultry production rejecting the null hypothesis that climate change adaptation strategies have no significant influence on technical efficiency of poultry production.

The γ- parameter shows the relative magnitude of the variance in output associated with

Technical efficiency. The coefficients of the variables derived from the Maximum Likelihood

Estimation (MLE) are very important for discussing results of the analysis of the data. These

Coefficients represent percentage change in the dependent variables as a result of percentage

change in the independent (or explanatory) variables. The coefficients presented in the upper segment of the table showed that, the parameter of chick was positive (0.29) and significant at 1%. This implied that a 100 percent increase in the number of chicks increased the value of revenue in poultry production by 29 percent. The coefficient of feed (0.68) was also positive and significantly related to poultry revenue value at 1%. The result meant that a 100% increase in quantity of feed increased poultry revenue value by 68 percent.

Climate change, farm and farmer specific variables influencing inefficiency of poultry production are contained in the inefficiency model of the lower section of Table 6. The following variables, raising of broods and sell, tree planting around poultry house, more water served had negative and significant relationship on economic inefficiency, while better hygiene had a positive and significant relationship with economic inefficiency. The climate change adaptation strategies, farm and farmer specific variables that had significant relationship with economic inefficiency were discussed below:

Raising of brood and sell was found to have a negative (-3.26) and significant relationship with farmers inefficiency in the study area. This implied that increase in raising of brood and sell would lead to reducing farmers' economic inefficiency in poultry production. This is expected because younger birds experience less heat stress and less effect of climate change hence reducing loss and increasing revenue in poultry production. The use of tree planting around poultry house was negative (-1.36) and significant to inefficiency. This implied that increasing the practice of tree planting around poultry house reduced inefficiency in the value of poultry output. Tree planting around poultry house provided ventilation and shade and also reduced the intensity of heat in poultry houses. It increased the value of poultry output by reducing the death rate of birds and other effects of climate change. More water served had a negative (-1.37) and significant relationship with farmers inefficiency in the study area. This implied that increase in the practice of serving more water would lead to reducing farmers' economic inefficiency in poultry production. This is because birds take in more water to conserve heat and reduce effects of heat on birds thereby increasing the value of poultry output. The use of better hygiene was found to have a positive (2.02) and significant relationship with farmers' inefficiency in the study area. This implied that increase in the use of better hygiene increased inefficiency in poultry production. This was not expected because better hygiene is supposed to reduce the spread of diseases and death rate.

Table 3: Maximum Likelihood Estimates of Parameters in the Stochastic Frontier Analysis for Effects of Climate Change Adaptation Strategies on Technical Efficiency of Poultry Production in Benue State

Independent variables	Coefficient	t-ratio
Constant	1.45	4.37
Chick	0.29	4.71***
Feed	0.68	10.35***
Labour	-0.002	-0.26

Fumigation	0.01	0.98
Water	0.0003	0.03
Miscellinous	0.06	1.31
Technical Inefficiency Model		
Constant	-6.18	-1.57
Farm size space	0.35	1.22
Keeping of resistant breeds	0.28	0.61
Prompt and extra vaccination of birds	0.71	1.12
Raising of broods and sell	-3.26	-1.75*
Tree planting around poultry house	-1.36	-1.74*
More space per bird	0.26	0.51
More water served	-1.37	-1.51*
Better hygiene	2.02	1.90*
Diagnostic statistics		
Sigma squared	1.23	2.43
Gamma	0.93	30.44
Likelihood function	-80.71	

*, *** = significant at 10% and 1% level respectively

Source: field survey, 2018

IV. CONCLUSION

The result on perception showed that majority of the farmers in the study area perceived intensity of climate change as very high temperature (Mean=3.3), excessive rainfall (Mean=2.2), drought (Mean=2.04), irregular relative humidity (Mean=2.1, excessive sunshine (Mean=3.0), wind effect (Mean=1.8), and long period of harmattan with each variable exceeding the cut-off mean score of 2.0 except wind effect which had a mean of 1.8.

The result also shows that majority (97.4%) of farmers agreed they were aware of climate change with 85.8% having 6 years of awareness. The analysis of data on climate change adaptation strategies revealed that majority of the respondents used better hygiene (75.8%), More space per bird (73.7%), More water served (72.1%), Prompt and extra vaccination of birds (68.4%), Keeping of resistant breeds (63.2%), regular practice of routine management (59.5%), installed cooling equipment (57.9%), Tree planting around poultry house (55.8%) and keeping of early maturing birds (55.3%) as climate change adaptation strategies on poultry production.

The result of the stochastic frontier analysis showing the climate change adaptation strategies influencing inefficiency of poultry output showed that, raising of broods and sell, tree planting around poultry house, more water served were found to reduce farmers' economic inefficiency of poultry output at 10% level of significance.

RECOMMENDATIONS

Based on the findings of this, the following recommendation are made:

i. There is need to improve on farmers' access to climate change information as well as to promote adaptation methods that are appropriate for specific climate change related risks.

ii. Effort should be made to provide credit facilities to help improve farmers' capacity to adapt to climate change.

REFERENCES

[1] Adejuwon, S.A. (2004). Impact of climate variability and climate change on crop yield in Nigeria.*Contributed Paper toStakeholders Workshop on Assessment of Impact andAdaptation to Climate Change (AIACC)*: 2-8.

[2] Adesiji G.B. Tyabo, I.S. Bolarin, O. Ibrahim, M. Baba, S.T. (2013). Effects of climate change on poultry production in Ondo State, Nigeria. *Ethiopian Journal of Environmental Studies and Management 6 (3):*242–248.

[3] Alade, O.A. Ademola, A.O. (2013). Perceived effect of climate variation on poultry production in OkeOgun area of Oyo State. *Journal of Agricultural Science*, 5(10):176–182.

[4] Chah, M. J., Odo, E., Asadu, A. N. and Enwelu, I. A. (2013). Poultry farmers' adaptation to climate change in Enugu North Agricultural Zone of Enugu State, Nigeria. Department of Agricultural Extension University of Nigeria Nsukka. Journal of Agricultural Extension Vol. 17 (1) June, 2013 ISSN 1119-944X. Page 105.

[5] Chavanapoonphol, Y., Battese, G.E. & Chang, H.C. (2005, February 9 – 11). The impact of rural financial services on the technical efficiency of rice farmers in the Upper North of Thailand. Paper presented at the Annual Conference of the Australian Agricultural and Resource Economics Society at Coffs Harbour.

[6] Demeke Solomon, (2004). Egg production and performance of local and White Leghorn hens under-intensive and rural household conditions in Ethiopia. LRRD 16: 2.

[7] Gueye E.F. (2003) Gender issues in family poultry production systems in low-income food-deficit countries. food and agriculture organization of the united nations. FAO Smallholder Poultry Production paper No.4

[8] Hoffmann, I. (2008). Livestock genetic diversity and climate change adaptation. *Livestock and Global Change Conference proceeding*. May 2008, Tunisia.

[9] Intergovernmental Panel on Climate Change(IPCC),(2001a). Climate Change 2001 – The Scientific Basis: Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change [Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K. and Johnson, C. A. (eds.)], Cambridge University Press, Cambridge, UK.

[10] Intergovernmental Panel on Climate Change (IPCC), (2007a). Climate change impacts, adaptation and vulnerability. In: Third Assessment report of the intergovernmental panel on climate change. Parry ML, Canziani OF, Palutikof JP, Vanderlinden PJ, Hasson CE. (eds.) Cambridge University Press, Cambridge, United Kingdom, pp. 80-96.

[11] Intergovernmental Panel on Climate Change(IPCC),(2007b).Impact, Adaptation and Vulnerability.Contribution of Working Group 1 of the Intergovernmental Panel on Climate Change to the Third Assessment Report of IPCC.

[12] Nnaji. A.O.(2001). Forecasting seasonal rainfall for utilization In agricultural decision making in Nigeria. Int. J Agric, 1(2):30–41.

[13] NPC, (2006). National Population Commission Report

[14] Onyenechere, E. Igbozurike, U.(2008). Women crop farmers, adaptation to rainfall variability in Amaraku, Imo State, Nigeria. InNwajiuba, C. (ed) Climate Change and Adaptation in Nigeria MargrafVerlag, Germany, pp: 65-72.

[15] Ozor, N. Madukwe, M. Enete A.A. Amaechina, E.C. Onokala, P. Eboh, E.C. Ujah, O. Garforth, C.J. (2010).Barriars to climate change Adaptation Among Farming Household of Southern Nigeria. *Journal of Agricultural Extension*, 14(1).

[16] Thornton, P.K. (2010). Livestock production: recent trends future prospects. Report to the Department for International Development, ILRI, Nairobi, Kenya, Pp. 2853-2867.

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An Analysis of Value-Added of Seaweed Products as An Efforts to Improve Public Welfare in Bantaeng District

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Abstract— *This study aims to quantify the revenue and analyze the value-added seaweed crackers product of SMEs A and SMEs B in Bantaeng District by using the income formula and the value-added revenue method. The results shows that SMEs A gains higher revenues amounted IDR 1.618.862 than SMEs B, which get the most revenue IDR. 885.250. It is influenced by the type of product, activities production, capital, and marketing. Meanwhile, the ratio of the value-added of SMEs A was also higher by 91.30%, with a profit rate of 98.54% compared to 88.43% for SMEs B by a margin of 98.43%. It can be assumed that the seaweed cracker business can improve the incomes and public welfare in Bantaeng.*

Keywords— ***products, seaweed, value-added, SMEs, production.***

I. INTRODUCTION

Seaweed is one of the leading commodities that have an attraction to be cultivated because it is simple and has a competitive market in the world. The seaweed demand increases in every year. The use of seaweed is also more extensive and varied due to increased knowledge of the commodity. At a time in the past, the process of seaweed only from post-harvest handling to drying. Meanwhile, nowadays, the empowerment and development of the seaweed can be re-processed into new products (Suryawati, 2017)

Changing seaweed into new products of higher economic value, it will be able to provide value-added due to the issuance costs of forming new higher prices and profits more excellent than without treatment processes. Seaweed so that the value-added would be higher than unprocessed seaweed It has excellent prospects mainly carried out during the production of seaweed is abundant and low price (Rahman, 2015).

Value added is increasing the price of the product because the functional input given on the product concerned. This input relates to the process of transformation of raw materials to the storage. This added value can be analyzed through the methods of calculation to estimate changes in the value of raw materials after getting treatment. Values obtained are the excess of the product's value with the cost of raw materials and other inputs (Hayami, 1987).

The term added value in line with the macro-economic concepts related to national income calculations using measurements productive. The meaning of value-added here is the difference between the output value of a business, the total revenue from sales, and the costs used to manufacture these products. This value can be determined from the difference between the input's value and the output of the business (Haller & Stolowy, 1995).

The added value of the seaweed can create distinctiveness of an area through culinary offerings. Seaweed can be developed into various types of derivative products with high health value. One is the seaweed crackers. Are made from seaweed that has an existence to develop. The culinary offerings in the field of business are very promising. They include business areas that will not be endless because, in everyday human life, is always associated with consumption. Seaweed crackers, especially high nutritional value that is expected to be a special place in the hearts of the people (Ernawati, 2020).

The existence of innovation in making crackers is one crucial strategy in increasing sales volume. It can provide value-added of an existing product and provides better solutions for consumers in selecting products. In buying a product, consumers do not merely look at the value or function of a product that needs it, but consumers also pay attention to whether the selected products add value or advantages compared with other similar products. It can be said that innovation is a decisive factor in the competitive industry and is a formidable weapon to face the competition. It can be reflected that winning the competition increased sales volume, which means improving coIDRorate profits, so the company's continuity can be assured. Conversely, if the company cannot innovate then able to make products that are misaligned (Russell, 1996)

Based on the description above, this study aims to determine how much revenue and value-added of crackers seaweed on Micro, Small, and Medium EnteIDRrises (SMEs) in Bantaeng Districts. Bantaeng has abundant raw materials potential of seaweed to be processed into crackers. This study's results are expected to provide input and suggestions as well as business opportunities that promise to increase social welfare in Bantaeng.

II. RESEARCH METHODS

The study was conducted in two Districts Bantaeng on SMEs. The research was conducted in November 2019 until February 2020. Primary data is obtained directly from the source and researched through interviews and surveys (questionnaires) with seaweed crackers businesses. A method of selecting respondents SMEs and MSMEs B was puIDRosive with consideration of business units that are still active in seaweed processing until the time of the study. The level of income can be used as a reference comparison. This study used two analyzes is the analysis of income and value-added analysis.

III. RESULTS & DISCUSSION

1. Revenue SMEs Crackers Seaweed

Revenue is the output of an entire amount of income received by a person as remuneration on the results of the efforts initiated. The aim of building a business is to generate revenue, which can be used for personal and business survival pioneered. The higher the income, the greater the potential for businesses to finance spending and business activity.

One of the essential things in running a business is the cost of production. The production is the total of all fees that are used from production to marketing preparation. In Table 2 are presented the total revenue earned SMEs SMEs A and B as follows.

SMEs	Product	Revenue (TR)(Rp)	Cost (TC) (Rp)	Income (TR-TC) (Rp)
A	Cheeses Stick	1.083.000	198.084	885.250
B	Chesses Paper	1.966.000	347.471	1.618.862

Fig 1. Average monthly Revenue During the Month (November to January) Crackers Seaweed Production

The table above shows that the average monthly income crackers A cheese sticks on SMEs were lower by IDR.885.250, compared to crackers cheese paper on SMEs B amounting IDR.1.618.862. From the research results obtained show that first, there is the influence of the product on the level of revenues—cheese crackers paper products preferred by the community. The second result showed that there are significant factors of production to the level of revenues. SMEs B performs an average of eight times the production in one month. At the same time, SMEs A does Ra on average three times the production in one month.

The third result shows that there is a significant capital factor in the level of revenues. Capital is referred to here as a material to conduct production activities, paid labor, and other costs. The results showed that there are four significant marketing factors on the level of revenues. SMEs B markets their products over to the showroom as a marketing center with the goal of outsiders who visit Bantaeng, entrust the product to the store's, and market directly to consumers. Most buyers purchase products SMEs B directly by several agencies that conduct activities. Meanwhile, SMEs A market their products only to entrust to the showroom and shops nearby. It leads to a take to recover the capital used for production again.

2. Calculation of the Value Added Production Seaweed Crackers

The value-added analysis is an estimation of the raw material method with a special treatment to get the value. The added value is influenced by the technology used in the processing. The basis of calculation used is the added value per unit of the central raw material kg. Tables value-added analysis calculation results in seaweed cracker production in SMEs SMEs A and B are presented as follows

Variable	Calculation	SMEsA	SMEsB
		Cheeses Stick	Chesses Paper
Output,Input, dan Price			
Output (kg)	A	25	30
Raw Materials (kg)	B	1	1
Labor (HOK)	C	1,125	1,125
Conversion Factor	D=A/B	25	30
Coefficient of Labor (HOK/Kg)	E=C/B	1,125	1,125
Output Price (Rp/Kg)	F	130.000	100.000
Average Labor Wage (Rp/HOK)	G	40.000	35.556
Revenues and Profit			
Raw Mateial Price (Rp/Kg)	H	11.000	11.000
Other Output Donation (Rp/Kg)	I	365.000	250.000
Output Value (Rp/Kg)	J= D*F	3.250.000	3.000.000
Value Added (Rp/Kg)	K=J-I-H	2.874.000	2.739.000
ValueAdded Ratio(%)	L=K/J*100	88,43	91,30
Labor remuneration (Rp/Kg)	M=E*G	45,000	40.000
Part of Labor (%)	N=M/K*100	1,57	1,46
Profit (Rp/Kg)	O=K-M	2.829.000	2.699.000
Profitability (%)	P=O/K*100	98,43	98,54
Reply Services for Production Factor			
Margin (Rp/Kg)	Q=J-H	3.239.000	2.989.000
Profit(%)	R=O/Q*100	87,34	90,30
Labor(%)	S=M/Q*100	1,39	1,34
Other Input (%)	T=I/Q*100	11,27	8,36

Fig 2. Table Calculation of Value Added

From the table above shows that the value of the conversion factor on crackers cheese sticks at 25. The value of a factor on paper cheese crackers at 30. The number of Working People's Day (HOK) in the processing of cheese sticks crackers and Papercheese crackers amounted to 1.125 HOK to cultivate seaweed as 1 kg. The coefficient of employment in SMEs and MSMEs A is the same B of 0.75 HOK/kg. The average wage for labor in the manufacture of cheese sticks crackers of IDR.26.667 per HOK, and the paper cheese crackers amounted to 20,000 per HOK.

In the manufacture of cheese sticks crackers have other input contribution amounting to IDR365.000 / kg, and on paper cheese crackers have other input amounting to IDR250,000 / kg. The output value in the manufacture of crackers or cheese sticks crackers is IDR 3.250.000 or 88.43%, while the paper cheese crackers are IDR 3.000.000 or 91.30% for 1 kg of seaweed. The value-added on cheese sticks crackers is IDR.2.874.000, while paper cheese crackers are IDR 2.739.000 with equal to 1 kg of seaweed.

Labor costs in cheese stick crackers are IDR 200.00, the profit level is 93.04% and the margin is IDR 3,239 million for 1 kg of seaweed. While the labor costs in cheese paper crackers are IDR 250,000, the profit rate is 90.87% and the margin is IDR 2,989,000 for 1 kg of seaweed.

IV. CONCLUSION

SMEs B has a higher revenue of IDR 1.618.862 compare to SMEs A, which only has IDR885.250 of the revenue. It is influenced by the type of product, production factors, capital factors, and marketing factors.

For added value generated by the paper cheese crackers on SMEs B is IDR.2.739.000 for 1 kg of seaweed with a gain of IDR.2.989.000, whenever the production process. As for the value-added generated by crackers, cheese sticks on SMEs A is IDR.2.874.000 for 1 kg of seaweed with a gain of IDR.3.239.000 whenever the production process.

Suggestions can be applied based on the above results is related institutions can perform the following activities as a form of follow-up measures for efforts to optimize product development seaweed crackers in Bantaeng

1. Building market access to increase the demand for cracker products outside Bantaeng constrained transportation away
2. Building business partnership pattern that allows access capital to increase production so the business capacity is more optimal.
3. Encourage Marine and Fisheries Agency's commitment to guiding businesses to a group formed to stand independently.

REFERENCES

[1] Djahidin, 1985. Financial Statement Analysis. Ghalia Indonesia, Jakarta. Jumingan, 2006. Financial Statement Analysis. Bumi Aksara, Jakarta

[2] Ernawati, C. (2020). Crackers existence Wet For Culinary Kapuas Hulu. Proceedings of the National Seminar on Educational Anthropology (SENASPA), Vol. 1, 133 - 141.

[3] Haller, A., & Stolowy, H. (1995). Value Added Accounting in Germany and France: A Conceptual and Empirical Comparison. Annual Congress of the European Accounting Association (pp. Of conference proceedings). Birmingham, United Kingdom: May 10-2. campus.hec.fr

[4] Hayami, Y., Kawagoe, T., Morooka, Y., & Siregar, M. (1987). Agricultural Marketing and Processing in Java Upland A Perspective From A Sundanese Village. Bogor: CPGRT Center

[5] Khairani, S., & Pratiwi, R. (2018). Turnover Increased Sales Through Diversification and Promotion Strategy In Palembang Typical Souvenir Craft SMEs. Journal of Community Services, 36-43

[6] Langitan, 1994. Analysis of Value Added Products Soy Milk Refreshments. Department of Social Economics of Agriculture, IPB Bogor
[7] Mane, A., & Menne, F. (2017). Seaweed Farmers Family Empowerment in Rural Pa'jukukang To Increase Revenue. XVII edition of the journal Research Unibos Makassar, 1-17
[8] Sugama, Ketut. 2013. Aquaculture Development Opportunities crab shell is soft To Increase Community Income Nangro Aceh Darussalam province. Presentation Material. Banda Aceh September 6th 2013
[9] Suryawati, S. H., & Erlina, M. D. (2017). Business Development Strategy Seaweed Cultivation in South Buton District. J. Socio-Economic KP Vol. 12 No. 1, 31-44
[10] Rahman, S. (2015). Analysis of the Value Added Agroindustri Corn Chips. Journal of Food Technology Application 4 (3), 108-111
[11] C Russel, R., & Taylor, B. (1996). Production and Operations Management: Focusing on Quality and Competitiveness. New Jersey: Prentice Hall

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Consumption and Effect of Artificial Sweeteners and Artificially Sweetened Products on Lebanese Population

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Abstract**—***Non-caloric or non-nutritive artificial sweeteners are chemically synthesized or extracted from natural sources to replace regular sugar. The main reasons behind using artificial sweeteners are weight loss, blood sugar control for diabetic people, and dental caries prevention. Although many studies support the use of sweeteners and artificially sweetened products, others focus on the long-term negative consequences of their usage. A survey was conducted to explore the effect and prevalence of non-caloric tabletop sweeteners and non-caloric sweetened products consumption among Lebanese population based on their Body Mass Index (BMI), sex, age, and health. The survey was completed by a sample of 457 subjects. The questionnaire included consumption pattern, brands used, and the frequency of consuming artificial sweeteners and sweetened products. Data showed that 30% of Lebanese population consumes artificial sweeteners as tabletop while 31% consumes artificially sweetened products. Sucralose was the most consumed sweetener; however, its daily intake range was far below its Acceptable Daily Intake (ADI). The highest percentage of consumers (41%) made the shift to sweeteners from regular tabletop sugar around one year prior to this study. With increasing age, there was a decrease in the percentage of respondents who observed "weight loss" due to replacing regular sugar with sweeteners. The study determined that the majority of respondents (66%) were unaware of the side effects of these sweeteners. Also, the majority of diabetic respondents (78%) and (57%) did not observe any decrease in HbA1c and blood sugar levels, respectively. In a complementary survey that targeted Lebanese dietitians, 78% recommended the use of tabletop sweeteners instead of tabletop sugar, while 52% did not support the consumption of artificially sweetened products. Also, the survey showed that "Nevella" was the most recommended brand by (57%) of dietitians.*

Keywords**—*****Lebanon, sweetener, effect, artificial, population, dietitians.***

I. INTRODUCTION

Human's affection for sweet-tasting food is inborn. Studies have shown preference for sweet-tasting products in newborns and infants [1]. Several studies confirm a tight correlation between the consumption of sucrose over a life-time and tooth decay, as well as other diseases such as obesity, diabetes, and cancer[2,3]. Obesity is a major problem throughout the world and could lead to other severe diseases such as type-2 diabetes and cardiovascular heart diseases. Rising rates of obesity require avoiding the over consumption of calories and searching for non-caloric alternatives to sugar [4].This has created an increased demand for innovative low-calorie foods; and as a result, non-nutritive sweeteners have become very popular [5]. "Sodium saccharin" is the oldest synthetic sweetener which was discovered in 1879, followed by "cyclamate" in 1937 [6]. Cyclamate was originally approved for commercial use by the U.S. Food and Drug Administration (FDA) in 1951, then after 18 years it was banned by the same agency[7]. These innovative sweeteners, which mimic the taste of sugar, can be thousands offolds sweeter than sugar (sucrose), and that it is why they are called "intense sweeteners"[8]. They are either "artificial sweeteners", which are chemically synthesized or "natural sweeteners", which are extracted from natural origin (Table 1). Until now, there were many concerns and debates regarding their safety; In 2014, a Nature study showed that saccharin directly modulates the composition and function of microbiome and induces dysbiosis accounting for the downstream glucose intolerance phenotype in the mammalian host [9]. Accumulating evidence suggests that frequent consumers of these sugar substitutes may be at increased risk of excessive weight gain, metabolic syndrome, type 2 diabetes, and cardiovascular disease [10]. Although many studies demonstrated the hazardous effects of the long-term consumption of artificial sweeteners, there are still others in the medical and health communitythat defend them[11]. In 2017, a Lebanese study showed that the brand "Canderel®" was the only NNS (non-nutritive sweetener) that caused an increase in insulin levels without any effect on blood glucose in healthy subjects, which putthe consumer at risk of developing diabetes or pre-diabetes [12]. The largest US cohort study of postmenopausal women found a positive correlation between higher intake of ASB (artificial sweetened beverages) (twice or more daily) and the incidence of ischemic stroke, especially the small artery occlusion subtype, coronary heart disease (CHD), and all-cause mortality [13]. With the increasing potential risks of sweeteners, further studies on the effect of their consumption are needed.

In Lebanon, there is little or no data about the consumption of artificial sweeteners (AS) or artificially sweetened products (ASP). The main objective of this study was to estimate the rate of consumption of artificial sweeteners and sweetened products among Lebanese population. The second objective was to highlight the dietitians' recommendations toward the consumption of AS. The data collected in this study argues if there is an emerging need to take urgent action to prevent the risk of overconsumption of such products and to collect additional data about the side effects of AS.

Table 1. Different sweeteners with their origin, corresponding ADI (Acceptable Daily Intake), sweetness intensity, calories they provide, and regulatory status [14].

Sweetener	Origin	ADI [a]	Sweetness [b]	Kcal/g	Regulatory Status
Acesulfame-K (E950)	synthesized	15	200 x	0	Approved (21 CFR 172.800)
Advantame (E969)	synthesized	32.8	20000 x	0	Approved (21 CFR 172.803)
Aspartame (E951)	synthesized	50	200 x	4	Approved (21 CFR 172.804)
Neotame (E961)	synthesized	0.3	4150 x[c]	0	Approved (21 CFR 172.829)
Saccharin (E954)	synthesized	15	450 x[c]	0	Approved (21 CFR 180.37)
Steviol (E960)	natural	4	300 x[c]	0	GRAS [d]
Siraitia-grosvenorii	natural	-	175 x[c]	2	GRAS [d]
Sucralose (E955)	synthesized	5	600 x	0	Approved (21 CFR 172.831)

[a] *mg/kg bodyweight/day*
[b] *Multiplier of sweetness intensity compared to table sugar (Sucrose)*
[c] *Average*
[d] *Generally Recognized as Safe*

II. METHODS

2.1 Study design, site and subjects

A cross-sectional study design was applied from August 11, 2019 till September 13, 2019 and the sample size was 457 Lebanese respondent aged 18+ of both sexes (170 males and 287 females) from different regions of Lebanon. For the second survey, the sample size was 54 dietitians distributed all over Lebanon aged 21-39 years.

2.2 Data collection and analysis

A food frequency questionnaire was used to estimate the knowledge and consumption pattern of artificial sweeteners. Survey had 24 questions close ended with general information such demographic characteristics (sex and age), medical history, and consumption pattern of artificial sweeteners (AS) and artificially sweetened products (ASP). In addition, the questionnaire includes questions about weight, blood sugar, and HbA1c level change as observed by respondents. Respondents' anthropometric data namely height and weight were asked about in the questionnaire. Body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of height in meters (kg/m^2) to get four categories: underweight: BMI<18.5 kg/m^2, normal weight: BMI 18.5-24.9 kg/m^2, overweight: BMI 25-29.9 kg/m^2, and obese: BMI≥30 kg/m^2. Dietitians' survey was conducted to get their opinion about AS consumption, products, and brands which they recommend. The questionnaires were sent to respondents via communicating applications.

2.3 Statistical Analysis

Data management was conducted using the Statistical Package for Social Science (SPSS) (IBM SPSS Statistics Version 23). For all analyses, P-value < 0.05 was used to detect statistically significant difference. Data were analyzed using chi-squared test for categorical data.

III. RESULTS AND DISCUSSION

The percentage of consumption of tabletop AS among Lebanese population was almost similar to that of ASP as illustrated in *Fig. 1A.* A total of 457 respondents participated in this survey (170 males, 287 females), where 30% of respondents consumed AS (artificial sweeteners) as tabletop and30%of respondents consumed ASP. The percentage obtained is very close to that observed in an Egyptian study that was done on Alexandria University students in 2017 where 31% of the respondents were consumers of tabletop artificial sweeteners [15].Likewise, these results matched a study aimed to assess the trends of artificial sweeteners consumption among young adults in the USA, and revealed that the prevalence of consumption of artificial sweeteners was up to 30% among the American population in 2014 [16]. Our results show that only 20% of the whole sample was consumers of both tabletop and products artificially sweetened. Figure 1B shows the percentage of consumption duration. Where a high percentage of respondents (41%)started using AS as early as one year or less prior to this study.

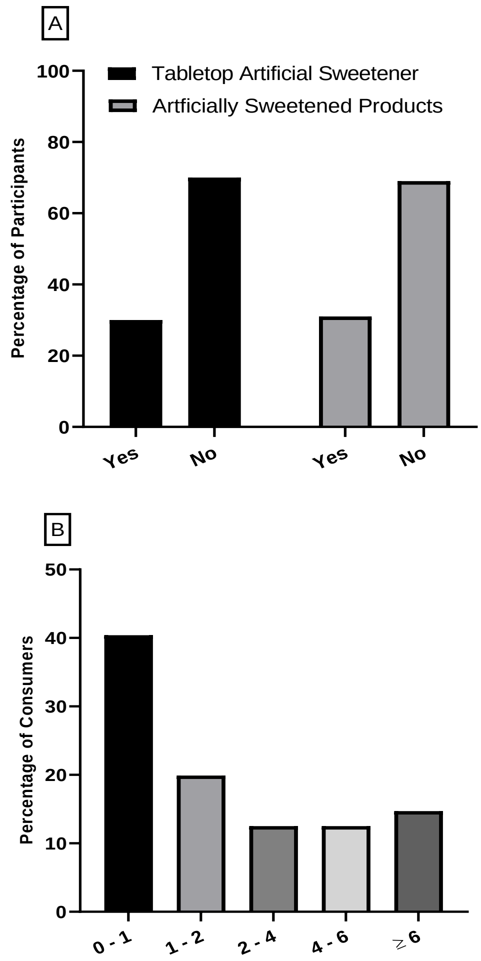

Fig.1. Percentage of participants using tabletop artificial sweeteners and artificially sweetened products (Fig. 1A).Percentage of AS consumers with different time intervals (years; Fig. 1B).

The percentages of male and female consumers are 28% and 31%, respectively, and both significantly differ ($p<0.0001$) from non-consumers (Table 2). Data in this study show that the percentage of diabetic male consumers is 21% with no significant difference ($p>0.05$) from diabetic female consumers which is 14%. Highest percentage of consumers belong to the 36-45 years age group (42%), whereas 48% of this age group reported that they suffered from diseases such as diabetes, cardiovascular heart disease, and blood pressure. Unlike the other age groups, the percentage of male consumers in the 36-45 years is (59%), which was significantly ($p<0.05$)

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higher than the female consumers (41%).Respondents who were18-20 years and 21-25 years of age showed the highest percentage of non-consumers (81% and 75%, respectively). This is related to their health condition where 91% of the 18-20 age group and 96% of the 21-25 age group reported that they did not suffer from any disease. These percentages differ from the Egyptian study where the highest percentage of consumers is of 20 to less than 22 years old (35%) [15]. Statistical analysis shows that 59% of 18-20 age group and 72% of 21-25 age group of Lebanese none consumers had normal body weight.

Table 2 shows that 23% of consumers reported that they do not suffer from any disease, but 45% of them consumed tabletop AS to control their body weight and 21% to enjoy the sweet taste. The statistical data shows that there is a significant difference (p<0.0001) among BMI (Body Mass Index) categories between consumers and non-consumers, where 31% of overweight and 50% of obese respondents were consumers of tabletop sugar, but there was no significant difference in consumption of sweetened products among age groups, gender or BMI categories (p>0.05).

Although there is a big debate regarding the benefits and risks of AS, our study determined that 53% of consumers believe that AS are healthier than tabletop sugar, 21% had no idea whether it is a healthier choice or not, but 26% thought that it is not a healthy alternative to tabletop sugar and yet they still continued to consume it any way. Sixty nine percent of overweight respondents and 56% of obese respondents considered AS to be a healthier option compared to regular tabletop sugar.

With respect to the purpose behind AS usage, respondents gave a variety of answers. The majority of respondents (62%)consumed AS to control their body weight, 25% to enjoy the sweet taste, 9% to keep blood sugar level low (diabetic respondents), and 4% to avoid dental caries. The highest percentage of those who consumed AS to control their body weight is in the 36-45 age group (82%). One third of the respondents within 18-20 and 26-35 age groups consumed AS to enjoy the sweet taste, while "diabetes" was the main reason for AS intake for 42% of the respondents in the 46+ age group.

Concerning the frequency of AS consumption, 29% consume it once daily and only 9% consume it more than two times per day (Table 3), while the rest consume it less frequently. Table 3 shows that the highest percentage (76%) of respondents consume 1 pill/sachet each time. With no significant difference among age groups (p>0.05) with respect to the number of pills consumed, the highest percentage (78%) of 1 pill/sachet consumers was for 26-35 years age group. Lebanese population mainly consume AS in hot beverages such as coffee and tea with a percentage of 87%. The percentage of males (92%) who consume AS in hot beverages is significantly higher than females (84%).

*Table 2. Distribution of consumers of Tabletop artificial sweeteners among gender, age groups, BMI categories and diseases suffering from. (***: very highly significant)*

	Consumers Count (%)	Non- consumers Count (%)	Total Count
Sex (p-value <0.0001 ***)			
Male	48 (28)	122(72)	170
Female	88 (31)	199(69)	287
Age (p-value <0.0001 ***)			
18-20	13(19)	56(81)	69
21-25	36(25)	106(75)	142
26-35	40(36)	71(64)	111
36-45	27(42)	37(58)	64
46+	20(28)	51(72)	71
BMI (p-value <0.0001 ***)			
Underweight	-	13(100)	13
Normal weight	49(22)	172(78)	221
Overweight	39(31)	88(69)	127

Obese	48(50)	48(50)	96
Disease (p-value <0.0001 ***)			
Obesity	28 (67)	14 (33)	42
Diabetes	22(71)	9(29)	31
Blood pressure	2(11)	17(89)	19
Other diseases	6(22)	21(78)	27
No disease	78(23)	260(77)	338

Different industrial and marketing companies have been investing in producing a substitute for sucrose. The most common brands of AS that are consumed among Lebanese population are: "Bloby", "Canderel", "Nevella", and "Splenda" which have sucralose, while "Marinas Lixol" and "Sussli" have a mix of sodium cyclamate and sodium saccharin as the active ingredients.

Table 3. Frequency and amount of consumption of tabletop artificial sweeteners.

	>2times/day	1 time/day	3-4 times/ week	2-3 times/week	2-3 times/month	Total
1 pill or sachet/time	5	32	16	20	30	103 (76%)
2-3 pills or sachets /time	6	6	4	6	6	28 (21%)
>3 pills or sachets/time	2	1	0	1	1	5 (3%)
Total	13 (9%)	39 (29%)	20 (15%)	27 (20%)	37 (27%)	136 (100%)

A high percentage (78%) of dietitians recommend the consumption of AS instead of regular sugar. This result was obtained through a separate survey that targeted Lebanese dietitians who are distributed over all Lebanese governorates. Fifty seven percent of dietitians recommended the brand "Nevella". The most probably reason for this recommendation could be the presence of probiotics in "Nevella" brand."Bloby" (sucralose) was the most consumed brand (25%) among Lebanese population, followed by Canderel tablets (sucralose) (21%) (*Fig.2A*). Results in this study are matched with the most consumed AS among Alexandria University students in Egypt which is sucralose [15]; moreover, sucralose was heavily consumed among overweight and obese Indians[11]. In this study, the intake amounts (mg/Kg) of sucralose, sodium saccharin and sodium cyclamate were much lower than the corresponding ADI of each sweetener. The mean daily intake of sucralose among the Lebanese population is 0.188 mg/kg with a range of 0.06-0.66 mg/kg. The sucralose daily intake formost of respondents is between 0.06-0.21 mg/Kg body weight/day, but all of these presented ranges are far below the ADI of sucralose which is stated by FDA as 5 mg/kg body weight (Fig. 2B). Similarly, sodium saccharin and sodium cyclamate intake amounts (mg/Kg) were below their ADI. The respondents mean daily intake of saccharin is 0.054 mg/kg and of cyclamate is 0.54 mg/kg.

One of the main reasons for the consumption of AS is body weight control, 27% of consumers noticed weight loss when they consumed AS as a sugar substitute while 40% of consumers did not notice any weight change. The remaining 33% of consumers were not sure about their weight change.

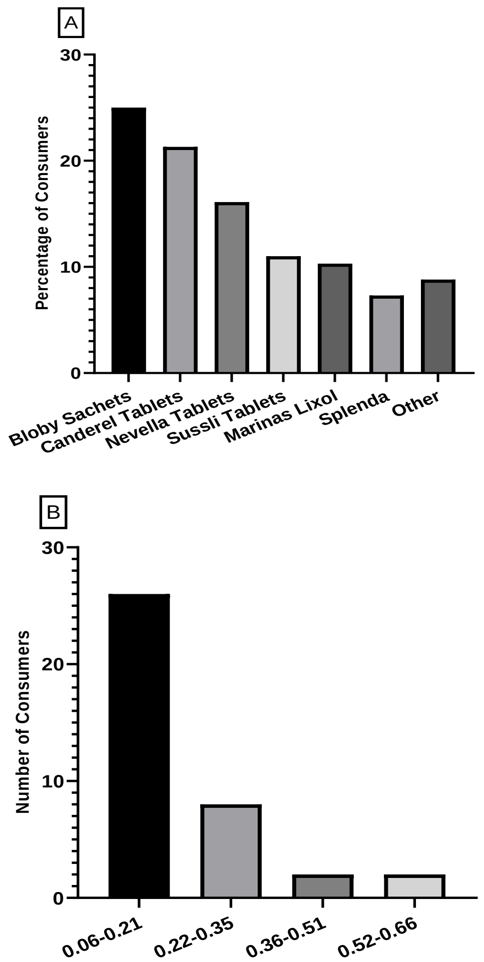

Fig.2. Percentage of most consumed tabletop artificial sweeteners by Lebanese population (Fig. 2A).Frequency of consumption of the four sucralose ranges (mg/kg body weight/ day; Fig. 2B).

The percentages of obese and overweight respondents that observed weight decrease upon using AS are 38% and 28% respectively, with no significant difference ($p>0.05$) (Table 4).

Figure 3A shows the frequency of consumption of artificially sweetened products. Where 51% of ASP consumers consume it 2-3 times/month and only 9% consume it on a daily basis. As well as, 52% of the lebanese dietitians do not recommend the consumption of AS products. Chocolate bars are the most consumed zero-sugar products (71%), followed by soft drinks (65%), and chewing gums (59%). Based on the responses, AS side effects are limited to stomach discomfort (10%) and headache (7%).

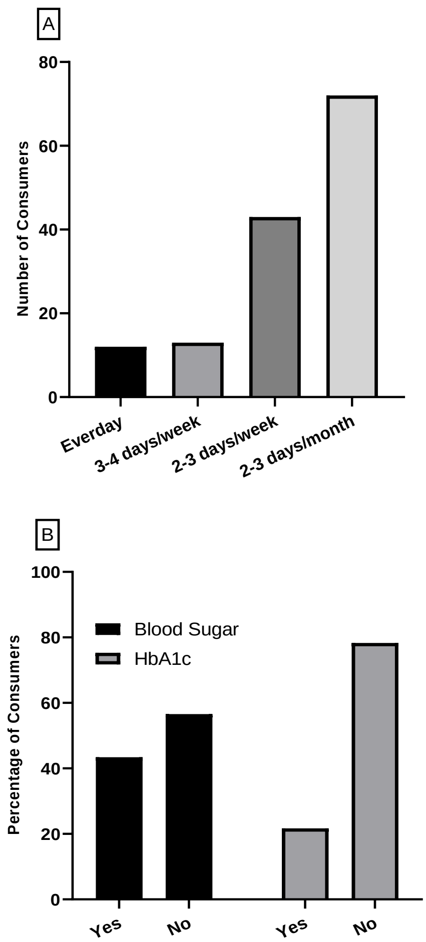

Fig.3.Frequency of consumption of artificially sweetened products (Fig. 3A).Answers of diabetic respondents about the decrease in blood sugar or HbA1c upon using artificial sweeteners (Fig. 3B).

As for the diabetic respondents, 57% and 78% did not observe a decrease in blood sugar and HbA1c, respectively, upon the consumption of AS (*Fig. 3B*).However, studying the duration of usage of diabetic respondents reveals that 4 to 6 years interval consumers observed significantly higher blood sugar decrease compared to 2-<4 years consumers. Whereas, less than one-year consumers did not observe any significant decrease in blood sugar. On the other hand, comparing the consumption duration of AS of HbA1cdid not show any significant differences.

Our data shows that the Lebanese population lack the knowledge of the side effects of AS consumption, where 20% of respondents linked AS intake to cancer and 66% did not state any possible side effects. The remaining 14% of consumers said that it causes problems in liver, kidney and heart. In comparison to the Indian study, only 7% of Indian respondents were able to state the later side effects [11].

Table 4. Respondents observations of weight decrease upon using artificial sweeteners with respect to sex, age and BMI categories.

	No Count (%)	Not sure Count (%)	Yes Count (%)
Sex (p-value <0.303)			
Male	15(31)	19(40)	14(29)
Female	39(44)	26(30)	23(26)
Age (p-value <0.0001 ***)			
18-20	5(38)	4(31)	4(31)
21-25	15(42)	5(14)	16(44)
26-35	22(55)	8(20)	10(25)
36-45	8(30)	14(52)	5(19)
46+	4(20)	14(70)	2(10)
BMI (p-value <0.096)			
Underweight	-	-	-
Normal weight	26(53)	15(31)	8(16)
Overweight	14(36)	14(36)	11(28)
Obese	14(29)	16(33)	18(38)

IV. CONCLUSION

In conclusion, the present study highlights, for the first time, the percentage of Lebanese population that consumes artificial sweeteners and artificially sweetened products. The highest percentage (42%) of consumers was determined to be within the 36-45 years age group. Even though a considerable high percentage of the population consumes AS, the mean intake was determined to be within the ADI (sucralose average intake was 0.188 mg/kg) as set by the FDA (sucralose ADI is 5 mg/kg bw/ day). However, some of the Lebanese sweeteners have cyclamate which was banned by FDA but not by Joint FAO/WHO Expert Committee on Food Additives (JECFA).According to this study, AS new consumers are on the rise probably due to an increase in obesity awareness and an increase in the number of dietitians who recommend AS intake. However, it was alarming to discover that a large portion of the Lebanese population is still unaware of the side effects of AS.A large number of AS consumers (40%) did not observe any decrease in body weight upon substituting tabletop sugar with AS. Also,the percentage of diabetics who did not observe a decrease in blood sugar and HbA1c was high. These results cast doubts on effectiveness of artificial sweeteners in controlling blood sugar levels. Further studies on the effect of AS and AS products on the body weight, blood sugar, and HbA1c are needed.

REFERENCES

[1] Maone, T. R., Mattes, R. D., Bernbaum, J. C., & Beauchamp, G. K. (1990). A new method for delivering a taste without fluids to preterm and term infants. *Developmental Psychobiology, 23(2), 179-191. doi:10.1002*

[2] Ludwig, D. S., Peterson, K. E., & Gortmaker, S. L. (2001). Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. *The Lancit, 357(9255), 505-508. doi:10.1016*

[3] Jiang, Y., Pan, Y., Rhea, P. R., Tan, L., *et al*, P. (2016). A Sucrose-Enriched Diet Promotes Tumorigenesis in Mammary Gland in Part through the 12-Lipoxygenase Pathway. Cancer Research, 76, 24-29.

[4] Kapadiya Dhartiben, B., & Aparnathi, K. (2017). Chemistry and Use of Artificial Intense Sweeteners. *International Journal of Current Microbiology and Applied Sciences, 6, 1283-1296. doi:10.20546*

[5] Tandel, R. K., 2011. Sugar substitutes: Health controversy over perceived benefits.. *Journal of Pharmacology and Pharmacotherapeutics*, 2, 236-243.doi: 10.4103/0976-500X.85936

[6] Khan, R., & Aroulmoji, V. (2018). Low Calorie High-Intensity Sweeteners. *International Journal of Advanced Science and Engineering, 5, 934-947. doi:10.29294*

[7] Weihrauch, M. R., & Diehl, V. (2004). Artificial sweeteners-do they bear a carcinogenic risk? *Annals of Oncology, 15, 1460-1465. doi:10.1093*

[8] Chattopadhyay, S., Raychaudhuri, U., & Chakraborty, R. (2011). Artificial sweeteners – a review. *J Food Sci Technol, 51, 611-621. doi:10.1007*

[9] Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, *et al.* Elinav, E. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature, 514, 181-197. doi:10.1038*

[10] Swithers, S. E. (2013). Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. *Trends in Endocrinology and Metabolism, 24. doi:10.1016*

[11] Bhagyasri, A., Naveen Kumar, R., Balakrishna, N., & Sudershan Rao, V. (2016). Exposure Assessment of Artificial Sweeteners among Type 2 Diabetic, Overweight and Obese Individuals. *The Indian Journal of Nutrition and Dietetics, 53, 268-276. doi:10.21048*

[12] Imad, J., Wehbe, T., & Abou Jaoude, E. (2017). A comparative study of three non-nutritive sweeteners effects on insulin and glucose in healthy, non-diabetic adults. *Insights in Nutrition and Metabolism, 1(2), 73-79.*

[13] Rahmani, Y. M., Kamensky, V., Mnson, J. E., *et al.* (2019). Artificially Sweetened Beverages and Stroke, Coronary Heart Disease, and All-Cause Mortality in the Women's Health Initiative. *Stroke, 50, 555-562. doi:10.1161*

[14] FDA. (2014). FDA. Retrieved april 13, 2020, from https://www.fda.gov/food/food-additives-petitions/additional-information-about-high-intensity-sweeteners-permitted-use-food-united-states

[15] Tayel , D. I., Khamis, N. A., & Darwish, O. A. (2017). Artificial Sweeteners Consumption among Alexandria University Students, Egypt. *Journal of High Institute of Public Health, 47(1), 1-7.*

[16] Drewnowski, A., & Rehm, C. D. (2014). Consumption of Low-Calorie Sweeteners among U.S. Adults Is Associated with Higher Healthy Eating Index (HEI 2005) Scores and More Physical Activity. *Nutrients, 6, 4389-4403. doi:10.3390*

Bacteriological and Physicochemical quality of Borehole water used for drinking at Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria

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***Abstract*— *The concern over exposure to drinking water contaminants and the resultant adverse effects on human health has prompted several studies evaluating the quality of drinking water sources at Olusegun Agagu University of Science and Technology (OAUSTECH). Six (6) Borehole water samples were collected from three (3) functional boreholes in OAUSTECH. Two water samples from each borehole were collected using standard techniques. The physicochemical parameters analyzed include, odor, color, taste, temperature, pH, conductivity, alkalinity, total dissolved solid, total hardness and chloride. All physicochemical parameters of the Borehole water samples were shown to be within recommended permissible limit of WHO and NAFDAC standard for potable drinking water. The bacteriological analysis of the three borehole water samples were carried out using the Most Probable Number technique (MPN). From the analysis BH1, BH2 and BH3 tested negative for total coliforms and fell within the recommended limit of WHO and NAFDAC standard for drinking water. This study has shown that water from the boreholes at Olusegun Agagu University of Science and Technology is safe for drinking. However, good and proper environmental and personal hygiene must be maintained especially by the users of these boreholes to prevent their contamination with bacteria pathogen. Boreholes should also be properly constructed with good understanding of site locations.*

Keywords*— Boreholes, Bacteriological analysis, physicochemical analysis, potable water.

I. INTRODUCTION

Water is essential for the existence of humans and all living things and hence a satisfactory (adequate, safe and accessible) supply must be available to all. Water used for domestic purposes must be clean and not contain any microorganism, parasites and other substances which can pose danger to human health [1], [2], [3]. Safe water supply and sufficient sanitation to secure health are among the essential human rights. However, many people do not have access to safe drinking water and risk to waterborne diseases is a critical public health problem in many developing countries [3]. So securing safe drinking water for all is one of the significant difficulties of the 21st century. In a recent report, close to a billion people mostly living in the developing world do not have access to adequate and safe drinking water [3].

Control of the microbial quality of drinking water should be a priority in all countries, given the immediate and potentially devastating consequences of waterborne infectious diseases [4]. Contaminated water serves as a mechanism to transmit communicable diseases such as cholera, typhoid and guinea worm infection [5] [3]. Around 88% of diarrhea cases worldwide are linked to unsafe water, inadequate sanitation or insufficient hygiene. Most cases of diarrheal illness and death occur in developing countries with an estimated annual incidence of 4,600 million episodes and cause 2.2 million deaths every year [1]. Almost 6.1 billion people (89% of the world population) have used an improved drinking water source. This is 1% more than the 88%

Millennium Development Goal target [6]. Improved sources of drinking water in developed countries has reached 99%. In contrast, in the developing world – especially in sub-Saharan countries – the coverage is still low (63%) with 51% and 84% coverage rates among rural and urban populations, respectively. Moreover, above 40% of all people globally who lack access to safe drinking water live in sub-Saharan Africa [6]

Water is a key component in determining the quality of our lives. Although water covers about 70 percent of the earth's total surface, only 0.3% of it can be used by humans [7]. Among the various sources of water, ground water is the largest reservoir of drinking water, it is that water reservoir found in the saturated part of the ground underneath the land surface. It normally accumulate there when the water seep into the ground and move downward due to gravity through the pore spaces found between soil particles and crack in rock. The water eventually reaches a depth where the soil and rock are saturated [8]. Ground water is an important source of water supply with a number of advantages;It is commonly free from pathogenic organism due to infiltration and requires little or no treatment; turbidity and colour are generally absent and Its chemical composition is almost stationary [9]. Despite this advantage, ground water is susceptible to pollution as a result of recent or past human activities [10]

Water from boreholes may be free from dangerous water - related human diseases such as cholera, typhoid fever, dysentery, guinea worm and many others. Borehole water is ground water available in an aquifer obtained by installing a pump to draw the water to the consumers. Any contaminated surface water with pathogen that infiltrates into the soil and becomes groundwater would be filtered by the soil profile before reaching the depth of aquifer. An aquifer is saturated water bearing stratum that is capable of holding, transmitting and yielding sufficient water in underground to well. The major problems of boreholes is chemical content of the ground water, which must be analyzed to ascertain if these dissolved products are within the permissible limits for consumption purpose by the authorities such as the World Health Organization [11]. Many diseases in developing countries are caused by drinking contaminated water [12]. Quality of drinking water is fast becoming an essential aspect of water quality studies [12]. Therefore, the provision of potable water to the rural and urban population is necessary to prevent health hazards [13]..Certain minerals are also toxic such as the heavy metals. Though some of the heavy metals such zinc, manganese, nickel and copper act as micro-nutrients at lower concentrations, but become toxic at higher concentrations [14].. Health risk due to heavy metal contamination of water through soil has been reported. Therefore, auditing and monitoring of physicochemical, minerals and microbial until very recently ground water has been thought of as being a standard of water purity in itself and to a certain extent that is indeed true [15] .Groundwater pollution which is a product of human activities had profound impacts on the quality of water bodies due to the introduction of various pollutants such as organic compounds, heavy metals, agricultural waste, etc [16] Various studies confirmed the effect of heavy metals on the human health[17] Suitability of water for various uses depends on the type and concentration of dissolved minerals and ground water has more mineral composition than surface water.[18] The quality of ground water is constantly changing in response to daily, seasonal and climatic factors. Continual monitoring of water is highly crucial because changes in the quality of water have far reaching consequences in terms of its effects on man and biota[19].Contamination of ground water also depends on the geology of the area especially in limestone where extensive cavern systems are below the water table [14]. The changes in quality of groundwater response to variation in physical, chemical and biological environments through which it passes [20]. To safeguard the health of people and to reduce to the barest minimum experiences of drinking and/or using of low quality waters, it is necessary that the quality of water should be monitored with the view to finding lasting solutions to health problems associated with the use and drinking of low quality waters. Both liquid and solid waste materials dumped either on soil surface or buried are known to decompose to produce leaches that penetrate aquifers and contaminate the ground water thereby raising the potential toxicity of the water to consumers [21]

Burying and surfaces dumping of both industrial and domestic wastes are a common practice among rural and urban dwellers [22].[12] have described bore hole as a source of potable drinking and domestic use water for rural and urban population. [23] have explained that in order to understand the quality of borehole water, the physical, chemical and microbiological parameters must be analyzed. The quality of ground water however, principally depends on the elements present in it while seeping down. The World Health Organization (WHO) has set a quality guideline for drinking water and recommends that the properties of every drinking water should fall within the acceptable limit set by it [1]. The quality of water is assessed in terms of its physical, chemical, biological characteristics, and it's intended uses. For example, although distilled water is physically, chemically and bacteriologically pure, its taste is rather bland and it is highly corrosive [24]. It has been demonstrated repeatedly that water containing some dissolved constituents is far more palatable than pure water. Water quality varies for different purpose in every daily activity [18]. Water quality standards are standards established to determine whether water of a certain quality is suitable for its intended use. All portable water must confirm to these standards [1].

At Olusegun Agagu University of Science and Technology Okitipupa, there is not enough data on the bacteriological quality of water from boreholes sources in the university. Thus, evaluation of the bacteriological quality of these water sources will provide benchmark data about the bacteriological status and might enable insight into the development of further protective and treatment measures. Furthermore, information obtained from the present study might help in maintaining borehole hygiene and determine the sources of contamination possible to prevent outbreak of water borne diseases among the students which might prove very fatal as the population of the University is getting higher with each admission year. Therefore, the evaluation of groundwater quality is essential for the development of civilization and to establish database for future water resources strategic planning and development. Studies on groundwater have been carried out in different parts of Nigeria; however, no comprehensive study has been conducted on the groundwater quality in Okitipupa, ondo state.

II. MATERIALS AND METHODS

2.1 Sample collection

Six (6) borehole water samples from OAUSTECH study area were collected. Two (2) samples were collected from each borehole. The samples were taken from borehole of average depth 25-85 meters. All samples were collected in clean one (1) liters plastics container stored and transported in a cool box at a very low temperature into the microbiology laboratory and analyzed within 2 hours of collection.

2.2 Physicochemical Analysis

The water samples collected were analyzed for pH, temperature, odour, colour, taste, turbidity, electrical conductivity, alkalinity, acidity, total suspended solid, total dissolved solid, total hardness and chloride

2.3 Temperature Determination

This is the degree of coolness and hotness of a system. It is an important physical parameter, determined because of its effect on living organism. Usually the temperature of polluted water is higher than the water supply due to eutrophication process and geographical location which heat up the water. This was conducted at the site of sample collection using mercury in glass thermometer

2.4 pH Determination

The pH of the water samples was determined using the Wagtech pH meter. 10ml of each of the samples was poured into a sterile beaker and the anode of the pH meter was dipped into it and readings were obtained when it was stable.

2.5 Determination of Colour, Odour and Taste

A 20ml volume of each water sample was poured into a clean beaker. The sample was then shaken vigorously to check for any frothing and allowed to settle. Colour was determined through visual examination and those odours were determined through the sensation of smell.

Taste test was determined by putting a small volume of the water sample in the tongue and then immediately raised with distilled water (taste free) after sample.

2.6 Determination of Turbidity

The digital turbidity meter (2100P) was switched on, allowed to warm up for 30 minutes. It was set at zero NTU (Neplometric turbidity unit). The cell (60ml) was rinsed with distilled water and filled with 60ml of the water sample. The side of the cell was wiped with a tissue paper to clean the digital meter and after shaking for 20 minutes. The samples were inserted in the compartment. The selection button was turned to the appropriate turbidity range 0-10. The turbidity was then read off directly on the digital meter. The value was obtained and recorded in Nepholometric turbidity units.

2.7 Determination of Total Hardness

Total hardness was analyzed by titration of 50ml water sample into conical flask and 0.1ml of buffer solution was added or with standard EDTA. The EDTA was added in drops at pH 10 using Erichrome black I indicator and was gently shaken until the colour change into purple, and the hardness was calculated by multiplying the average number of drops of EDTA used for the sample by the calibration factor of 20.

2.8 Mohr Method Determination of Chloride

The Mohr method uses chromate ions as an indication in the titration of chloride ion with a silver nitrate standard solution. After all the chloride has been precipitated as white sulphur chloride, the first excess of titrant results in the formation of a silver chromate precipitate which signal the end points.

The reactions are:

$Ag^{+} + Cl \rightarrow AgCl_{(s)}$ White precipitate

$2Ag^{+} + CrO_4^{2-} \rightarrow Ag_2CrO^{-}_{4(s)}$ Yellow precipitate

25ml of the Bore Hole water sample was measured and put into a conical flask and the pH of the sample was measured or adjusted, 1.0ml of the potassium chromate indicator solution was added and the solution were titrated with standard silver nitrate solution (0.02N until the pinkish yellow and point was observed. The normality of the silver nitrate end point was observed. The normality of the silver nitrate used here is 0.0141N (blank) [25].

2.9 Determination of Electrical Conductivity

A 20ml of volume of each sample was poured into a clean beaker at room temperature. The conductivity meter (Wagtech Electrode) was switched ON for 10 minutes to standardize. The Wagtech Electrode was then rinsed with distilled water and was placed in the beaker containing the Bore Hole sample, and following stirring, the electrical conductivity values was determined after the pointer and the selector knob were adjusted, the reading was taken and recorded.

2.10 Determination of Total Dissolved Solid (TDS)

This is the term used for material residue that dissolves in water. Water with suspended solid may be aesthetically unsatisfactory for domestic purpose such as laundry, bathing, also water with high concentration of dissolved solid is unsuitably not potable for drinking and as well as for agriculture and industrial application or purpose. The total dissolved solids were determined by submersing the Wagtech probe into 100ml of each sample which the reading is taken and recorded.

2.11 Bacteriological Analysis

The coliform count was obtained using the 5 tube assay of the Most Probable Number (MPN) technique as described by [25]. The MPN technique for faecal coliform testing is useful in determining the faecal coliform density in most water. The technique is based on the most probable number of bacteria present in a sample [25].

2.12 Presumptive Test

Presumptive coliform test was performed using Macconkey broth. The first set of 5 test tubes had sterile 10ml double strength broth and the second 5 tubes and third sets of 5 tubes had 10ml of single strength broth. The test tubes are arranged in a row in three test tube racks. Each of the tubes contained Durham tubes before sterilization process was carried out using the autoclave.

Before sterilization all the test tubes containing the broth and Durham tubes were closed with non-absorbent cotton wool and then sealed with aluminum foil paper. After autoclaving for 15 minute at a temperature of $121^{0}C$ and pressure of approximately 1.1 kg/cm^3. The test tube were removed from the autoclave and allowed to cool to room temperature before the borehole water samples were aseptically transferred to the sterilized tubes. The first sets of test tubes for the double strength received 10ml each of the borehole water sample and for the second and third sets of test tubes for the single strength received 1ml and 0.1ml of the borehole water sample respectively using sterile pipettes. The tubes were incubated at $15^{0}C$ for 24-48h for estimation of total coliform and at $44.5^{0}C$ for faecal coliform for 24-48h and examined for acid and gas production. Acid production was determined by colour change of the broth from reddish purple to yellow and gas production was checked for by entrapment of gas in the Durham tubes. A combination of positive tubes at each inoculum was compiled and the MPN index/ 100ml of water samples were estimated using the MPN table.

2.13 Confirmed Test

Confirmed test was carried out by transferring a loopful of culture from the positive tube from the presumptive test into a tube of Brilliant Green Lactose Bile (BGLB) broth (Oxide) with Durham tubes. The tubes were incubated at $37^{0}C$ for 24-48h for total coliform and $44.5^{0}C$ for faecal coliforms and were observed for gas production.

2.14 Completed Test

To establish the presence of coliform bacteria and to provide quality control data, the positive tube from the confirmed test was carried out by streaking a loopful of broth from a positive tube onto prepared Eosine methylene Blue (EMB) agar and prepared nutrient agar plate for pure colonies. The plates were inoculated at $37^{0}C$ for 24-48h. Colonies developing on EMB agar, were further identified as coliform or faecal coliform, using cultural characteristics, morphology and biochemical tests. For faecal coliform, colonies with green metallic sheen were Gram stained and the IMVIC test was carried out on Nutrient agar stock cultures and used to identify the colony.

2.15 Identification of Bacterial Isolates

Stock cultures of the isolates with different cultural characteristics were made on nutrient agar slants. Gram staining was used to check for morphology and biochemical tests were performed to aid in identification. Various tests performed and used in probable identification of isolate.

III. RESULTS AND DISCUSSION

3.1 Results

The sanitary assessment of the boreholes used are as shown in Table 1 while the result obtained from the physicochemical and bacteriological analysis of borehole water in OAUSTECH are shown in Table 2.

Table 1: Sanitary Assessment of the Sampling Location

Sample Site	Physical Appearance	Proximity To Drainage	Proximity To Sewage Tank	Proximity To Refuse Dump Site
BH1	Clean	Far	Far	Close
BH2	Clean	Far	Far	Far
BH3	Clean	Close	Close	Far
BH4	Clean	Close	Far	Far

Keys:-

BH - Borehole
BH1 - Borehole at Car park
BH 2 - Back of ICT block
BH 3 - Microbiology laboratory

close ≤ 15 meters from pollution source
far ≥ 20 meters from pollution source

Table 2: Bacteriological and Physicochemical Parameters of water from sampled Boreholes.

	Parameters							
	pH	Electrical Conductivity (µS/cm)	Temperature (^{0}c)	Total dissolved solid(ppm)	Alkalinity Mg/L	Total hardness (ppm)	Chloride Mg/L	Most probable number (MPN/100ml)
Borehole 1	6.50	50.7μs/ cm	35.8^{0}c	3.52mg/L	0.20mg/L	10.77mg/L	69.00	0
Borehole 2	7.20	179.6μs/ cm	32.1^{0}c	0.10mg/L	53.40mg/L	151.06mg/L	1.37	0
Borehole 3	6.80	11. 2μs/ cm	30.7^{0}c	22.77mg/L	28.50mg/L	151.00mg/L	5.10	0
WHO guideline	6.8–8.5	≤ 400 µS/cm	25 - 39	<1000	20 - 200	< 500	≤ 250	0

3.2 Discussion

Table 1 revealed the sanitary assessment of the sampling locations, all the locations of the borehole water samples were clean. However, serious efforts should be made to maintain and sustain this level of hygiene as dirty environment can pollute the underground water and also lead to high prevalence of diseases like cholera, typhoid fever etc. which lead to high mortality rate. The table also showed that the appearance of all the borehole water sample is clear, it was also odourless, tasteless and colourless and they fell within the World Health Organization and National Agency for Food and Drug Administration and Control (NAFDAC) standard for drinking water. These could be due to the absence of particles and non- accumulation of solid and slits. Colour in natural water usually result from leaching of organic materials and is primarily the result of dissolved and colloidal humic substance, primarily humic acid and fluvic acid. However the overall colour do not pose any health threat to those that use the water.

Table 2 showed the various biological and physico-chemical parameters measured to ascertain the potability of water from sampled boreholes. The pH value ranged from 6.5 to 7.9, which falls within the recommended standard for drinking water standard. Water is said to be safe of the concentration of the substance do not exceed the level set by the regulatory bodies.this is consistent with results obtained by [27]. The World Health Organization [28] recommends a pH value of 6.5 or higher for drinking water to prevent corrosion. Although, a pH above 8.0 would be disadvantageous in the treatment and disinfection of drinking water with chlorine [29]. However, pH values between 6.5 and 8.5 usually indicate good water quality and this range is typical of most drainage basins of the world [30]

The Temperature of any water body affects the rate of proliferation of micro- organisms[16]. The Temperature values ranged from 30.7 to 35.8^{0}c, falling within the recommended permissible level of WHO and NAFDAC standard for drinking water standard of 25 – 39^{o}C as the temperature varies across different regions of the world. high temperature negatively impact water quality by enhancing the growth of micro-organisms which may increase taste, odour, colour and corrosion problems [28]. Therefore, it is important that groundwater temperature is not too high in order not to have microbial proliferation. Temperature affects biological, chemical and physical activities in the water [30]. Besides, increase in temperature of water decreases solubility of gases such as O2, CO2, N2 and CH4 [30]

The conductivity value ranged from 11.2 to 179.6$\mu s/$cm falling within the recommended standard, of WHO and NAFDAC for drinking water standards, indicatives of the absence of dissolved minerals and presence of some ions in low levels, thus conductivity was well below the standards of the regulatory bodies and therefore fell within the permissible limit for potable drinking water. Conductivity of the groundwater for the entire study area stands at an average of 80.5 µS cm-1 and it is in accordance with the findings of [31] which submit that values within the permissible WHO standards give a picture of very little solute dissolution generally in the groundwater, rapid ion-exchange between the soil and water, or basically a poor and rather insoluble geologic rock and mineral types.

The values for Alkalinity ranged from 0.20 to 53.4 mg/L which fell within the WHO and NAFDAC recommended standard for drinking water. Total dissolved solid values ranged from 0.27 to 22.77mg/L which fell within the permissible level recommended standard of WHO and NAFDAC. The total suspended solid values ranged from 0.02 to 3.52mg/L, fell within the recommended standard by the regulatory bodies. The Total Hardness of water is not a specific constituents but is a variable and complex mixture of cations and anions. Principally the water hardness are changed by ions such as calcium and magnesium. The total hardness from the bore hole water samples analyzed ranged from 3.30 to 151.06 mg/L fell within the permissible level of WHO and NAFDAC standard for potable drinking water from the result the total hardness analyzed is either soft or moderately hard .Soft water are associated with rickets in children and have been found to be statistically related to high mortality from cardio vascular diseases. Very hard water is not good for drinking and is associated with rheumatic pains and goiter [32]

The bacteriological analysis using Most Probable Number technique (MPN), showed that only all the Borehole water samples were free from the presence of coliforms which probably might have resulted from the clean environment. Potable water should be totally devoid of coliform. Borehole water samples from BH1a, BH1b, BH2a, BH2b, BH3a and BH3b all fell within the recommended standard of WHO and NAFDAC standard for drinking water standard have no presence of coliform. This results corroborates the finding of [17] that the MPN coliform index per 100ml of water sample collected from selected Bore Holes in Ilorin metropolis ranged from 0 to 16 MPN/ 100ml. The presence of total coliforms in groundwater indicates that the groundwater sources may be vulnerable to contamination by more harmful microorganisms hence the water samples are all fit for human consumptions as they have no such coliforms. This result coincides with the results of [33]; [34] [34]]8] who all posited that except groundwater is contaminated, it should be naturally free from faecal coliform and fall to the 0MPN/100ml standard recommendation by the WHO.

IV. CONCLUSION

Temperature, pH, conductivity, total dissolved solids, alkalinity and electrical conductivity, of groundwater in OAUSTECH were studied. Temperature values are consistent with tropical belt, it can be considered as being ambient relative to the geographical region and good enough in terms of supporting microbial growth. Average pH is slightly acidic and indicates susceptibility of metals and pipes to easy corrosion problems. The values of Electrical conductivity and total dissolved solids give a measure of the ionic load and contaminants in the water. Hence, from the EC and TDS values, the groundwater of this study area can be said to have low salt concentration and good for drinking and crop production. Furthermore, the pH and EC values infer that the water is clearly not saline and suggest its possible likelihood for irrigation agriculture. Although, other factor like Sodium Absorption Ratio (SAR) and Residual Sodium Carbonate (RSC) of the groundwater will have to be studied. Meanwhile, findings suggest that the groundwater in this aquifer is fresh. All parameters clearly fell below WHO international best standards for water quality.

Therefore, from results of this study, the groundwater in the study area can be regarded as being of good quality for drinking and agriculture purposes with reference to the parameters under consideration, although with little pH treatment because of its slight acidity. However, further studies with reference to the chemical and microbial analyses will have to be done to have a broader picture of this water quality.

REFERENCES

[1] WHO (2011). Guidelines for Drinking-Water Quality, Vol. 38, 4th edn. World Health Organization, WHO, Geneva, Switzerland, pp. 104–108

[2] Alvarez-Bastida C., Martínez-Miranda V., Solache-Ríos M., Hernández I. L., Teutli-Sequeira A. & Mejía G. V. (2018): Drinking water characterization and removal of manganese. from water. *J. Environ. Chem. Eng*. 6, 2119–2125

[3] Khalid S., Murtaza B., Shaheen I., Ahmad I., Ullah M. I., Abbas T., Rehman F., Ashraf M. R., Khalid S. & Abbas S. 2018 Assessment and public perception of drinking water quality and safety in district Vehari, Punjab, *Pakistan. J. Clean. Prod*. 181, 224–234

[4] WHO/UNICEF (2017): Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG Baselines. World Health Organization, Geneva, Switzerland and UNICEF, New York, USA.

[5] WHO (2013): Water Quality and Health Strategy 2013–2020. World Health Organization, Geneva, Switzerland

[6] UNICEF (2012): Progress on Drinking Water and Sanitation: 2012 Update. UNICEF, New York, USA

[7] Eniola, K. I.T., Obafemi, D.Y., Awe, S.F., Yusuf, I.I., and Falaiye, O.A. (2007). Effects of container and storage conditions of bacteriological quality of borehole water. *Nigeria Journal of Microbiology* 21(3):1578-1585.

[8] Samie A., Makonto T., Odiyo J., Ouaboi-Egbenni P., Mojapelo P. & Bessong P. (2011): Microbial quality, diversity and antibiotic susceptibility profiles of bacterial isolates from borehole water used by schools in Greater Giyani Municipality, Mopani District, South Africa. *Afr. J. Microbiol. Res.* 5, 198–210

[9] Al-Kasimi, S.M. (2002). Existence of Ground vapour-flux up flow: profit and utilization of planting the Desert using Reflective carpet Dahran. Journal of Environmental Science 3: 105-119.

[10] Adogo, I.Y., Ajiji,M.A., Anyanwu, N.O.J. and Ajide, B.(2016): Bacteriological and Physicochemical Analysis of Borehole water in Auta Balefi Community ,Nassarawa State ,Nigeria. *British Microbiology Research Journal* 11(4):1-7.

[11] World Health Organization-WHO (2006). Guideline for drinking water quality. Volume 1 Geneva Addendum to the 3rd volume 1 Recommendations WHO. 23-48.

[12] Asuguo, J.E. and Etim, E.E. (2012).Physicochemical and Bacteriological studies of selected Borehole water in Uyo, Akwa Ibom State, Nigeria. *International Journal of Modern Chemistry* 2 (1):7-14.

[13] Chilton, J. (1996).Aquifers as Environments for microbial activity. In: proceedings of the International Symposium on Environmental Aspects of Microbiology, Sigtuna, Sweden 2: 293-304.

[14] Ibekwe, A.M., Murinda, S.E. and Graves, A.K. (2011). Microbiological Evaluation of Water Quality from Urban Watersheds for domestic water supply improvement. *International Journal of Environmental.* Res. Public Health 8(12): 466076.

[15] Asante, F. (2013). Bioaccumulation of heavy metal on water, sediments and tissues of some selected fishes from the Red Volta of Ghana. *British journal of Applied Science and Technology* 6(2): 594-803.

[16] Adekunle, M.T., Gbadebo, A.M. and Banyoko, O.B. (2009). Assessment of Ground water quality in a typical rural settlement in south west Nigeria. *International Journal of Environmental Research and Public Health* 6:307-318

[17] Agbabiaka, T.O. and Sule, I.O. (2011). Bacteriological assessment of selected borehole water sample in Ilorin metropolis. *International Journal of Applied Biological Research* 2(2):31-37.

[18] Boulding and Rusell, J. (1995). Ground water contamination; assessment, prevention and remediation; Practical Handbook of Soil, Vadose Zone, Boca Raton, Fl: Lewis Publishers 25-13.

[19] Balakrishan, P., Saleem, A. and Malikarjun, N.D. (2011). Ground Water Quality Mapping using Geographic Information System (GIS). A case study of Gulbarga City, Karnataka, India. *African Journal of Environmental Science Technology* 5(12):1069-1084.

[20] Anim, A.K., Duodu, G.O. and Ahialey, E.K. (2011). Assessment of surface water quality. The perspective of the weija dam, Ghana. *International Journal of Chemistry* 3(2):32-39.

[21] Edema, M.O., Omemu, A.M. and Fapeju, O.M. (2001). Microbiology and Physicochemical Analysis of Different Sources of Drinking water in Abeokuta, Nigeria. *Nigeria Journal of Microbiology* 15(1):57-67.

[22] Adeyemi I.A**,** Alhassan A, Temitayo A.A, Muhammed O.A and James O.A (2014). A study of *Aspergillus niger* hydrolysed cassava peel as a carbohydrate source on the

histology of broiler chickens.*Springerplus* 3: 31. doi: 10.1186/2193-1801-3-31

[23] Chidinma, L.U., Emmanuel, C.N. and Nennaya, R.T. (2016). Physicochemical and Bacteriological Assessment of some Borehole waters in the Federal Capital Territory, Nigeria. *International Research Journal of Public and Environmental Health* 3(6):140-145.

[24] De-simone, L.A.M., Hamilton, P.A. and Gillion, R.J. (2009). Quality of water from domestic wells in principal aquifers of the United States. Overview of major findings. United States geological survey circular 1: 1332-48.

[25] APHA., 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edn., American Public Health Association/American Water Works Association, Washington, DC., USA.

[26] Ahaneku, I.E. and P.A. Adeoye, 2014. Impact of pit latrines on groundwater quality of Fokoslum, Ibadan, Southwestern Nigeria. *Br. J. Applied Sci. Technol*., 4: 440-449.

[27] WHO, 2010. Guideline for Drinking Water Quality. 3rd Edn., World Health Organization, Geneva, Switzerland.

[28] UNICEF, 2008. UNICEF Handbook on Water Quality. United Nations Children's Fund (UNICEF), New York, USA., Pages: 179.

[29] UNEP/GEMS, 2007. Water quality outlook. United Nations Environmental Programme (UNEP)/Global Environmental Monitoring System (GEMS), Burlington, Ontario, Canada.

[30] Yilmaz, E. and C. Koc, 2014. Physically and chemically evaluation for the water quality criteria in a farm on Akcay. *J. Water Resour. Prot*., 6: 63-67.

[31] H.H. Oyem, I.M. Oyem and D. Ezeweali, (2014). Temperature, pH, Electrical Conductivity, Total Dissolved Solids and Chemical Oxygen Demand of Groundwater in Boji-BojiAgbor/Owa Area and Immediate Suburbs. *Research Journal of Environmental Sciences, 8: 444-450.***DOI:** 10.3923/rjes.2014.444.450,
URL: https://scialert.net/abstract/?doi=rjes.2014.444.450

[32] Bahar, M. and M.S. Reza, 2010. Hydrochemical characteristics and quality assessment of shallow groundwater in a coastal area of Southwest Bangladesh. Environ. Earth Sci., 61: 1065-1073.

[33] Eltahir Y. M. & Abdelrahman A. A. 2013 Bacterial contamination of drinking water in the internally displaced people camps in South Darfur, Sudan. *Comput. Water Energy Environ. Eng*. 2,10.

[34] Bello O., Osho A. & Bello T. 2013 Microbial quality and antibiotic susceptibility profiles of bacterial isolates from borehole water used by some schools in Ijebu-Ode, Southwestern Nigeria. *Sch. Acad. J. Biosci.* 1, 4–13.

Effect of Fertigation Levels and Different Spacings on Yield and Yield Attributes of Bell Pepper (*Capsicum annuum* L.var.grossum sendt.) in Polyhouse condition

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Abstract— *An experiment was carried out in bell pepper (Capsicum annuum L.var.grossum sendt.) to study the influence of fertigation levels and different spacings on yield and yield attributes under polyhouse condition.The perusal of the data revealed that widely spaced (45 cm x 60 cm) plants produced the highest number of fruits plant^{-1} (13.75), fruit girth (25.23 cm) and fruit yield plant^{-1} (1.53 kg) while fruit yield m^{-2} (8.92 kg) was the highest for closely spaced (45 cm x 30 cm) plants. Yield and yield attributes were significantly influenced by fertigation levels. Among the fertigation levels, 100 % adhoc recommendation of KAU for precision farming (230: 25: 250 N, P_2O_5 and K_2O kg ha^{-1}) registered the highest number of fruits plant^{-1}(15.15), fruit girth (26.24 cm), fruit yield plant^{-1} (1.72 kg) and fruit yield m^{-2} (8.30 kg).*

Keywords— ***bellpepper, fertigation, spacing, foliar nutrition, yield and yield attributes.***

I. INTRODUCTION

Bell pepper (*Capsicum annuum* L.var.grossum sendt.) also known as capsicum, sweet pepper or shimla mirch is one among the most popular vegetables grown in polyhouses worldwide. The bell pepper fruits are available in different attractive colours and they have great demand in markets. Bell pepper is rich in proteins, vitamin A, ascorbic acid, riboflavin, thiamin, niacin and minerals like potassium, magnesium and calcium (Joshi and Singh, 1975). Being a cool season crop, year round production of quality bell pepper fruits is not possible in open field condition. Crops are more vulnerable to weather fluctuations in open field (Ochigbu and Harris, 1989) with more pests and diseases incidence leading to low productivity as well as quality. Protected cultivation techniques can be effectively utilized for the production of good quality produce with high productivity. Compared to open field cultivation, polyhouse cultivation resulted in 2-3 times yield enhancement in bell pepper (IIHR, 2011). Efficient and optimum application of fertilizer under protected condition ensures improved growth, yield and quality of bell pepper along with minimized loss of inputs and increased economic benefits. Adoption of appropriate plant spacing is one of the important aspects of successful crop production. Optimum plant spacing ensures proper growth and development of plants resulting in maximum yield of crop and economic use of land.

II. MATERIALS AND METHODS

A field trial was done to study the influence of fertigation levels and different spacings on yield and yield attributes of bell pepper under polyhouse condition. The soil of experiment plot was sandy clay loam, acidic in reaction, high in organic carbon and available phosphorus, low in available nitrogen and potassium. The study was laid out in split plot design with 12 treatment combinations replicated three times. The main plot treatments consisted of four levels of fertigation viz., 100 % *adhoc* recommendation of KAU for precision farming (F_1), 50 % *adhoc* recommendation of KAU for precision farming (F_2), 50 % *adhoc* recommendation of KAU for precision farming + foliar spray of combined solution of urea and potassium chloride each at 1.25 % at 30 and 60 DAT (F_3) and 25 % *adhoc* recommendation of KAU for precision farming + foliar spray of combined solution of urea and potassium chloride each at 1.25 % at 30 and 60 DAT (F_4). Farmyard manure @ 25 t ha^{-1} was given as basal to all the treatments. The KAU *adhoc* recommendation of bell pepper for precision farming is 230:25:250 N, P_2O_5 and K_2O kg ha^{-1}as fertigation along with 24 kg ha^{-1} rajphos as basal. The details of nutrients used for fertigation as per KAU adhoc recommendation of bell pepper for precision farming are given in Table 1. For treatments requiring foliar nutrition combined solution of urea and potassium chloride each at 1.25 % was given. The subplot treatments consisted of three spacings viz., 45 cm x 30 cm (S1), 45 cm x 45 cm (S2) and 45 cm x 60 cm (S3).

Table 1. Fertigation schedule as per KAU adhoc recommendation of bell pepper for precision farming

Time of application	Fertilizer	Kg ha^{-1}
Basal	Rajphos	24
3 DAP to 18 DAP	19:19:19	3.150
	13:00:45	3.700
	46:00:00	10.100
	12:61:00	0.000
21 DAP to 54 DAP	19:19:19	1.580
	13:00:45	14.700
	46:00:00	3.700
	12:61:00	0.490
57DAT to 120 DAT	19:19:19	1.580
	13:00:45	14.700
	46:00:00	9.500
	12:61:0 0	0.490

III. RESULTS AND DISCUSSION

Among different fertigation levels F_1 (100 % *adhoc* recommendation of KAU for precision farming) recorded the highest number of fruits $plant^{-1}$ (15.15) followed by F_3 (50 % *adhoc* recommendation of KAU for precision farming + foliar spray at 30 and 60 DAT) (13.49) and F_2 (50 % *adhoc* recommendation of KAU for precision farming) (12.37). Maximum fruit girth (26.24 cm) was recorded from F_1 and it was on par with F_3 (25.24 cm) and significantly superior to F_2 (23.89 cm) and F_4 (22.25 cm). F_1 also recorded higher fruit yield $plant^{-1}$ (1.72 kg) and fruit yield m^{-2} (8.30 kg). Similar findings of increased yield in bell pepper with 252 kg N and 240 kg N was reported by Hartz *et al*. (1993) and Aliyu (2002) respectively. The increased availability of nutrients for the treatment F_1 might have increased the photosynthate accumulation enhancing the yield attributes and yield. Similar finding of increased fruit weight, yield plant $^{-1}$ and yield ha^{-1} with higher dose of NPK (250:200:200 kg ha^{-1}) was reported by Shrivastava (1996).

Yield and yield attributes were significantly influenced by different plant spacings. The treatment S_3 (45 cm x 60 cm) recorded the highest number of fruits $plant^{-1}$ (13.75), fruit girth (25.23 cm) and fruit yield $plant^{-1}$ (1.53 kg). The number of fruits $plant^{-1}$ at 45 cm x 30 cm (S_1) and 45 cm x 45 cm (S_2) were 12.58, 12.67 respectively which were on par The lowest fruit girth was reported by S_1 (23.33 cm) and it was on par with S_2 (24.66 cm). Similarly lowest fruit yield $plant^{-1}$(1.30 kg) was obtained from S_1 (45 cm x 30 cm) and it was on par with S_2 (45 cm x 60 cm) (1.36 kg). Higher yield and yield attributes for S_3might be due to higher availability of sunshine, more space and less competition for nutrients which in turn promoted more number of flowers and increased photosynthate accumulation. Similar results of higher yield and yield attributes with wider spacing in bell pepper were reported by Alam *et al*. (2011) and Biradar *et al*. (2014). Maximum total fruit yield m^{-2} was obtained from closely spaced plants (45 cm x 30 cm) (8.92 kg) followed by S_2 (6.74 kg) and both the treatments were significantly superior over S_3 (5.67). The higher plant population in closer spacing resulted in higher fruit yields m^{-2} compared to wider spacing. Similar findings of increased fruit yield ha^{-1} with closer spacing were reported by Zende (2008) and Shivakumar *et al*. (2012).

Table 2. Effect of fertigation levels on yield and yield attributes of bell pepper under polyhouse condition.

Treatments	Number of fruits plant^{-1}	Fruit girth (cm)	Total Fruit Yield Plant^{-1} (kg)	Total Fruit Yield m^{-2} (kg)
F_1	15.15	26.24	1.72	8.30
F_2	12.37	23.89	1.32	6.84
F_3	13.49	25.24	1.40	7.19
F_4	11.00	22.25	1.15	6.10
CD (0.05)	0.909	1.274	0.120	0.656

Table 3. Effect of different spacings on yield and yield attributes of bell pepper under polyhouse condition.

Treatments	Number of fruits plant^{-1}	Fruit girth (cm)	Total Fruit Yield Plant^{-1}(kg)	Total Fruit Yield m^{-2}(kg)
S_1	12.58	23.33	1.30	8.92
S_2	12.67	24.66	1.36	6.74
S_3	13.75	25.23	1.53	5.67
CD (0.05)	0.797	0.979	0.936	0.502

IV. CONCLUSION

The study revealed that for high yield and yield attributes the bell pepper plants should be treated with 100 % adhoc recommendation of KAU for precision farming. Among different spacing closer spacing of 45 cm x 30 cm is ideal for profitable cultivation of plants under polyhouse. Even though interaction of two treatments was not significant, higher net returns (₹ 1,88,956 10 cents-1) and B: C ratio (3.30) were recorded from the plants treated with 100 % adhoc recommendation of KAU for precision farming along with closer spacing of 45 cm x 30 cm (f_1s_1).

REFERENCES

[1] Alam, M. S., Saha, S. R., Salam, M. A., and Alam, M. K. 2011. Effect of sowing time and plant spacing on the yield and yield attributes of bell pepper (*Capsicum annuum* L.). *Bangladesh J. Agric. Res.* 36(1): 271-278.

[2] Aliyu, L. 2002. Growth and yield of pepper (*Capsicum annuum* L.) as affected by nitrogen and phosphorus application and plant density. *Crop Res. Hisar* 23(3): 467-475.

[3] Biradar, M. S., Patil, A. A., Mantur, S. M., and Mannikeri, I. M. 2014.Influence of growing environment and planting geometry on yield and yield attributes of bell pepper (*Capsicum annuum* L.var. grossum Sendt.) genotypes. *Karnataka J. Agric. Sci.* 27 (2): 202-207.

[4] Hartz, T. K., Legstrange, M., and Mary, D. M. 1993. Nitrogen requirement of drip irrigated pepper. Hortic. Sci. 18(11): 1097-1099.

[5] IIHR [Indian Institute of Horticulture Research]. 2011. Protected Cultivation of Bell pepper. Technical bulletin No.22. IIHR, Bengaluru, 28p.

[6] Joshi, M. C. and D. P. Singh, 1975. Chemical composition in bell pepper. *Indian Hortic*. 20: 19-21.

[7] Ochigbu, A. A. and Harris, G. P. 1989. Effect of film plastic cover on the growth and yield of bush tomato grown in a bed system. *J. Hortic. Sci*. 64(1): 300-302.

[8] Shivakumar, S., Hussain, A., Kurubar, A. R., and Patil, M. G. 2012. Yield and yield parameters of bell pepper under different spacingss and fertilizers levels in shade net. *Asian J. Hortic.* 7(2): 246-249.

[9] Srivastava, A. K. 1996. Effect of fertilizer levels and plant spacings on flowering, fruitset and yield of bell pepper (*Capsicum annuum* var. grossum L.) cv. Hybrid Bharat. *Advan. Plant Sci*. 9(2): 171-175.

[10] Zende, U. M. 2008. Investigation on production techniques in bell pepper under protected cultivation. MSc (Ag) thesis, University of Agricultural Sciences, Dharwad, Karnataka, India, 160p.

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Impact of cluster frontline demonstration programme on the yield of chickpea (*Cicer arietinum* l.) in Mehsana district of Gujarat, India

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Abstract— *Cluster Front Line demonstrations (CFLDs) is a unique approach to provide an direct interface between researcher and farmers as the scientists are directly involved in planning, execution and monitoring of the demonstrations. The present study was conducted to assess the impact of cluster frontline demonstrations of chickpea crop in the Mehsana district of Gujarat state. Chickpea (Cicer arietinum L.) is a highly nutritious grain legume crop and is widely appreciated as health food as well as high return crop. Cluster Front line demonstrations were conducted at 93 farmers' fields under 37.2 ha, to demonstrate production potential and economic benefits of improved technologies. Study revealed that improved cultivation practices comprised under CFLDs viz., improved varieties, proper tillage, proper seed rate, line sowing using seed cum fertilizer drill, seed treatment with chemical fungicide, dual inoculation of Rhizobium + PSB, RDF as per STV, water management at critical stages, weed management and application of IPM module for the management of insect (Specially on gram pod borer) resulted in increase in yield in gram crop over the check plots. The improved technologies gave higher yields and recorded a mean yield of 20.60 and 21.45 q/ha chickpea yield during 2017-18 and 2018-19, respectively which was 22.26 and 16.39 percent higher compared to prevailing farmers practice. The benefit: cost ratios of chick pea cultivation under improved practices were 4.10 and 3.82 as compared to 3.20 and 3.43 under farmer practices for the two consecutive years.*

Keywords— ***Chickpea, Economics, GJG-3, Yield, technology gap, extension gap, technology index, CFLDs.***

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the premier pulse crop widely consumed in India. It is an important *rabi* season food legume having extensive geographical distribution and contributing 39 percent to the total production of pulse in the country (Singh *et al.*, 2013). It is the cheapest source of protein and is the inseparable part of the daily diets of every Indians. It also plays an important role in sustainable agriculture enriching the soil through biological nitrogen fixation (BNF). It is a good source of protein (18-22%), carbohydrate (52- 70%), fat (4-10%), minerals (calcium, phosphorus, iron) and vitamins (Singh *et al.*, 2014). It is an excellent animal feed. Its straw also had good forage value. Chickpea is grown in more than 50 countries (89.7% area in Asia, 4.3% in Africa, 2.6% in Oceania, 2.9% in Americas and 0.4% in Europe). In India, the area under chickpea was 8.39 million hectares with a production of 7.81million tons and productivity of 931kg/ha during rabi-2016-17 (FAOSTAT, 2017). The major chickpea producing states are Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharastra, Andhra Pradesh, Gujarat, Karnataka, Haryana, Bihar and West Bengal. In Gujarat, area under chickpea was 0.295 million hectares with a total production of 0.364 million tones and productivity of 1235 kg/ha during 2017-18 (Anon., 2017). Mehsana district of Gujarat occupies 597 hectares of land and 7670 qt. production with average productivity of 1285 kg/ ha of chickpea(Anon., 2017). Its productivity is far below the potential yield. Abiotic stresses are responsible for declining of yield potential (Singh *et al.*, 2013). For making the nation self- sufficient in pulses, the productivity levels need to be increased substantially from 598 kg per ha to 1,200 kg per ha by 2020 (Ali and Kumar, 2005).Through much progress has been made in the field of agriculture research and education, but benefits of these developments could not be realized by the farming community because of low adoption of technologies at the farmers level. Cluster Front line demonstration (CFLDs) is introduced by the Indian Council of Agricultural Research, New Delhi with inception of technology mission of pulse and oil seed crops during mid eighties. The field demonstration took place under the close supervision of scientist of the KVKs.

Through survey, farm diagnostic visit and farmers meeting it was realized that the reason behind the lower productivity was due to lack of improved variety, no seed treatment, imbalance use of inorganic fertilizers, lack of knowledge about IPM practices etc. Among the biotic stress, the gram pod borer is a major pest occurring for 75 per cent pod damage in the crop (Krishan Kant *et al.*, 2007).To combat the causes of yield reduction and technology gap, dissemination of recommended technologies of chickpea through cluster frontline demonstration were organized at farmer’s field during 2017 -18 and 2018-19.

II. MATERIALS AND METHODS

Krishi Vigyan Kendra, Mehsana of Gujarat state conducted cluster frontline demonstrations on chickpea at farmers' field to assess its performance during Rabi seasons of the year 2017-18 and 2018-19 in different villages of Mehsana district. During these two years, 37.2 hectares with 93 number of demonstration under chickpea were laid out with improved management practices using improved variety GJG-3. In general, the soil of the area under study was sandy loam with low to medium fertility status. Each demonstration was of 0.4 ha area and the components of demonstration comprised of improved varieties, proper tillage, proper seed rate, line sowing using seed cum fertilizer drill, seed treatment with chemical fungicide, dual inoculation of Rhizobium + PSB, RDF as per STV, water management at critical stages, weed management and application of IPM module for the management of gram pod borer and other pests. In the demonstration one control plot was also kept in which the farmers practices were carried out. The sowing was done during Mid November under irrigated conditions and harvested during last fortnight of March. The difference between demonstration package and existing farmers practice are given in Table 1. Full gap was observed in case of use of HYVs, seed treatment, use of micronutrient and pest management and partial gap was observed in fertilizer dose, irrigation and weed management, which definitely was the reason of not achieving potential yield. Farmers were not aware about recommended technologies. Farmers in general used local or old-age varieties instead of the recommended high yielding resistant varieties. Unavailability of seed in time and lack of awareness were the main reasons. Farmers applied higher seed rate than the recommended.

Table 1: Gap analysis between recommended practices and farmer’s practices in chickpea.

Technology	**Improved practices**	**Farmers practice**	**Gap (%)**
Use of HYVs	GJG - 3	Local	Full gap
Land preparation	Ploughing and harrowing	Ploughing and harrowing	Nil
Seed rate	60 kg/ha	65 kg/ha	High seed rate
Sowing method	Line sowing	Line sowing	No gap
Seed treatment	Bio fertilizers and Trichoderma	No seed treatment	Full gap
Fertilizer dose (NPK kg/ha)	20:40:00	Use only DAP	Partial gap
Macronutrient	Sulphur 20 kg/ha	No Macronutrient	Full gap
Weed management	Pre-emergence application of Pendimethalin (0-3 DAS) followed 2 hand weeding at 25 DAS and 55 DAS	Hand weeding	Partial gap
Irrigation	One at branching, flowering, pod	1 – 2 irrigation	Partial gap

	development stage and grain filling stage		
Pest Management (pod borer)	HNPV – 450 LE, Pheromone trap – 10 nos, Neem oil (10000ppm) – 1.8 lit and Beauveria bassiana 2.4 kg per ha.	Indiscriminate application of Mix/different pesticide to control pest	Full gap

Before conducting the demonstration, Krishi Vigyan Kendra is conducted training to the selected farmers on sowing and nutrient management, pest management and post harvest management aspect. The demonstrations on farmers' fields were regularly monitored by scientists of Krishi Vigyan Kendra, Mehsana right from sowing to harvesting. The yield data were collected from both the demonstration and farmers practice using random crop cutting method and analysed by using simple statistical tools. Selection of site and farmers' selection were considered as suggested by Choudhary(1999). The observation on grain yield (qtl/ha) and straw yield (qtl/ha) were recorded. Other parameters like increasing in yield (%), technology gap(%),extension gap(%) and technology index were worked out as suggested by Kadian *et al.*,(1997).The gross return, net return, cost of cultivation and benefit cost ration were also calculated. The data output were collected from both RP as well as farmers practices and finally the extension gap, technology gap, technology index along with benefit cost ratio were workout (Samui *et al.*,2000) as given below:

Increasing yield (%) = Demonstration Yield – Farmers Yield X 100 / Farmers Yield

Technology gap= Potential Yield – Demonstration yield

Extension gap = Demonstration Yield – Farmers yield

Technology index= Potential Yield - Demonstration Yield X 100 / Potential Yield

III. RESULTS AND DISCUSSION

Yield

Cluster Frontline demonstrations (CFLD) are effective tools in introducing various new technologies to the farmers and educational them and to increase the farmer's knowledge and confidence level by comparison of productivity levels between improved production technologies in demonstration trials. The data (Table 2) indicated that the cluster front line demonstration has given a good impact over the farming community of Mehsana district as they were motivated by the new agricultural technologies applied in the demonstrations. Results of 93 frontline demonstrations indicated that the cultivation practices comprised under CFLD viz., use of improved variety (GJG-3), balanced application of fertilizers @ 20:40:0:20 kg NPKS per ha, line sowing, timely weed management, water management at critical stages and control chickpea pod borer through IPM module, produced on an average 20.60 and 21.45 q/ha chickpea yield during 2017-18 and 2018-19, respectively which was 22.26 and 16.39 percent higher compared to prevailing farmers practice (Table 2). The results indicated that the cluster front line demonstrations have given a good impact over the farming community of Mehsana district as they were motivated by the new agricultural technologies applied in the CFLD plots (Table 1). This finding is in corroboration with the findings of Poonia and Pithia (2011) and Raj *et al.* (2013).The data presented in Table 2 indicated that the average yield of chickpea under package demonstration was 21.03 q/ha whereas that the yield under farmers practice was 17.64 q/ha. This indicated that use of improved technology for chickpea production contributed 19.33 per cent higher production than the local practice. The above findings were also similar to the findings of Singh (2002), Poonia and Pithia (2011), Patel *et al.*,(2013) and Raj *et al.*,(2013).

Table 2 : Productivity,Technology gap, Extension gap and Technology Index of Chickpea as grown under CFLD and existing package of practices

Year	**Variety**	**Area (ha)**	**No. of Demo**	**Grain Yield (q/ha)**			**% increase**	**Technology Gap (q/ ha)**	**Extension Gap (q/ ha)**	**Technology Index (%)**
				Potential	**Demo**	**Local**				
2017 - 18	GJG-3	7.20	18	26.25	20.60	16.85	22.26	5.65	3.75	21.52
2018 - 19	GJG-3	30.0	75	26.25	21.45	18.43	16.39	4.80	3.02	18.29
Average	-	-	-	**26.25**	**21.03**	**17.64**	**19.33**	**5.23**	**3.39**	**19.91**
Total	-	**37.20**	**93**	-	-	-	-	-	-	-

Technology gap

The technology gap in the demonstration ranged from 4.80 to 5.65 q/ha yields over potential yield. and average technological gap during the period of study is 5.23 q/ha.(Table 2). The technology gap observed may be attributed to the dissimilarity in soil fertility, salinity and erratic rainfall and other vagaries of weather conditions in the area. Hence, variety wise location specific recommendation appears to be necessary to minimize the technology gap for yield level in different situations and similar finding were found by Mukherjee (2003) and Mitra and Samajdar(2010).

Extension gap

The yield gaps presently ranging between 3.02 to 3.75 q/ha. The average extension gap during the period of study was 3.39 q/ha(Table 2). This emphasized the need to educate the farmers through various means for the adoption of improved agricultural production technologies to reverse this trend of wide extension gap. More and more use of latest production technologies with high yielding variety will subsequently change this alarming trend of galloping extension gap. The new technologies will eventually lead to the farmers to discontinue the old technology and to adopt new technology (Table 1). This finding is in corroboration with the findings of Hiremath and Nagaraju (2010).

Technology Index

The technology index shows the feasibility of the evolved technology at the farmer's fields and the lower the value of technology index more is the feasibility of the technology (Jeengar *et al.* 2006). As such, fluctuation in the technology index was ranged from 18.29 % in 2018 - 19 to 21.52 % in 2017-18 and average technology index during the period of study is 19.91% (Table2) These findings corroborate with the findings of Mokidue *et al.*, (2011) and Tomar (2010).

Economic

The input and output prices of commodities prevailed during each year of demonstration were taken for calculating cost of cultivation, gross return, net return and benefit cost ratio (Table 3). The net return from recommended practices was Rs. 66023 to Rs. 76994 while the net return from farmer practices was Rs. 48951 to Rs. 63199.It means that net return from demonstration was higher than the farmer practices. The average additional cost during the period of study was Rs.137 per ha and additional net return was Rs.15434 per ha. The increase benefit: cost ratio was also calculated. The benefit cost ratios of under recommended practices were higher (4.10 and 3.82) as compared to farmers practice (3.20 and 3.43). This may be due to higher yield obtained under recommended practices compared to farmer's practices. Thus, it was clearly showed that the demonstration of chickpea with full package was better than farmer's practices. Similar result has been reported by earlier by Teggelli *et al.*(2015), Tomar; (2010) and Mokidue *et al* ;(2011), Prajapati *et al.(2019),* Upesh Kumar *et al.(2019)*

Table 3: Economics evaluation of demonstrated package of practices

Year	**Grain Yield (q/ha)**		**Biological Yield(/ha)**		**Gross Expenditure (Rs/ha)**		**Gross Return (Rs/ha)**		**Net Returns (Rs/ha**		**B:C Ratio**	
	Demo	**Local**	**Demo**	**Local**	**Demo**	**Local**	**Demo**	**Local**	**Demo**	**Local**	**Demo**	**Local**
2017 - 18	20.60	16.85	19.70	15.25	21302	22262	87325	71213	66023	48951	4.10	3.20
2018 - 19	21.45	18.43	20.75	16.45	27292	26060	104287	89259	76994	63199	3.82	3.43
Average	**21.03**	**17.64**	**20.23**	**15.85**	**24297**	**24161**	**95806**	**80236**	**71509**	**56075**	**3.96**	**3.32**

IV. CONCLUSION

The productivity enhancement under cluster front line demonstration over traditional method of rabi chickpea cultivation created greater awareness and motivated the other farmers to adopted appropriate production technology of chickpea in district. The selection of specific technology like use of improved variety (GJG-3), balanced application of fertilizers (N:P:K:S @20:40:0:20 kg NPKS per ha) , line sowing, timely weed management, water management at critical stages and control chickpea Pod borer through IPM module were undertaken in a proper way. Cluster Frontline demonstration was effective in changing attitude of farmers towards pulse cultivation. Cultivation of demonstrated plots of rabi chickpea with improved technologies has increased the skill and knowledge of the farmers. Cluster Front line demonstration also helped in replacement of local varieties with improved recommended varieties. This also improved the relationship between farmers and scientist and built confidence between them. These technology maybe popularize through enhancing awareness among the farming community by regular campaigning of the technology, conduct large scale/ cluster demonstration, distribution of literature in local language, develop success cases/ model cases, use of ICT media like- Video conferencing, Kisan Mobile Sandesh, Whats app etc.

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REFERENCES

[1] Ali, M. and Kumar, S. 2005. Yet to see a breakthrough. In: Survey of Indian agriculture. The Hindu, Chennai, India, pp. 55-56.
[2] Anonymous.2017. Second advanced estimates of area, production and yield of major *kharif/rabi*crops of Gujarat State for the year 2017-18, Director of Agriculture, Gujarat State, Gandhinagar,2017.
[3] Choudhary, B. N. (1999). KrishiVigyan Kendra- a guide for KVK manager. Division of Agriculture Extension, ICAR, pp. 73-78.
[4] FAOSTAT, 2017. FAOSTAT Agriculture data. Available at http://faostat.fao.org/>accessed 11 March,2019.
[5] Hiremath, S. M. and Nagaraju, M. V. 2010. Evaluation of on-farm front line demonstrations on the yield of chilli. *Karnataka J of Agricultural Sciences*,**23(2):**341-342.
[6] Jeengar K. L., Panwar P., Pareek O.P. 2006. Front line demonstration on maize in Bhilwara District of Rajasthan. Current Agriculture. **30(1/2):**115-116.
[7] Kadian, K. S., Sharma, R. and Sharma, A. K. 1997. Evaluation of front line demonstration trials on oilseeds in KangraVally of Himachal Pradesh. *Ann. Agric. Res.***18**:40.
[8] Mitra, B. and Samajdar, T. 2010. Field gap analysis of rapeseed-mustard through front line demonstration. *Agricultural Extension Review*, **22:** 16-17.
[9] Mokidue, I., Mohanty, A. K. and Sanjay, K. 2011. Correlating growth, yield and adoption of uradbean technologies, *Indian Journal of Extension Education*, **11(2):**20-24.
[10] Mukherjee, N. (2003). Participatory, Learning and Action. Concept Publishing Company, New Delhi, pp. 63-65.
[11] Patel, M. M., Jhajharia, A. K., Khadda, B. S. and Patil, L. M. 2013. Front-line Demonstration: An effective communication approach for dissemination of sustainable cotton production technology. *Indian Journal of Extension Education and Rural Development*, **21:**60-62.
[12] Prajapati, P. J., Joshi, N. S., Patel, M.L., Parmar, V.S., Gadhiya, K.K. and Hadiya, N.J. 2019. Impact of frontline demonstrations on yield of chickpea (*Cicer arietinum L.*) in Amreli district of Gujarat state. J. of Pharmacognosy and Phytochemistry; **8(2):** 1431-1433.

[13] Poonia, T.C. and Pithia, M.S. 2011. Impact of front line demonstrations of chickpea in Gujarat. Legume Research.**34(4):**304-307.
[14] Raj A.D., Yadav, V. and Rathod, J. H. 2013. Impact of Front Line Demonstrations (FLD) on the Yield of Pulses. International Journal of Scientific and Research Publications. **3(9):**1-4.
[15] Samui, S.K., Mitra, S., Roy, D.K., Mandal, A.K. and Saha, D. 2000. Evaluation of front line demonstration on groundnut. Journal of the Indian Society Costal Agricultural Research.**18(2):**180-183.
[16] Singh, A. K., Manibhushan, Bhatt, B.P., Singh, K.M. and Upadhyaya A. 2013. An Analysis of Oilseeds and Pulses Scenario in Eastern India during 2050-51. *Journal of Agril. Sci.*5 **(1) :** 241- 249.
[17] Singh, D., Patel, A.K., Baghel, S.K., Singh, M.S., Singh, A. and Singh, A.K. 2014. Impact of front line demonstration on the yield and economics of chickpea (*Cicer arietinum* L.) in Sidhi district of Madhya Pradesh. *J. Agric. Res.,* **1(1):** 22-25.
[18] Singh, PK. 2002. Impact of participation in planning on adoption of new technology through FLD. *MANAGE Extension Research Review*, July-Dec.45-48.
[19] Teggelli, Raju G., Patil,D.H., Naik, Ananda, Zaheer Ahamed,B. and Patil,M.C. 2015. Impact of frontline demonstration on the yield and economics of pigeonpea in Kalaburgi district of Karnataka State. *I.J.S.N.*,**6(2):**224-227.
[20] Tomar, R. K. S. 2010. Maximization of productivity for chickpea (*Cicer arietinum L*.) through improved technologies in farmer's field. *Indian Journal of Natural Products and Resources* **1(4):** 515-517.
[21] Upesh Kumar, G.A. Patel, H.P. Patel, R.P. Chudhari and S.S. Darji. 2019. Impact of front line demonstration programme on the yield of chickpea (*Cicer arietinum L.*) in Patan District of Gujarat,India. Agricultural Research Communication centre Journals, DOI:10.18805/LR4081.

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Effect of Different Planting Time on *Tomato Yellow Leaf Curl Virus* (TYLCV) of Tomato and Its Impact on Yield in Bangladesh

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***Abstract*— *Tomato yellow leaf curl virus (TYLCV) is infamous for tomato-infecting viruses and causes a huge loss of the yield irrespective of the planting time. So, in order to find out the effect of different planting time on TYLCV disease of tomato and its impact on yield. A study was carried out at the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh during the period of October 2016 to May 2017. Two popular BARI released variety namely BARI Tomato-14 and BARI Tomato-16 were used in this study that was transplanted at three different planting times ($1^{s}t$ planting time on 1^{st} November 2^{nd} planting time on 15^{th} November and 3^{rd} planting time on 1^{st} December 2016). The lowest percent TYLC disease incidence (12.42%) and percent disease severity (15.37%) were found in 3^{rd} planting (1^{st} December) in BARI Tomato-16 variety, respectively. Under the present study, considering the percentage of TYLC disease incidence and severity, BARI tomato-16 on 1^{st} December showed better performance comparing others. The 3^{rd} planting (1^{st} December) and BARI Tomato-16 variety were found to have the highest yield (77.23 ton/ha) and promising the lowest level of percentage of disease incidence (12.42%) and percent disease severity (15.37%) against TYLCV. A strong positive correlation was obtained between the whitefly population and the percent disease incidence of TYLCV. The whitefly population and the yield of tomato were negatively correlated with each other. The yield was also found significant and negatively correlated with the percentage of TYLCV disease incidence*.

***Keywords*— TYLCV, tomato, disease incidence, severity, planting time, yield.**

I. INTRODUCTION

Tomato (*Solanum lycopersicum L.*) is a popular vegetable crop in Bangladesh as well as many countries in the world. According to FAO [1], in world vegetable production, it ranks 2nd in the world's vegetable production next to potato. Wilcox *et al*. [2] studied the significant role of tomato in human nutrition, because of its rich source of lycopene, minerals and vitamins such as ascorbic acid (Vitamin-C) and β-carotene (Vitamin-A) which are antioxidants and promote good health. About 178 million tons of tomatoes were produced in the world in 2016 among which only 368,121 tons produced in Bangladesh, as reported by FAO [1]. The low yield of tomato in Bangladesh is, however, not an indication of the low yielding potentiality of this crop, but the fact that the lower yield may be attributed to a number of reasons like use of low yielding variety, unavailability of quality seeds of improved varieties, disease infection, improper irrigation and fertilizer management etc. Among the factors Lukyanenko [3] has reported that tomato is susceptible to more than 200 diseases and losses of the yield due to the disease as high as 71- 95%. By all, *Tobacco mosaic virus* (TMV), *Tomato leaf curl virus* (TLCV) and *Tomato yellow leaf curl virus* (TYLCV) caused 80, 90 and 100% yield loss of tomato, respectively, as investigated by Martelli and Quacquarelli [4].

The *Tomato yellow leaf curl virus*, better known as TYLCV, has reported to be a major menace, which limits the tomato cultivation in all tomato growing areas of the world, as stated by Green and Kalloo [5], Brunt *et al*. [6] and Kalloo [7]. In Bangladesh, the prevalence of TYLCV was first noted by Akanda [8] and the damage may reach even up to 100% depending on the varieties and stage of infection. The virus is mechanically non-transmissible, graft transmitted, transmitted by whitefly (*Bemisia tabaci*) in the field. Since it appeared in epidemic form for the last few years, various strategies have been pursued to control the disease. The efforts have been made to characterize the virus systematically, manage the disease through manipulation of sowing dates, growing seedlings in net house and application of insecticides, as reported by Paul [9], Rahman [10], Gupta [11], Azam [12], Akhter [13] and Sultana [14]. Nevertheless, developing resistant variety is the best option for the control of TYLCV, but none of the tomato varieties cultivated in our country is found to have resistance or tolerance to the virus, as noted by Rahman *et al*. [15]. Hence, the management of TYLCV in Bangladesh is of immensely important to reduce the crop loss and to minimize the deterioration quality, so that the cultivation of tomato could be profitable for farmers.

As the disease caused heavy loss to tomato in many countries, the development of suitable management practices is of utmost importance. It needs an in-depth investigation of the prevalence of the virus in different tomato varieties, the crop damage with respect to the stage of plant infected by the virus, etc. Considering the importance of the above background, the present research program was designed to know the effect of different planting times on the prevalence of TYLCV and whitefly in tomato.

The proposed research work will be carried out to achieve the following specific objectives:

- To evaluate the incidence and severity of *Tomato yellow leaf curl virus* (TYLCV) against two popular cultivars of tomato.
- To find out a suitable planting time of tomato for the reduction of TYLCV incidence.
- To see the effect of Tomato yellow leaf curl disease on the impact of the yield of tomato.

II. MATERIALS AND METHODS

The experiment was conducted at the research field of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207. For fulfilling the objectives of the experiment, the seeds of BARI Tomato-14 (2007) and BARI Tomato-16 (2015) were collected from Vegetable Division, Horticulture Research Centre (HRC), BARI, Joydebpur, Gazipur-1701, Bangladesh. Later the seeds were raised in 6 seedbeds of 1 Sq. Meter and 10grams of each variety's seeds were sown in the seedbed on 01st October 2016, 15th October 2016, and 01st November 2016 as 1st, 2nd, and 3rd planting time respectively. After sowing, seeds were then covered with finished light soil. Then light watering, shading, weeding, and mulching were done as necessary to provide seedlings a good environment for vigorous growth and development. The field was plowed, cross-plowed, cleaned, leveled, Furadan10G application for controlling soil-borne pathogen and fertilizer application (as per recommended dose by BARI, 2005) were done in a sequential manner.

The layout of the experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The experimental site was refuted into 3 blocks each containing 6 plots of 2.45m x 2.30m in size, having a total of 18 plots. The drain was kept 1.0 m between the blocks and 0.5 m between the plots. The distance between row to row and plant to plant was 65 cm and 60 cm, respectively. Healthy and uniform seedlings were transplanted in the experimental plot allowing an accommodation of 16 plants per plot.

In the field, intercultural operations like irrigation, drainage, stalking, weeding, top-dressing, etc. were accomplished to enhance the growth and development of the tomato seedlings. Fruits were harvested at 5 days intervals during maturity to ripening stage started from 20th February 2017 and completed by 30th May 2017. The identification of *Tomato Yellow Leaf Curl Virus* (TYLCV) was based on typical field symptoms as described by Akanda [8], Alam [16], and Gupta [11]. The plants were inspected at 20, 30, 40, 50, and 60 DAT to observe the appearance and development of the symptoms of TYLCV at three planting time (Figure 1).

A: Healthy Plant | *B: Early TYLCV Symptoms* | *C: Mid TYLCV Symptoms* | *D: Severe TYLCV Symptoms*

Fig.1: (A) Healthy Tomato Plant, (B-D) TYLCV Symptoms at Tomato Plant

Collecting data on the following parameters-

- Number of leaves/plant
- Number of infected leaves/plant
- Number of infected plants
- Number of branch/plant
- Number of flowers/plant
- Number of fruits/plant
- Fruit weight/plant (kg)
- Fruit weight/plot (kg)*
- Plant height (cm)
- Fruit yield (kg/ha)

All these entities were documented from 20, 30, 40, 50, and 60 DAT for better observation of the occurrences.

As for Disease Incidence (%), the calculation was followed by the formula which was used by Ashrafuzzaman [17]:

$$\text{Disease Incidence (\%)} = \frac{\text{Number of diseased plant /leaves}}{\text{Number of total plants/leaves observed}} \times 100$$

Likewise, the Disease Severity (%) was calculated using the following formula which was used by Ashrafuzzman [17]:

$$\text{Disease Severity (\%)} = \frac{\text{Amount of tissue infected}}{\text{Total area inspected}} \times 100$$

The scaling was done based on the Disease Rating Scale given by Ali *et al*. [18].

Table-1: Disease Rating Scale of TYLCV

Rating	Scale	Severity Range (%)
0	Immune	0
1	Highly Resistant	1-10
2	Moderate Resistant	11-25
3	Tolerant	26-50
4	Moderate Susceptibility	51-60
5	Susceptibility	61-70
6	High Susceptibility	71-100

Source: Ali et al., (2005)

The data obtained for different characters were statistically analyzed using MSTAT-C software. To calculate the level of significant difference and to separate the means within the parameters Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) test were performed at a 5% level of significance. Graphs and charts were also done to see the interpretation of different parameters.

III. RESULTS

1. Effect of planting time on disease incidence (%) and disease severity (%) of TYLCV between tomato varieties

There were significant differences found among different planting times between two tomato varieties as shown in table 2. The TYLCV disease incidence (%) ranged from 12.417 to 76.833 among three planting time. The highest TYLCV incidence was observed on 1st November in BARI Tomato-14 (76.833%) followed by BARI Tomato-16 (62.50%). Then again, the lowest TYLCV incidence (%) was found in the 1st December planting time in BARI Tomato-16 (12.417) followed by the same planting time in BARI Tomato-14 (21.67). On the other hand, TYLCV severity (%) ranged from 15.370 to 69.217. The highest TYLCV severity (%) was observed in 1st November in BARI Tomato-14 (69.217) followed by BARI Tomato-16 (57.193) and the lowest TYLCV severity (%) was found in 1st December in BARI Tomato-16 (15.370) followed by same planting time in BARI Tomato-14 (23.557).

Table-2: Effect of Three Planting Time on TYLCV Disease Incidence (%) and Disease Severity (%) Between Two Varieties

Treatment	Variety	Disease Incidence (%)	Disease Severity (%)
1st November	BARI Tomato-14	76.833 a*	69.217 a
	BARI Tomato-16	62.500 b	57.193 b
15th November	BARI Tomato-14	39.333 c	42.050 c
	BARI Tomato-16	30.000 cd	35.283 d
1st December	BARI Tomato-14	21.667 de	23.557 e
	BARI Tomato-16	12.417 e	15.370 f

LSD (0.05)	10.006	5.7692
CV (%)	8.74	5.04

*Means followed by same letters not significantly different at 5% level by LSD

2. Effect of growth characters due to *Tomato yellow leaf curl virus* (TYLCV) disease infection between two tomato varieties

Growth contributing characters were affected due to TYLCV infection at three planting time between two tomato varieties. The effects of growth and growth contributing characters due to TYLCV are shown in table 3. The maximum number of leaves per plant (80.33) was observed at 2nd and 3rd planting time in BARI Tomato-16 followed by 1st planting in the same variety (79.00). Whereas, the minimum number of leaves/plant (73.67) was found in 1st planting (1st November) in BARI Tomato-14. The highest number of branch/plant (10.667) was observed at 3rd planting in BARI Tomato-16 followed by BARI Tomato-14 (79.000). The lowest number of branch/plant (5.333) was found at 1st planting in BARI Tomato-14. In the case of the number of flower number per plant, the maximum number of flower/plant (76.000) was observed in 3rd planting in BARI Tomato-16 followed by 2nd planting in BARI Tomato-16 (70.000). The minimum number of flower/plant (41.000) was found in 1st planting in BARI Tomato-14 followed by BARI Tomato-16 (54.333) in the same planting. Likewise, the plant height ranged from 68.167 cm to 95.200 cm, while the tallest plant (95.200 cm) was found in 3rd planting in BARI Tomato-14 followed by the same planting time in BARI Tomato-16 (88.767 cm). The lowest plant height (68.167 cm) was recorded in 1st planting of BARI Tomato-16 variety followed by BARI Tomato-14 (73.800 cm) variety.

Table-3: Effect of Three Planting Time on Growth and Growth Contributing Character Between Two Tomato Varieties Against Tomato Yellow Leaf Curl Virus (TYLCV)

Treatment	Variety	Leaves/ plant (No.)	Branch/ plant (No.)	Flower/ plant (No.)	Plant height (cm)
1st November (1st Planting)	BARI Tomato-14	73.667 b*	5.333 d	41.000 e	73.800 e
	BARI Tomato-16	79.000 a	5.667 b	54.333 d	68.167 f
15th November (2nd Planting)	BARI Tomato-14	75.333 b	7.667 c	57.000 cd	84.500 c
	BARI Tomato-16	80.333 a	8.667 bc	70.000 b	78.933 d
1st December (3rd Planting)	BARI Tomato-14	75.333 b	10.333 ab	60.667 c	95.200 a
	BARI Tomato-16	80.333 a	10.667 a	76.000 a	88.767 b
LSD (0.05)		2.5323	1.6882	4.2937	2.5141
CV (%)		1.16	7.40	2.53	1.09

*Means followed by same letters not significantly different at 5% level by LSD

3. Effect of yield and yield characters due to *Tomato yellow leaf curl virus* (TYLCV) disease infection between two tomato varieties

Yield and yield contributing characters of the tomato were affected due to TYLCV infection at different planting times. The effects of the yield and yield contributing characters due to TYLCV are shown in table 4. The highest number of fruits per plant (40.00) was observed in 3rd planting in BARI Tomato-16 followed by 2nd planting in BARI Tomato-16 (37.333) and the lowest number of fruits/plant (21.333) was found in 1st planting in BARI Tomato-14 followed by same planting time in BARI Tomato-16 (28.333). As for the range of fruit weight/plant, the maximum fruit weight/plant (2.72 kg) was observed in 3rd planting in BARI Tomato-16 followed by BARI Tomato-14 (2.52 kg) and the minimum fruit weight/plant (1.6667 kg) was found in 1st planting of BARI Tomato-14 followed by same planting time in BARI Tomato-16 (1.9267 kg). Fruit yield between two tomato varieties at three planting, the highest fruit yield (43.52 kg/plot = 77.23 ton/ha) was found in 3rd planting in BARI Tomato-16 followed by 2nd planting in BARI Tomato-16 (40.64 kg/plot = 72.12 ton/ha). On the contrary, the lowest fruit yield was recorded in 1st planting in BARI Tomato-14 (26.667 kg/plot = 47.32 ton/ha) followed by the same planting time in BARI Tomato-16 (30.827 kg/plot = 54.71 ton/ha).

Table-4: Effect of Different Planting Time on Yield and Yield Contributing Character between Two Tomato Varieties against Tomato Yellow Leaf Curl Virus (TYLCV)

Treatment	Variety	Fruits/ plant (No.)	Fruit weight/ plant (Kg)	Yield (kg/plot)	Fruit yield (ton/ha)
1st November (1st Planting)	BARI Tomato-14	21.333 e*	1.6667 e	26.667 e	47.323 e
	BARI Tomato-16	28.333 d	1.9267 d	30.827 d	54.706 d
15thNovember (2nd Planting)	BARI Tomato-14	29.667 d	2.3133 c	37.013 c	65.685 c
	BARI Tomato-16	37.333 b	2.5400 b	40.640 b	72.121 b
1st December (3rd Planting)	BARI Tomato-14	32.333 c	2.5200 b	40.320 b	71.553 b
	BARI Tomato-16	40.000 a	2.7200 a	43.520 a	77.232 a
LSD $_{(0.05)}$		2.0020	0.1456	2.3300	4.1351
CV (%)		2.24	2.25	2.25	2.26

*Means followed by same letters not significantly different at 5% level by LSD

4. Relation between average whitefly population and TYLCV disease incidence (%)

The relationship between the whitefly population and disease incidence (%) of TYLCV in the field is shown in figure 2. A strong positive correlation exists between the incidence (%) of TYLCV infection and the whitefly population. It means that with the rise of the whitefly population, TYLCV infection also increases. A regression line was fitted between the whitefly population and % incidence of TYLCV. The correlation coefficient (r) was 0.9435227** and the contribution of the regression ($R2$= 0.8902) indicated that 89.02% TYLCV infection increased by whitefly.

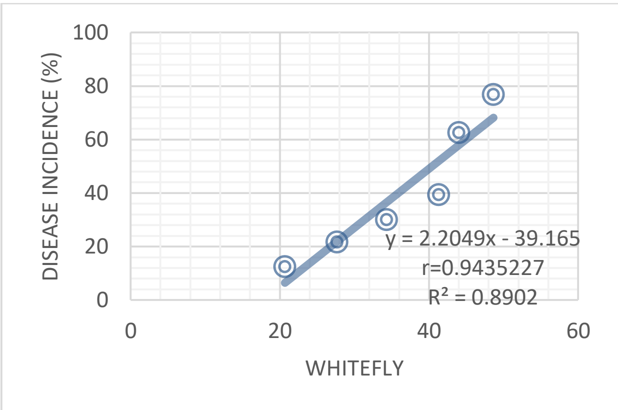

Fig.2: Relation Between Whitefly Populations Build up and % TYLCV Incidence

5. Relation between TYLCV disease incidence (%) and yield (ton/ha) of tomato

A significant negative correlation was found between the incidence of TYLCV (%) and the yield of tomato is shown in figure 3. It means that with the increase of incidence of TYLCV (%), the yield of tomato reduced. A regression line was fitted between % incidence of TYLCV and the yield of tomato. The correlation coefficient (r) was -0.991826366** and the contribution of the regression (R^2 = 0.9837) indicates that 98.37 % yield in tomato would be affected by TYLCV infection.

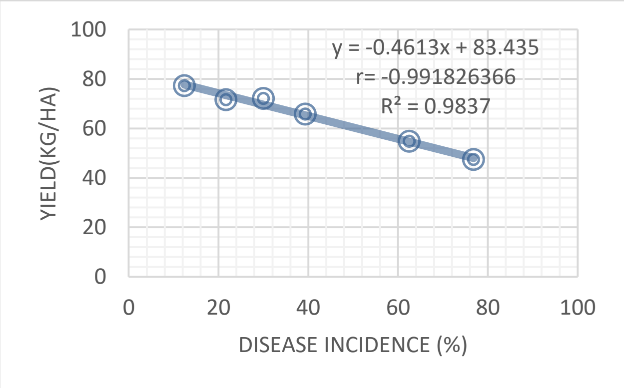

Fig.3: Relation Between Incidence of TYLCV (%) and Yield of Tomato

6. Relation between average whitefly population and yield (ton/ha) of tomato

Following a negative correlation was found between the whitefly population and yield of tomato is shown in figure 4. This indicates that with the rise of the whitefly population, the yield of tomato decreased. A regression line was fitted between the whitefly population and yield of tomato. The correlation coefficient (r) was -0.913954412** and the contribution of the regression (R^2 = 0.8353) indicates that 83.53% yield in tomato would be affected by whitefly.

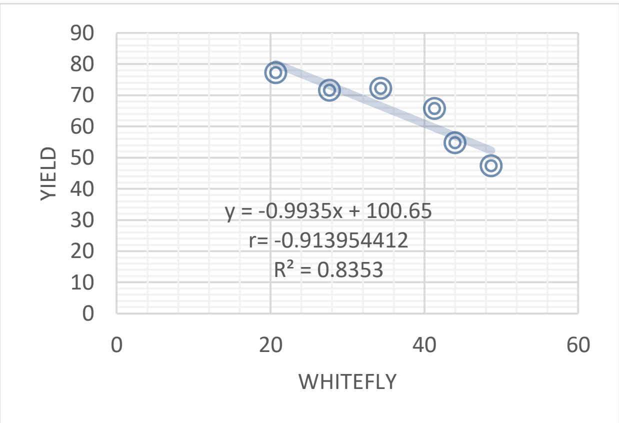

Fig.4: Relation Between Whitefly Population and Yield of Tomato

IV. DISCUSSION

Tomato (*Solanum lycopersicum*) belongs to the Solanaceae family which is a popular vegetable crop in Bangladesh as well as many countries around the world. Among the virus diseases of tomato, TYLCV is the major one with respect to prevalence, severity, and damage to the crop in all tomato growing areas in the world which alone could cause 100% yield loss of the crop. To study the effect of different planting time on *Tomato yellow leaf curl virus* (TYLCV) and its impact on the yield of tomato a field experiment was conducted at the Research farm of Sher-e-Bangla Agricultural University, Dhaka, during the Rabi season from October' 2016 to March' 2017. There were two factors in the experiment comprising three levels of planting time (1st November 15th November and 1st December) and two levels of variety as BARI Tomato-14 and BARI Tomato-16.

1. Effect of TYLCV disease incidence (%) and severity (%) between tomato varieties

The results of the present study indicated that tomato yield was seriously affected by the date of planting because it has a high sensitivity towards temperature for fruit set. High temperature brings down fruit setting and ultimately effect on yield. So, during flowering time tomato needs optimum temperature for better yield. As we get that, the 3rd planting (1st December planting) and the BARI Tomato-16 between two varieties performed better in the case of TYLCV disease incidence (12.417%) and severity (15.37%). On the other hand, the highest TYLCV disease incidence (76.833%) and severity (69.217%) was found in 1st planting of BARI Tomato-14. Almost such type of investigation on different planting time and varietal performance against the TYLCV disease incidence (%) and severity (%) in tomato field was obtained by Mazyad *et al.* [19], Pilowsky *et al.* [20], Gupta [11], Azam [12], Paul[9] and Rashid *et al.* [21].

2. Outcome of yield and growth of tomatoes on three planting time

Likewise, the highest number of branch/plant (10.67), fruit/plant (40.00), flower/plant (76.00), fruit weight/plant (2.72 Kg), fruit yield (77.23 ton/ha) was observed in 3rd planting of BARI Tomato-16 and as for the lowest number of branch/plant (5.33), fruit/plant (21.33), flower/plant (41.00), fruit weight/plant (1.67 Kg), fruit yield (47.32 ton/ha) was recorded in 1st planting of BARI Tomato-14. These results go in agreement with Sinisterra *et al.* [22] findings which signify that branch number reduced more in the early planting than the late planting and the fruits number reduced more in the early planting than the late planting due to TYLCV infection. In agreement with Gupta

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[11], the late planting produced more flowers than the early planting. The late planting had more fruit weight/plant than the early planting and the higher severity of TYLCV was one of the reasons for reduction of fruit yield in tomato plants, in agreement with Lukyanenko [3] and Polston *et al*. [23] as they reported that TYLCV is caused a reduction of the yield and pointed out that TYLCV transmitted by whitefly is the most serious disease of tomato in tropical and subtropical Asian countries and parts of Africa, respectively. The highest number of leaves/plant (80.333) was observed in the 2nd planting of BARI Tomato-16 and the lowest number of leaves/plant (73.667) was found in 1st planting in BARI Tomato-14. From this result, it can be concluded that the number of leaves/plants reduced more in the early planting compared to the late planting. However, the tallest plant (95.20cm) was found in the 3rd planting of BARI Tomato-14 and the lowest plant height (68.167cm) was recorded in the 1st planting of BARI Tomato-16. This result indicated that there were significant differences between the late planting and the early planting. Results indicated that the late planting obtained maximum heights than the early planting these types of findings were also reported by Rahman [10]. The results indicated that the yield of tomato was positively influenced by the number of leaves, number of flowers, plant height, number of fruits, fruit weight. The results of the study agree with the findings of Mohanty [24-26].

The highest number of whiteflies per plant (48.667) was observed in the 1st planting of BARI Tomato-14. The lowest number of whitefly/plant (20.667) was found in the 3rd planting of BARI Tomato-16. The results of this present study revealed that the whitefly number reduced more in the late planting than the early planting. Verma *et al*. [27] stated that the incidence of TYLCV on tomato was directly related to the population density of the vector developed when the incidence of the disease also began to increase. The increase of the whitefly population was also found to be positively correlated with the spread of TYLCV in the field as reported by Mehta *et al*. [28], Gupta [11], and Paul [9].

Cohen and Nitzany [29] reported that TYLCV shows great regional and seasonal variations mainly because of fluctuations in the population density of the whitefly vector. For this, the relationship between the whitefly population and incidence of TYLCV was investigated. A positive correlation between the incidence of TYLCV and 46 whitefly population (0.9435227) was recorded which was supported by Saikia and Muniyappa [30], Polizzi *et al*. [31] and Aboul-Ata *et al*. [32]. The present study also revealed the relationship between the whitefly population and yield of tomato. A negative correlation (r = -0.913954412) between the whitefly population and yield of tomato was recorded which is an accordance with the findings of Gupta [11]. A negative correlation (r = -0.991826366) between the incidence of TYLCV and the yield was also obtained that has also been supported by Gupta [11].

V. CONCLUSION

The results indicated that the percentage of TYLCV disease incidence and disease severity was lowest in 3rd planting (1st December) of BARI Tomato-16 variety. It also indicated that higher incidence and severity of TYLCV were one of the reasons for the reduction of fruit yield in tomato plants which are greatly dependent on planting time. Not to mention, among all the 3rd planting of BARI Tomato-16 varieties had maximum growth and growth contributing characters like as the number of leaves, branch flower, and plant height, indicated that the yield of tomato was increased by a number of leaves, branch flower, and plant height. As for yield and yield contributing characters like as number of fruits, fruit weight/plant, fruit yield (kg/plot), fruit yield (kg/ha), and fruit yield (ton/ha) were highest in the 3rd planting of BARI Tomato-16 varieties.

Other results also revealed that the whitefly population reduced more in the late planting than the early planting showing a positive correlation between the whitefly population and disease incidence (%) of TYLCV (0.9435227**) with temperature. A negative correlation (r = -0.991826366**) between the incidence of TYLCV and yield was also obtained in this study giving us a negative correlation (r = -0.913954412**) between the whitefly population and yield of tomato was also recorded. Considering the outcome of the study, there is still a need for a resistant variety and better cultural management to inhibit the growth of the disease and for farmers, late planting is the best to get high yield amid the TYLCV.

REFERENCES

[1] FAO., 2016. FAOSTAT database. Food and Agriculture Organization of the United Nations, Geneva. http://www.fao.org/faostat/en/#data/QC/visualize.

[2] Willcox, J.K., Catignani, G.L. and Lazarus, S. 2003. Tomatoes and cardiovascular health. Critical Reviews in Food Science and Nutrition, 43(1). pp.1-18.

[3] Lukyanenko, A.N. 1991. Disease Resistance in Tomato. In: Kalloo, G. (Ed.). Genetic Improvement of Tomato. 14, Monographs on Theoretical and Applied Genetics, Germany: Springer-Verlag Berlin Heidelberg, pp. 99-119.

[4] Martelli, G.P. and Quacquarelli, A. 1983. The present status of tomato and pepper viruses. Acta Horticulture, 127, pp.39-64.
[5] Green, S. K. and Kalloo, G. 1994. Leaf curl and yellowing viruses of pepper and tomato: an overview. Technical Bulletin No. 21. Asian Vegetable Research and Development Center, Taiwan. p.51.
[6] Brunt, A., Crabtree K., and Gibbs. A. (Edt.). 1990. Tomato yellow leaf and Gemini virus. In: Viruses of Tropical Plants. CAB International, Wallingford, Oxon, OX-10 eight DE, UK., pp. 578-580.
[7] Kalloo, G. (Ed.). 1991. Genetic Improvement of Tomato Series: Monographs on Theoretical and Applied Genetics. Germany: Springer-Verlag Berlin Heidelberg.
[8] Akanda, A.M. 1991. Studies on the virus and mycoplasma disease of vegetable crops in Bangladesh. A thesis for the partial fulfillment of Doctor of Agriculture. Submitted to the Plant Pathology Laboratory. Department of Agronomy, Faculty of Agriculture, Kyushu University, Fukuoka, Japan. p.181.
[9] Paul, S.K. 2002. Management of TYLCV in respect to growth and yield of tomato using net house grown seedlings and insecticidal spray. A thesis submitted to the Department of Plant Pathology, BSMRAU, Gazipur-1706 for the partial fulfillment of M.S. in Plant Pathology.
[10] Rahman, A. H. M. A. 2003. Growth and yield performance of eight tomato varieties infected with *Tomato yellow leaf curl virus* (TYLCV) under field condition. An MS thesis submitted to the Department of Plant Pathology, BSMRAU, Salna, Gazipur, Bangladesh. p.140.
[11] Gupta, N.D. 2000. Occurrence of *Tomato yellow leaf curl virus* (TYLCV) and *Tomato purple vein virus* (TPVV) and their effect on growth and yield of tomato. M.S. Thesis, Department of Plant Pathology, BSMRAU.
[12] Azam, M. R. 2001. Prevalence and effect of *Tomato yellow leaf curl virus* (TYLCV) on growth and yield of net house grown seedlings of tomato. M.S. Thesis, Department of Plant Pathology, BSMRAU, Gazipur. p.69.
[13] Akhter, K. 2003. Effect of date of sowing on the prevalence of *Tomato yellow leaf curl virus* (TYLCV) and impact of TYLCV infection on growth and yield contributing characters of tomato varieties. An M.S. Thesis submitted to the Department of Plant Pathology, BSMRAU, Gazipur-1706.
[14] Sultana, N. A. 2001. An investigation to some aspects of TYLCV and TPVV infecting tomato. An MS thesis submitted to the Department of Plant Pathology, BSMRAU, Salna, Gazipur, Bangladesh. p.90.
[15] Rahman, A.H.M.A., Akanda, A.M. and Alam, A.K.M. 2008. Relationship of Whitefly Population Build up with the Spread of TYLCV on Eight Tomato Varieties. Journal of Agriculture and Rural Development, 4(1 & 2). pp. 67-74.
[16] Alam, N. 1995. Studies on the virus diseases of tomato in Bangladesh. An M. Phil thesis submitted to the Department of Botany, Jahangirrnagar University, Savar, Dhaka. pp. 54-77.
[17] Ashrafuzzaman, 2016. Management of early blight of tomato through different botanical and chemical fungicides. An M.S. Thesis submitted to the Department of Plant Pathology, SAU, Dhaka-1207.
[18] Ali, S., Khan, M.A., Habib, A., Rasheed, S. and Iftikhar, Y. 2005. Correlation of environmental conditions with *Okra yellow vein mosaic virus* and *Bemisia tabaci* population density. International Journal of Agriculture and Biology, 7(1). pp. 142–144.
[19] Mazyad, H.M. Omar, F., Al-Taher, K. and Salha. M. 1979. Observations on the epidemiology of *Tomato yellow leaf curl virus* disease on tomato plants. Plant Disease Reporter, 63(8). pp. 695-698.
[20] Pilowsky M., Cohen S., Ben-Joseph R. and Nahon S., 1993. Effect of *Tomato yellow leaf curl virus* on tolerant and susceptible cultivars. Proceedings of the XIIth Eucarpia meeting on tomato genetics and breeding, Plovdiv, Bulgaria, 27-31 July 1993, pp. 31-32.
[21] Rashid M. H., Hossain I., Hannan A., Uddin S. A. and Hossain M. A. 2008. Effect of Different Dates of Planting Time on Prevalence of *Tomato yellow leaf curl virus* and Whitefly of Tomato, Journal of Soil and Nature, 2(1). pp. 1-6.
[22] Sinistera, X., Patte, C.P., Siewnath, S. and Polston, J.E. 2000. Identification of TYLCV-Is in the Bahamas. Plant Disease, 84(5). p. 592.
[23] Polston, J. E., McGovern, R. J., Sherwood, T. and Kelly, R. 1999. New developments in *Tomato yellow leaf curl virus* in Florida. In: 1999 Proceedings of the Florida Tomato Institute, Naples, FL, USA, PRO-516. Gainesville, FL, USA: University of Florida, IFAS, pp. 2-5.
[24] Mohanty, B.K. 2002a. Variability, heritability, correlation and path coefficient studies in tomato. Haryana Journal of Horticultural Sciences, 31(3-4). pp. 230-233.
[25] Mohanty, B.K. 2002b. Studies on variability, heritability, interrelationship and path analysis in tomato. Annals of Agricultural Sciences, 23 (1). pp. 65-69.
[26] Mohanty, B.K. 2003. Genetic variability, correlation and path coefficient studies in tomato. Indian Journal of Agricultural Research, 37(1). pp. 68-71
[27] Verma. A.K., Basu, D., Nath, P.S., Das. S., Ghatak. S.S. and Muthapadhayak. S. 1989. Relationship between the population of whitefly, *Bemisia tabaci* and the incidence of *Tomato yellow leaf curl virus*. Indian J. of Myco. Res. Plant Virus Research Centre, Department of Plant Pathology. Bidhan Chandra Krishi Viswsvidyalaya, Kalyani, West Bengal.
[28] Mehta, P., Wyman, J.A., Nakhla, M.K. and Maxwell, D.P. 1994. Transmission of *Tomato yellow leaf curl* geminivirus by Bemisia tabaci (Homoptera: Aleyrodidae). Journal of Economic Entomology, 87(5). pp. 1291–1297.
[29] Cohen, S. and Nitzany, F.E. 1966. Transmission and host range of the *Tomato yellow leaf curl virus*. Phytopathology, 56. pp. 1127-1131.
[30] Saikia, A.K. and Muniyappa, V. 1989. Epidemiology and control of *Tomato leaf curl virus* in Southern India. Tropical Agriculture, (Trinidad and Tobago), 66(4). pp. 350-354.

[31] Polizzi, G. and Asero, C. 1994. Epidemiology and incidence of *Tomato yellow leaf curl virus* (TYLCV) in green house protected by screen in Italy. Acta Horticulture, 366. pp. 345-352.

[32] Aboul-Ata, A. E., Awad, M. A. E., Abdel-Aziz, S., Peters, D., Megahed, H., and Sabik, A. 2000. Epidemiology of *Tomato yellow leaf curl begomovirus* in the Fayium area, Egypt. EPPO Bulletin, 30. pp. 297-300.

Nitrogen Use Efficiency (NUE) in tomato (*Solanum lycopersicum*) seedlings in response to treatment with extract of *Cymbopogon citratus* and mineralization of *Tithonia diversifolia* leaves and cow dung

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***Abstract*— *The aim of this work was to study the effect of Cymbopogon citratus extract on nitrogen metabolism in relation to the increase of Nitrogen Use efficiency (NUE) in tomato plants. The culture substrates (δ) were prepared with fertilizations of 15g N and 5g N following the formula: δ + tomato plants + treatments (2%). Treatments included, Hydro Ethanol Extract (HEE) of C. citratus (2%), 2% Ridomil (R) and Control (C). The tomato seedlings were transplanted 32 days after sowing and (δ) sampled 12th, 24th, 36th and 48th days after transplanting and the following parameters determined: Total nitrogen, Electrical Conductivity (EC (dS/m)), Total Mineral content (TM (ppm)), pH water, nitrate (NO3- (ppm)), ammonium (NH4+ (ppm)) and NUE (kg^{-1} DM), using these techniques: Kjedahl, Electrochemistry, Spectrophotometry. The results from the dosage of N revealed that Tithonia diversifolia (Ti), Cow dung (Cd), soil/sand (2:1) mixture and NPK contained 3.32%, 2.13%, 0.23 %, and 23.00% of N respectively. The kinetics of mineralization in the δTi, δCd showed a primary mineralization while that in the δNPK and δC showed a secondary mineralization. The values of NUE_{NPKHEE}, NUE_{NPKR}, NUE_{TiHEE}, NUE_{TiR}, NUE_{CdHEE}, and NUE_{CdR} increased by 38.49%; 37.45%; 27.74%; 52.07%; 93.93%; 70.52%, respectively.*

The combination of plant spray with HEE of C. citratus and soil amendment with T. diversifolia or cow dung improved significantly the NUE of tomato plants confirming that T. diversifolia and cow dung are slow mineralization nitrogenous biofertilisers.

Keywords*— Nitrogen use efficiency, Mineralization, Inputs from plants, Physicochemical soil parameters, Nitrogen release pathway.

I. INTRODUCTION

From the second green revolution which is dedicated to increasing productivity while using sustainable farming methods and driven by significant advances in agricultural research and technology, was born the notion of Nitrogen Use efficiency (NUE) (Zeigler and Mohonty, 2010). However, the last decades have been marked by a large use of nitrogen fertilisers, which has had a significant impact on agricultural yields, causing an increase in nitrogen fertiliser costs, and leading to growing needs in NUE (Mulvaney et al., 2009, Zeigler and Mohonty, 2010). Similarly, nitrogen fertilisers once applied to the soil are directly mineralized by beneficial soil microorganisms

(Sherlock and Goh, 1985). The nitrogenous minerals thus liberated will be used by the plant, of which it represents 2 to 5% of its dry weight and is the structural component of proteins, nucleic acids, cofactors and secondary metabolites (Miller and Cramer, 2005). The mineral nitrogen available to the plant is in the form of ammonium and nitrate (Herrera, 1999).

World consumption of fertilisers amounted to 196.4 million tons in 2016: 68.6% nitrogen, 19.2% phosphates and 12.3% potassium (Faostat, 2016). It is estimated that 50 to 70% of nitrogen fertiliser losses in the plant-soil-plant system recorded each year is caused by rapid fertiliser mineralization, soil type, plant species, and soil physico-chemical properties (Hogdes, 2002; Good et al., 2004). However, losses of these minerals have serious impacts on ecosystems, stratosphere, groundwater and agricultural yields. This leads to great economic losses for farmers (Peoples et al., 1995). The challenge is that of optimizing the use of nitrogenous minerals available in the soil by plants, while trying to optimize the nitrogen uptake provided by the mineralization of biofertilisers.

Chemical and engineering approaches have been used on nitrogen metabolism in plants and despite their limits and ethical issues among ecologists, they have shown effectiveness in raising the NUE in plants by 2% (Hongmei et al., 2008, Swain and Abhijita, 2013).However, it was stated that the NUE should be raised by 20% to save $ 4.1 billion every year (Raun and Gordon, 1999). Kaho et al. (2011), evaluated the combined effectsof *Tithonia diversifolia* (FTd) and inorganic fertilisers (NPK and Urea) on maize grain yields and soil properties. With the acceptability index (AI) of more than 2 in T4 (5 t/ha FTd, 0NPK and 0Urea) and T5 (2.5 t/ha FTd, 75 kg 20-10-10 and 75 kg of Urea), they found that *T. diversifolia* has a potential for improving plant nutrient availability in these soils for the cultivation of maize without chemical fertilisers. The leaves of *T. diversifolia* (Nuraini and Sukmawatie, 2014), and cow dung have fertilizing properties (Tanimu et al., 2013). Sakakibara (2002), stated that congestion of certain phytohormones communicates the availability of nitrogenous minerals to the root system of a plant, by demonstrating that algae extracts are rich in phytohormone that can stimulate absorption and the use of nitrogenous minerals in wheat plants. These previous research works constitute motivation of the present study.

The aim of this work was to study the effect of *Cymbopogon citratus* hydro ethanol extract on nitrogen metabolism in combination with*Tithonia diversifolia* (Ti), Cow dung (Cd) and an inorganic fertiliser(NPK) in relation to the increase of Nitrogen Use efficiency (NUE) in tomato plants.

II. MATERIAL AND METHODS

2.1. *Experimental site*

The study was carried out in a greenhouse at Institute of Agricultural Research and Development (IARD), Nkolbisson, Yaoundé, Cameroon at latitude 3º 51 Northand longitude 11º 40 East, with an altitude of 759 m. The annual distribution of rains is bimodal with pics in May and October. The average annual precipitation varies from 1134-2112 mm. Average temperature is around 24.7ºC. Relative humidity varies between 50-80% in dry season and 70-90% in raining season.

2.2. *Materials*

2.2.1. *Biofungicide plant*

Whole plants of *Cymbopogon citratus* (D.C.) Stapf were harvested at Nkolbisson, Yaoundé around households and identification of the plant species was conducted by the Cameroon National Herbarium in Yaoundé. The fresh plants were air dried at room temperature (25-27°C) for 10 to 14 days and milled into powder.

2.2.2. *Synthetic fungicide*

Ridomil (4% w/w Metalaxyl-M or Mefenoxam, CGA329351; 60% w/w Copper hydroxide) used as synthetic fungicide was bought at the phytosanitary shop at Nfoundi market.

2.2.3. *Synthetic fertiliser*

The synthetic fertilizer used, was NPK 23.10.5, bought in the market place.

2.2.4. *Biofertilisers*

Fresh leaves of *Tithonia diversifolia* L. were harvested in the city at Nkolbisson, Yaoundé and air dried at room temperature (25-27°C) for 10 to 14 days and milled into powder.

Cow dung was collected at Institute of Agricultural Research and Development (IARD) Nkolbisson-Yaoundé farm, dried at open air and milled into powder.

2.2.5. *Tomato seeds' cultivar*

Seeds of the tomato cultivar "Roma VF" bought at local shop at Nfoundi market, Yaoundé were used for the experiment.

2.3. *Methods*

2.3.1. *Preparation of Ridomil suspension*

Twenty grams (20g) of mefoxan powder and copper oxide (Ridomil) were weighed and introduced into one literof distilled water and the mixture stirred for a few minutes.

2.3.2. *Preparation of the hydro ethanol extract of C. citratus*

Dried plants were milled and freed from lipids by mixing 100 g of powder with 100 ml of hexane for 24h. After filtration, the residue was displayed for complete evaporation of the solvent. The hydroethanol extract was obtained by adding the residue to 100 ml of 70% ethanol and the mixture allowed standing for 24h. The supernatant was passed through Whatman nº1 filter paper. The ethanol was totally evaporated using a rotary evaporator and the water residue adjusted to 100 ml with distilled water and freeze dried for later use in tomato plants treatment.

2.3.3. Extraction of the essential oil of C. citratus

The essential oil (EO) was extracted from dried plant material by hydrodistillation for five hours using a Clevenger-type apparatus. The EO collected was dried on anhydrous sodium sulphate (Na_2SO_4) column and kept in the refrigerator at 4°C into airtight brown bottles. Yields of the oils were calculated as percent of dried plant material weight (% w/w).

2.3.4. Experimental Design

Thirty two-day-old tomato plants raised in nursery in the greenhouse were used for the experiment. Three different nursery beds were fertilised as followed: to 5 Kg mixture of soil/sand (2: 1) were incorporated, 22 g of NPK in the first tray, 150 g of *T. diversifolia* powder in the second tray and 200 g of cow dung powder for the third tray; for a reasoning of the fertilization with 5 g of nitrogen. Tomato seeds were treated with 0.2% essential oil of *C. citratus* before seeding at the rate of 200 seeds per tray.

2.3.5. Physicochemical parameters experimental design cultural and substrates

-Experimental design

The trial was conducted in a completely randomized blocks design. The main block was represented by seedlings sprayed with 2% hydroethanol extract of *C. citratus*, a positive control block was sprayed with 2% Ridomil and the negative control block was sprayed with water. Each block consisted of 12 pots, divided into 3 amendments with 3 repetitions each. The fertilisers were: *T. diversifolia* powder, NPK, cow dung. Unfertilized sand/soil was used as control.

-Cultural Substrates preparation

Amended soils were prepared according to the method described by Henrickson, 2005. To 5kg soil/sand mixture (2:1), was incorporated 67.5g of NPK, 600g cow dung powder and 450g of *T. diversifolia* powder, making a reasoning of fertilization of 15g of nitrogen. Amended soils distributed in pots of 50cm high and 30cm diameter included: cow dung potting soil (δCd), soil *T. diversifolia* (δTi), soil NPK (δNPK), control potting soil (δC). The 32-day old tomato seedlings were transplanted into the previously prepared pots. The seedlings were treated once after transplantation with 250 ml of solution, by spraying with 2% Ridomil, 2% hydroethanol extact *C. citratus* and distilled water for the control. The plants were kept for an adaptation period of 20 days. After that, culture substrates were sampled periodically each 12^{th} day after transplantation for 48 days. Part of substrates was dried for one week; the samples were re-milled and sieved with less than 2 mm mesh size. The upper fraction was used to determine pH_{water}, and the finest fraction used to measure the electro conductivity (EC), total minerals, nitrate and ammonium minerals. The results allowed to establish hthe kinetics of pH_{water}, EC; and Turnovers of TDS, nitrates and ammoniums concentration.

The pH of the culture substrates was measured by electrochemistry using HANNA pH meter instruments after preparing culture substrate suspensions in distilled water at a ratio of 1:5 (NF ISO 10390, 2005). Electro conductivity and total minerals concentrations of substrates were determined in cultural substrates suspension in distilled water at a ratio of 1:5 using an INOLAB brand conductivity meter (NFISO 11265, 2005). For the determination of nitrate minerals, the USDA formula was used by assigning to the EC determined in a ratio of 1:5 in Ds/m the coefficient 140 if and only if the water pH was below 7.2 (NF ISO 11265, 2005; USDA, 2014).

For the determination of ammonium minerals, samples of untreated cultural substrates were extracted with distilled water in a ratio 1:5. Four milliliters (4 ml) of the soil/water suspension of each sample were transferred to 40ml flasks; into which 1ml of the Nessler reagent was added and the orange-yellow colors are allowed to develop for 30 minutes. A standard was prepared using increasing concentration of ammonium minerals of 0, 1, 2, 3, 4, 5ppm; and 4ml of each solution were transferred into 40ml flasks to which 1ml of Nessler reagent was added and the orange-yellow colors were allowed to develop for 30 minutes. The optical density (OD) was determined spectrophotometrically at a λ of 410nm. Ammonium mineral concentrations were obtained graphically by referring to the calibration curves (NF ISO14256-2., 2007).

2.3.6. Nitrogen Optimization experiment

-Experimental design

The trial was conducted in a completely randomized blocks design. The main block was represented by seedlings sprayed with 2% hydro ethanol extract of *C. citratus*, a positive control block was sprayed with 2% Ridomil and the negative control block was sprayed with water. Each block consisted of 12 pots, divided into 3 amendments with

3 repetitions each. The fertilisers were: *T. diversifolia* powder, cow dung and NPK. Unfertilized sand/soil was used as negative control.

-Cultural Substrates preparation

Amended soils were prepared according to the method described by Henrickson, 2005. To 5kg soil/sand mixture (2:1), is incorporated 25g of NPK, 200g cow dung powder and 145g of *T. diversifolia* powder, making a reasoning of fertilization of 5g of nitrogen. The 32-day-old tomato seedlings were transplanted into the previously prepared pots and were treated once with 250 ml of solution, by spraying with solution of 2% Ridomil, 2% hydroethanol extact *C. citratus* and distilled water for the control. The plants were kept for an adaptation period of 20 days and uprooted after 35 days. Fresh/dry weights were weighed using a METTLER PC 2200 precision scale. Nitrogen contents in dry tomato plants, cow dung powder, *T. diversifolia* powder, and the experimental soil were determined by digesting 0.1g of each sample into a mixture of H_2O_2, selenium, $LiSO_4H_2O$ and 5ml of a 7N sulfuric acid solution. The mixtures are transferred to a digestion device which is programmed at a temperature of 400°C. for 12 hours. The nitrogen contents were determined spectrophotometrically at a λ of 655nm (NF ISO 11260 2005). NUE was expressed as the amount of dry matter produced by nitrogen content in the plant, as written by Masclaux-Daubresse et al., 2010 by the formula following:

NUE= DM÷N witch DM (Dried Matter in mg); N (Nitrogen in mg/Kg or ppm)

2.4. Statistical analyzes of the results

The results obtained were subjected to a descriptive analysis for the calculation of means, standard deviations and the search for significant differences using Statistical Package for Social Sciences (SPSS) software version 22.0. The Analysis of Variance (ANOVA) test coupled with the Newman Keuls t-Student test was used to evaluate the smallest significant difference at the 0.05 probability level. The graphs were built from the Microsoft Office 2013 Excel software.

III. RESULTS

3.1. Nitrogen (N) content in cultural substrates

The nitrogen contents as percent in the cultural substrates are given in Table 1. *Tithonia diversifolia* powder showed higher nitrogen proportions than the cow dung powder, with 3.32%, and 2.13%, respectively. The soil-sand mixture in a ratio of 2:1, contained 0.21% nitrogen and NPK, 23% of nitrogen.

3.2. Physicochemical parameters of soil substrates in pots with tomato plants

3.2.1. Kinetics of mineralization of fertilisers in experimental blocks

The results obtained from the determination of the EC (dS/m) in the δ of the three blocks(HEE of *C. citratus;* Ridomil and Control*)* allowed to establish the curves expressing the kinetics of mineralization by measuring the EC (dS/m) as a function of time (Fig. 1).

The comparison of the different mean ECs as function of time in days, showed very significant differences ($p \leq 0.05$). δTi and δCd showed mineralization kinetics that followed progressive dynamics over distributed time for the 48-day experiment; the EC δTi varied from EC_{12} = 351. 46 x 10^{-3}dS/m to EC_{48} = 521.87 x 10^{-3}dS/m with a slight decrease on the 24th day, whereas in the δCd the EC ranged from EC_{12} = 212.55 x 10^{-3}dS/m at EC_{48} = 521.87 x 10^{-3}dS/m; δTi mineralized more rapidly than δCd. In contrary, the δNPK and δC showed mineralization kinetics that followed regressive dynamics on the distributed time for the 48-day experiment. EC in δNPK ranged from EC_{12} = 1315.03 x 10^{-3}dS/m to EC_{48} = 680.78 x 10^{-3}dS/m, δC had ECs varying from EC_{12} = 134.01 x 10^{-3}dS/m to EC_{48} = 46.60 x 10^{-3}dS/m.

3.2.2. Turn Over of total minerals (TDS (ppm)) in each substrate in the experimental blocks

The total mineral concentrations in substrate in block treated with HEE of the three blocks (HEE of *C. citratus;* Ridomil and Control*)* were recorded, values from which the total mineral Turn Over (TDS (ppm)) were generated are presented in Table 2.

The determination of the mean values of total mineral concentrations (TDS (ppm)) of the substrates recorded in table 2, resulted from the averages of the different values of (TDS (ppm)) of each substrate, taken from each block, with the corresponding interpretations.In general the mineralization process was highlighted by the content in total minerals in different blocs. δCd and δTi showed progressive mineral congestion over time and the values ranged from TDS_{12} = 302.23 ppm to TDS_{48} = 743.26 ppm for δCd, and from TDS_{12} = 502.22 ppm to TDS_{48} = 741.22 ppm for δTi. In contrary the δNPK and δC blocks showed regressive mineral congestion, with TDS ranging from TDS_{12} = 2049.93 ppm to TDS_{48} = 973.88 ppm for δNPK and from TDS_{12} = 191.57 ppm to TDS_{48} = 65.00 ppm for δC.

Summary of interpretation of the reactions (pH_{water}) of each substrate of the three blocks over time

The determination of the mean values of the reactions (pH_{water}) as shown in Table 3, resulted from the averages of the different values of the pH_{water} of each substrate, taken from each block, with the corresponding interpretations.

In general, the reactions of the different substrates showed a highly significant difference ($p \leq 0.05$) over the days. The δTi and δCd showed an improvement of the reactions in the direction of the optimal reaction of absorption of the minerals which had respective values between $pH_{watre12} = 7.00$ to $pH_{water48} = 6.33$ and $pH_{water12} = 6.12$ to $pH_{water48} = 6.18$. The δC had similar reaction with values varying from $pH_{water12} = 6.31$ to $pH_{water48} = 6.08$. The δNPK reactions showed an improvement in the direction of the immobilization reaction in comparison with the reactions of δBv, δTi, and δC,with values ranging from $pH_{water12} = 5.65$ to $pH_{water48} = 5.37$.

3.2.3. Turn Over of Nitrogenous Minerals From Fertilisers of Each substrate In Experimental Blocks

*-Concentration in Nitrate (*MNO_3^-(ppm)*) of pot substrates in experimental blocks*

The determination of the concentration in MNO_3^-(ppm) in substrates of the three blocks (HEE of *C. citratus;* Ridomil and Control*)* allowed to obtain of Turn Over of MNO_3^- (ppm) as illustrated in Fig.2.

From Fig.2, it can be seen that the concentrations of nitrogenous nitrate minerals in the substrates showed a significant difference ($p \leq 0.05$) as function of time in days. The δTi andδCd over the 48-day period showed mineral availability NO_3^-(ppm) which followed progressive dynamics; while in the δNPK and δC, mineral availability NO_3^-(ppm) followed regressive congestion dynamics after 48 days. Thus, with a mineral regression NO_3^-(ppm) ranging from $NO_3^-{}_{12}$ = 168.28ppm to $NO_3^-{}_{48}$ =134.82ppm and $NO_3^-{}_{12}$ =20.83ppm to $NO_3^-{}_{48}$ =8.52ppm.The δNPK, δC were denitrified with respect to δTi, δCd which were nitrified. TheδTi showed the highest nitrification, with concentrations NO_3^-(ppm) ranging from $NO_3^-{}_{12}$ =47.79ppm to $NO_3^-{}_{48}$ =77.23ppm, and δC with $NO_3^-{}_{12}$ =25.68ppm to $NO_3^-{}_{48}$ =68.75ppm. The biofertiliser, *T. diversifolia* was better at nitrification than cow dung.

**Summary of interpretation the Turn Over minerals nitrates in substrates of the three experimental blocks for the trial period*

The determination of mean mineral concentrations NO_3^- (ppm) reported in Table 6, resulted from the averages of the different values of the mineral concentrations NO_3^- (ppm) of each substrate, taken from each block, with the corresponding interpretations.

The Turn Over of nitrate (NO_3^-) in blocks showed a significant difference ($p \leq 0.05$) as function of time in days. After 48 days δTi and δCd showed nitrate Turnovers (ppm) that followed progressive congestion dynamics, with values ranging from $NO_3^-{}_{12}$ = 49.13 ppm to $NO_3^-{}_{48}$ = 73.2 ppm for δTi and $NO_3^-{}_{12}$ = 29.90 ppm to $NO_3^-{}_{48}$ = 63.59 ppm for δCd. For the δNPK and δC, the Turn over of NO_3^- (ppm) followed regressive congestion dynamics after 48 days. Thus, the Turn over values of NO_3^- (ppm) decreased $NO_3^-{}_{12}$ = 181.2 ppm $NO_3^-{}_{48}$ = 95.27 ppm for δNPK and $NO_3^-{}_{12}$ = 18.79 ppm to $NO_3^-{}_{48}$ = 6.55 ppm for δC.

*- Concentration in Ammonium (*MNH_4^+(ppm)*) of pot substrates in experimental blocks*

The determination of the concentration in MNH_4^+(ppm) in substrates of the three blocks (HEE of *C. citratus;* Ridomil and Control*)* allowed to obtain of Turn Over of MNH_4^+ as illustrated by Fig.3.

The statistical analysis of MNH_4^+ (ppm) data showed a significant difference ($p \leq 0.05$) over days. The δTi, δCd, δNPK and δC, over the 48-day period, showed the Turn Over availability of MNH_4^+ (ppm) that followed regressive congestion dynamics; with a regression in NH_4^+ (ppm) ranging from $NH_4^+{}_{12}$ = 75.07ppm to $NH_4^+{}_{48}$ = 43.06ppm for δNPK, $NH_4^+{}_{12}$ = 5.725ppm at $NH_4^+{}_{48}$ = 3.43ppm, for δTi $NH_4^+{}_{12}$ = 5ppm at $NH_4^+{}_{48}$ = 2.82ppm for δCd and $NH_4^+{}_{12}$ = 3.09ppm at $NH_4^+{}_{48}$ = 1.44ppm for δC.

3.3. Nitrogen Use Efficiency (NUE) of tomato seedlings 35 days after transplanting in experimental blocks

The data reported in Figure4, represent the Nitrogen Use Efficiencies (NUE) of tomato seedlings 35days after transplanting, values by which the NUE graph ($Kg^{-1}DM$) according to the treatments was constructed. The evolution of NUE for each treatment during the incubation period was higher in the amended and treated substrates than in the non-treated and non-amended control substrates. Seedlings obtained from combined amendment and treatments, NPK-HEE and NPK-R, showed an improvement in NUE of 38.49% and 37.45% respectively, as compared to seedlings from only amended plot NPK-C. The NUE values of 35-day-old seedlings from following treatment combinations Ti-HEE, Ti-R, Cd-HEE, Cd-R increased respectively by 27.74%, 52.07% and 93.93%, 70.52% as compared to the controls Ti-C and Cd-C (Fig. 4).

IV. DISCUSSION

4.1. Nitrogen (N) content in cultural substrates

With the increase in the use of synthetic nitrogen fertilisers and the repercussions caused on the environment by their

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mineralization, researchers have turned to new sources of nitrogenous sources to improve the use of available nitrogen minerals in the soil for crops. The results from the nitrogen content determination of the fertilisers gave the respective values of: 3.32% N, 2.13% N, 0.23% N, 23% N, in *Tithonia diversifolia*, cow dung, Soil/Sand and NPK. These results are in accordance with the findings of Roy and Kashem (2014).These authors reported that cow dung contained nitrogen and can be used as biofertilisers. Jama et al., 2000, reported on *T. diversifolia*'s ability to improve soil fertility. Roy and Kashem (2014), have shown the ability of cow dung to change the properties of a soil at different times and obtained similar results with the nitrogen content in cow dung of 2.13% and in the experimental soil of 0.52%. Jama et al., 2000 and Moke et al., 2013, obtained slightly different results for nitrogen content in *T. diversifolia* with respective values of 3.5% and 4.2%. Nitrogen contents in biofertilisers are elucidated by the state of the organic matter (OM), the effectiveness of the mineralization during the experiment, the parts of the plant used (leaves only, or whole plant) and the origin of the soil used.

4.2. Physicochemical parameters of soil substrates in pots with tomato plants

The mineralization kinetics in δTi and δCd over 48 days followed progressive dynamics, confirmed by total mineral congestion dynamics. The kinetics of mineralization of δNPK and δC followed regressive dynamics, confirmed by the Turn over of TMs that followed regressive congestion dynamics. These results are similar to those of Roy and Kashem (2014), which demonstrated the dynamics of mineralization of cow dung and poultry manure by determining the EC of the cultural substrates. In fact, Ti and Cd undergo primary mineralization, which in its execution process uses the young organic matter, in which the nutrients are incorporated in molecular form. The action of biotic and abiotic factors allows the partial release of minerals such as NO_3^-, NH_4^+, CO_2, PO_4^{2-}, SO_4^{2-} (Smith and Doran 1996). Some is used in the biochemical cycle to form humus, which combines with clay to form the Clay–Humic Complexe with a negative overall charge. This complex captures the positively charged minerals to form the adsorbent complex hence, for a slow mineralization of the OM; the minerals will have a longer hold time in the soil (Bationo et al., 2007, Duhanet al., 2005). The NPK and the non-treated control have undergone secondary mineralization, which in its execution process uses stable minerals or pre-existing adsorbent complex. This mineralization depends on the quantity, nature and composition of elements of the different fertilisers (De neves et al., 2004). This mineralization accounts for leaching, volatilization, and uptake and plant utilization (USDA 2014). Chibane (1999) showed that the favorable EC for tomato cultivation is 0.625dS / m. The EC values obtained at 48th day in δTi and δCd were 0.5229dS / m and 0.5218dS / m, respectively, very close to the optimal value for tomato cultivation. The δNPK, at the same date, revealed an EC of 0.68078dS / m instead of 1.315dS / m at the 12th day. During this period, this high concentration of minerals was phytotoxic for the tomato plants and the losses in biomass were observed, revealing the need to split in time fertilization with NPK. The soil reaction depend on the optimal pH of mineral absorption pH (6.5) and a water pH of immobilization of minerals water pH (4.5). Water pH kinetics in δTi and δCdcompared to δC revealed an increase towards 6.5; unlike water pH in the δNPK, the kinetics of water pH compared toδC revealed an improvement in the direction of 4.5. These data were in accordance with the findings of Azeez and Van Azerbeke, 2012 and Roy and Kashem 2014, who reported that the study of soil responses may vary over time depending on the type of amendment.

The results and illustrations obtained in δTi and δCd find their elucidations in the degree of mineralization; the primary mineralization that undergoes Cd and Ti contributes to the formation of Clay–Humic Complexe (CHC) molecules as described by Duhan et al., 2005, which revealed that any organic matter incorporated into the soil disseminated the CHC molecules. These CHC molecules have a global negative charge, which gives it the capacity to potentiate the free protons in the medium (UNIFA, 1999), which will improve the water pH towards neutrality. The results and illustrations obtained in the δNPK find their elucidations in the degree of mineralization, the NPK being an already mineralized fertiliser makes less available the CHC molecules in the soil.

The residual CHC is rapidly saturated with the protons, leaving the free proton concentrations in the medium capable of immobilizing all life in the soil. The water pH values in the δTi and δCd corroborate with the interval recommended by Hendrickson, 2005, who revealed that for tomato, the pH of the soil should vary between 6 and 7. The pH values obtained in the δC are characteristic of the soil pH in the region.

The results from our experience revealed that the M NO_3^-Turn Overs (ppm) in δTi and δCd followed progressive congestion dynamics. Turnovers of M NO_3^-(ppm) in δNPK and δC followed regressive congestion dynamics. These results are accordance with the observations of Khalil et al., 2005, who reported from a

90-day experiment on the rate of mineralization of nitrogen in different soil types under aerobic conditions of an organic material, a quality index on the transformation of organic nitrogen. The results obtained in δTi and δCd find their elucidations in nitrification; this nitrifying activity is favored by the pH of the soil, which reaches its optimum for water pH values greater than 6 with good availability of nitrate minerals. In addition, is the improvement of the soil texture that by dissemination of the CHC leads to the sequestration of nitrate minerals (Zaman et al., 2008).

The results obtained in the δNPK and δC found their elucidations in denitrification. This denitrification is favored by the water pH, which, when close to 5, amplifies volatilization (Zaman et al., 2007), the nature of the starting fertiliser, which can be in the form of a stable or residual product coupled with a well oxygenated soil, the daily watering frequency and with a less potential M NO_3^- texture only contribute to denitrification, which results in leaching, volatilization, immobilization by organic matter (OM) and a part used by the plant (Barton et al., 1999, Bowman et al., 2002, Bationo et al., 2007 and Baoqing et al., 2014).

The results from our experience with Turn OverM NH_4^+ (ppm) in δ revealed decongestion dynamics. These results are in agreement with those of Eigenberg et al., 2002, who evaluated the availability of MNH_4^+ (ppm) in soils amended to animal waste. The results obtained in the δTi, δCd and δC are not in accordance with those obtained by Roy and Kashem (2014), who revealed that soils amended with organic fertilisers were influenced by dynamics of progressive congestion of MNH_4^+ (ppm) for the duration of experiment. By referring to the nature of the fertilisers, the same processes would have occurred in the organic δ. These differences could be explained by the extraction efficiency. In fact, Roy and Kashem (2014), in their experiments suggested the use of 1N KCl solution to extract MNH_4^+ (ppm).In this work MNH_4^+ was extracted with water. These results confirm the importance of primary mineralization on the potentiation of MNH_4^+ (ppm). The decongestion observed in the δC and δNPK reflected either an extension of the MNH_4^+ (ppm) mineralization, which under the action of nitrifying microorganisms progressively oxidizes ammonium to nitrate, or losses by volatilization or immobilization by microorganisms. This mineralization of molecular nitrogen is influenced by the nature of the fertiliser, the watering regime, the volume of fertiliser applied, the type of soil and the duration of the experiment (Rahman et al., 2013).

4.3. Nitrogen Use Efficiency (NUE) of tomato seedlings 35 days after transplanting in experimental blocks

The results from the NUE optimization trial of the (2%) hydro ethanol extract (HEE) of *C. citratus* and 2% Ridomil in 35-day-old tomato plants and at the beginning of flowering period, revealed 38.49% improvement in the NPK_{EHE}, and 37.45% in the NPK_R combinations compared to the NPK_C combination. An improvement of 27.74%, 52.07% in the Ti_{HEE} and Ti_R combinations compared to the Ti_C combination and of 93.933% in the Cd_{HEE} combinations, and 70.52% in the Cd_R combinations compared to the Cd_C combination. These results corroborated those of Mérigout (2006), who demonstrated that green algae extracts are able to optimize the use of nitrogen in wheat plants. Smil (2001), revealed that nitrogen is the most important nutrient for agricultural production, because it is the yield' determinant. The high values of NUE obtained here, could be explained by the fact that NUE has been determined at the biomass level and according to Masclaux-Daubresse et al., (2010) who showed that the use of nitrogen in plants takes place in several stages: absorption, assimilation, translocation and remobilization. During assimilation, nitrogen integrates carbon chains to form proteins, cofactors, nitrogen bases and secondary metabolites. This assimilation leads to the formation of biomass and the high proportions in NUE at this stage positively influences the yields.This assimilation leads to the formation of biomass and the high values of NUE at this stage positively influence the yields since the assimilated nitrogen will be remobilized to allow the filling of the fruits or the seeds.

The NUE can be determined at fruiting stage and is influence by, the reasoning of nitrogen fertilization, the nature of the fertiliser and the degree of mineralization, the species of the plant, and the initial nitrogen status of the tomato plants from the nursery. The results obtained also find explanations for the climatic conditions, the soil type and the optimization approach used.

When compared the values of the NUE from the natural approach (biofertilisers and biopesticide) to the work of Lewandowsky and Schmidt (2006), Zub and Brancourt (2010), who used the genetic approach to optimize NUE in *Miscanthus* plants, the NUE obtained were lower than those reported in this work.

V. FIGURES AND TABLES

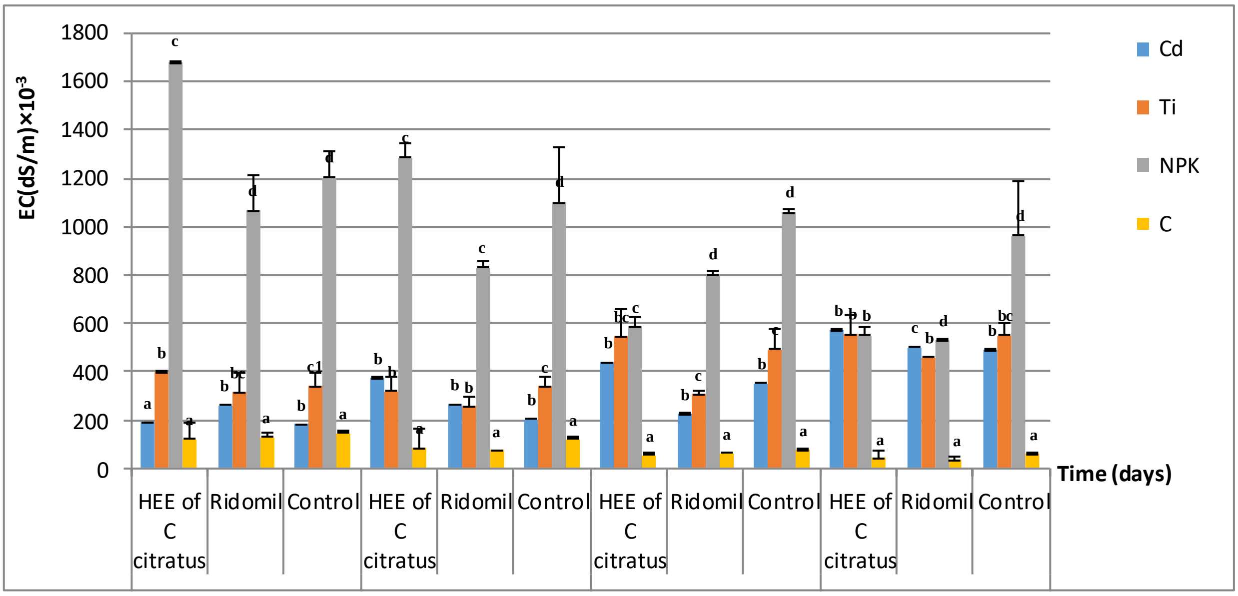

Fig. 1 Electro Conductivity EC ((dS/m)×10^{-3}) as function of time in bloc treated with 2% hydro ethanol extract (HEE) of C. citratus, Ridomil 2% and non-treated control.

[a…d] : Different letters indicate significant difference at $p \leq 0,05$ (n =3). Ti: *T. diversifolia*; Cd : Cow dung; NPK 23.10.5; C: Control

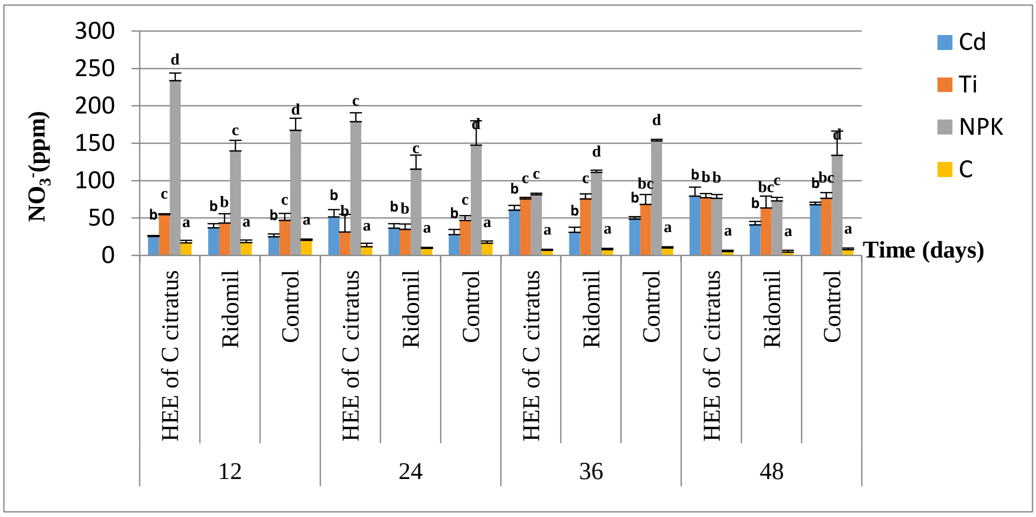

Fig. 2 Turn Over of NO_3^- (ppm) as function of time in block treated with 2% hydro ethanol extract (HEE) of C. citratus, Ridomil 2% and non-treated control.

[a…d] : Different letters indicate significant difference at $p \leq 0.05$; Bars denote mean +SD (n =3).Ti: *T. diversifolia*; Cd : Cow dung; NPK 23.10.5; C: Control

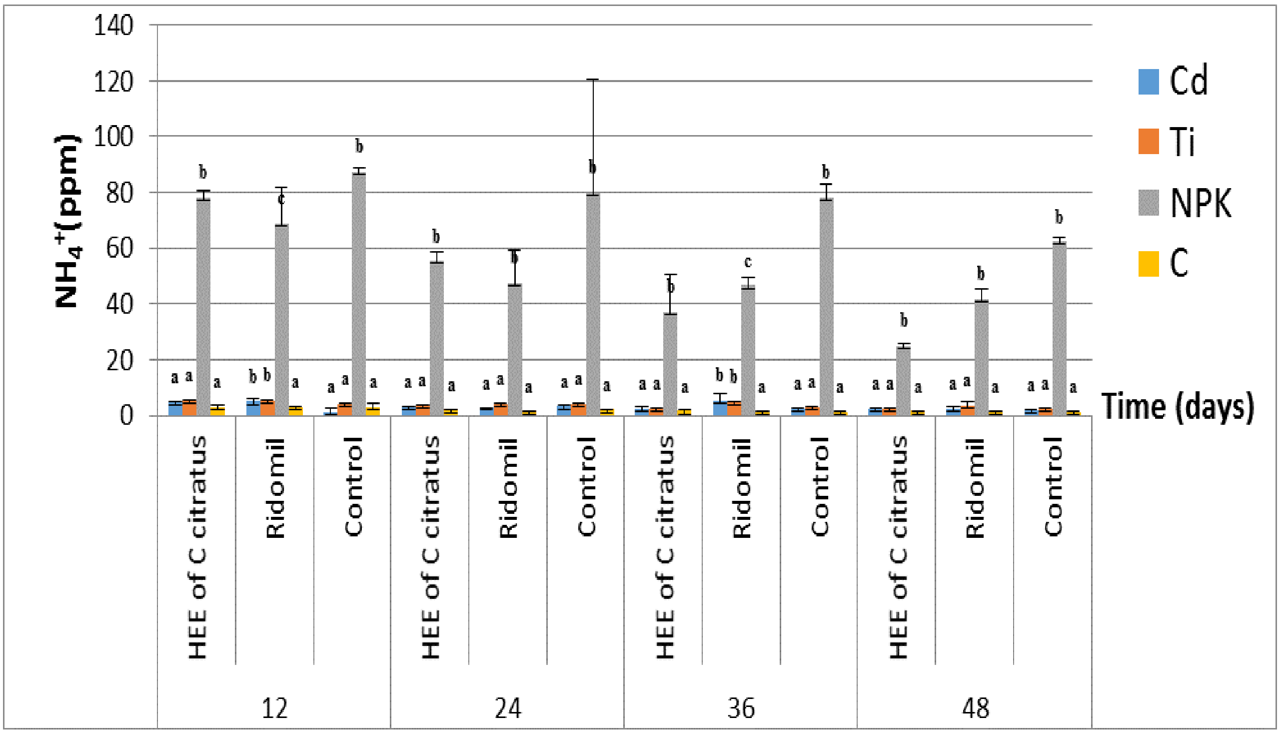

Fig. 3 Turn Over of NH_4^+ (ppm) as function of time in block treated with 2% hydro ethanol extract (HEE) of C. citratus, Ridomil 2% and non-treated control.

[a...c] : Different letters indicate significant difference at $p\leq 0.05$; Bars denote mean +SD (n =3).Ti: *T. diversifolia*; Cd : Cow dung; NPK 23.10.5; C: Control

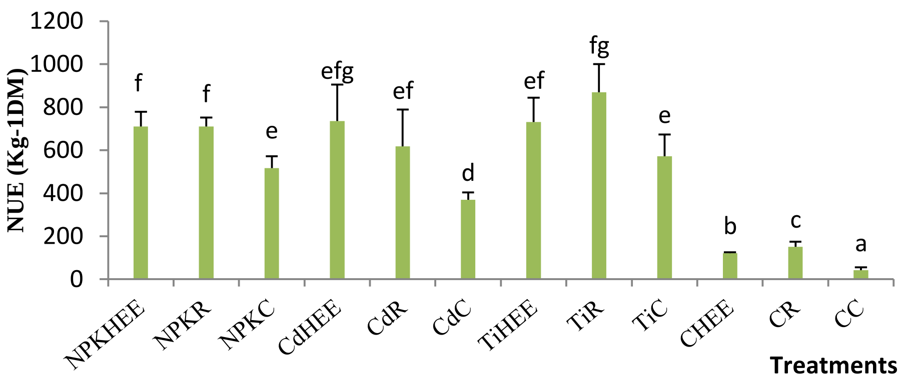

Fig. 4 NUE in 35-day-old tomato plants grown in non-treated and treated with 2%, HEE C. citratus and 2%), Ridomil 2% blocks.

[ag*] : Different letters indicate significant difference at $p\leq 0.05$; Bars denote mean +SD (n =3). Ti: *T. diversifolia*; Cd : Cow dung; NPK 23.10.5; C: Control; HEE: Hydro Ethanol extract of *C. citratus*; NUE (Nitrogen Use Efficiency)

Table 1 Nitrogen (N) content as percent in substrates

Substrates	**soil:sand (2:1)**	**cow dung**	***T. diversifolia***	**NPK**
N (%)	**0.21**	**2.13**	**3.31**	**23**

Table 2 Mean Turn Over of total minerals (TDS (ppm)) in blocks non-treated and treated with 2%HEE C. citratus and 2% Ridomil

	Mean TDS (ppm) in all blocks				
Time (days)	12	24	36	48	Interpretations
Fertiliser					
Cd	302.23^a ±136.14	387.27^a ±162.19	482.11^a ±81.38	743.26^b±53.11	Minerals' congestion
Ti	502.22^a ±69.55	425.4^a ±63.75	634.91^b ±66.47	741.22^b±88.96	Minerals' congestion
NPK	2049.93^c±79.69	1494.17^c ±201.5	1185.53^c ±16.18	973.88^b±133.37	Minerals' decongestion
C	191.57^a±19.08	135.5^a±19.95	92.24^a±4.51	65.00^a±14.77	Minerals' decongestion
Test	(0.05) **	(0.05) **	(0.05) ***	(0.05) **	

ac *: the values in the same columns followed by the different letters are significantly different at a probability p≤ 0.05. Each value is a mean of three repetitions±SD. Ti: *T. diversifolia*; Cd: Cow dung; NPK 23.10.5. ; C: Control.

Table 3 Mean reactions (pH_{water}) in different cultural substrates over time in days

	EHE *C. citatus*(2%). Ridomil(2%). Témoin				
	$pH_{water12}$	$pH_{water24}$	$pH_{water36}$	$pH_{water48}$	Interprétations
Cd	6.12^a ±0.23	6.54^b ±0.08	6.30^b ±0.06	6.18^a ±0.14	Improved of pH_{water} to 6.5
Ti	7.00^d ±0.19	6.72^d ±0.15	6.44^c±0.11	6.33^b ±0.13	Improved of pH_{water} to 6.5
NPK	5.65^a±0.315	5.67^a ±0.10	5.32^a±0.11	5.37^a ±0.09	Improved of pH_{water} to 4.5
C	6.31^b ±0.22	6.27^b ±0.08	5.92^a±0.14	6.08^b ±0.02	Improved of pH_{water} to 6.5
Test	(0.05) ***	(0.05) ***	(0.05) ***	(0.05) **	

ac *: the values in the same columns followed by the different letters are significantly different at a probability p≤ 0.05. Each value is a mean of three repetitions±SD in each block. Ti: *T. diversifolia*; Cd: Cow dung; NPK 23.10.5. ; C: Control; HEE: hydro ethanol extract.

Table 4 Mean Turn over of NO_3^- (ppm) in non-treated and treated with 2%, HEE C. citratus and Ridomil 2%

Time (days)	12	24	36	48	Interpretations
Fertiliser					
Cd	29.90^a ±2.57	39.41^a ±6.58	47.45^a ±8.23	63.59^a ±5.7	progressive nitrification
Ti	49.13^a ±6.56	38.49^a±11.51	74.01^b±7.78	73.2^b ±8.83	progressive nitrification
NPK	181.2^c±12.36	150.07^c±10.1	114.07^b±11.7	95.27^b±13.06	regressive denitrification
C	18.79^a ±1.88	13.39^a ±1.91	9.11^a ±0.34	6.55^a ±1.20	regressive denitrification
Test	(0.05) **	(0.05) **	(0.05) **	(0.05) **	

ac *: the values in the same columns followed by the different letters are significantly different at a probability p≤ 0.05. Each value is a mean of three repetitions±SD in each block. Ti: *T. diversifolia*; Cd: Cow dung; NPK 23.10.5. ; C: Control.

VI. CONCLUSION

The aim of this study was to evaluate the influence of hydro ethanol extract of *C. citratus* on nitrogen metabolism in relation to the increase of the NUE in tomato plants. The nitrogen content in the soil/ sand mixture, cow dung, *T. diversifolia*, and NPK were, respectively, 0.21% N, 3.32% N, 2.13% N, and 23.00% N. These levels guided to reason the fertilization at 15g N for the first test according to the complete needs of the tomato and at 5g N for the optimization test. The results of the mineralization of the various amendments led to the selection of two levels of mineralization. *Tithonia diversifolia* and cow dung showed primary mineralization by improving the pH in the direction of the optimal pH of mineral use of pH6.5. The kinetics of the EC, the Turn Over of the TM followed a progressive dynamic with progressive congestion dynamics in the δTi, δCd. NPK and Control showed secondary mineralization by improving water pH in the direction of the pH of immobilization of minerals. The kinetics of the EC, the Turn Over of the TM followed a regressive dynamic with a regressive dynamic of congestion. Results from the MNO_3^- (ppm) revealed two changes in Turn Over over 48 days. δTi and δCd showed MNO_3^- (ppm) Turn Over that followed a progressive congestion dynamic. δNPK and δC showed MNO_3^- (ppm) Turn Over that followed a regressive congestion dynamic. The results obtained from the determination of M NH_4^+ (ppm) revealed a significant evolution and disproportionality between the δNPK and the other treatments. The determination of NUE at the biomass level in tomato plants treated with 2% HEE of *C. citratus* and ridomil, showed an increase of 38.49% in NPK_{EHE} combinations, and 37.45% in NPK_R combinations. An increase of 27.74%, 52.07% in the Ti_{HEE} and Ti_R combinations and an increase of 93.93% in the Cd_{HEE} combinations, and 70.52% in the Cd_R combinations.

We conclude that the hydro ethanol extract of *C. citratus* justified the importance of the use of the natural approach to optimize the use of nitrogen by plants. The biofertilisers, *T. diversifolia*, cow dung showed a slow release nitrogen property, which is a challenge to the synthetic fertilisers like NPK. In fact, to formulate slow release synthetic chemicals, agro companies tend to incorporate the minerals into biodegradable films. The combined use of plant sprays with hydro ethanol extract of *C. citratus* and soil amendment with *T. diversifolia* or cow dung, improved significantly the nitrogen use efficiency (NUE) of tomato plants and could be used as alternative to conventional inputs.

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REFERENCES

[1] Azeez, J.O., Van Averbeke, (2012) Dynamics of soil pH and Electrical Conductivity with the application of three animal manures. *Communications Soil Science and Plant Analysis*. 43:865-874.Baoqing, C., Enke, L., Qizhuo, T., Changrong, Y., Yanqing, Z., (2014) Soil nitrogen dynamics and crop residues.*A review. Agronomy for Sustainable Development*.34: 429-442.

[2] Barton, L., McLay, C., Schipper, A.,Smith, C., (1999) Annual denitrification rates in agricultural and forest soils. *Australian Journal of Soil Research*.37:1073-1093.

[3] Bationo, A., Kihara, J., Vanlauwe,B., Waswa, B., Kimetu, J., (2007) Soil organic carbon dynamics, functions and management in West African agro-ecosystems. *Agricultural Systems.* 94: 13-25.

[4] Bouwman, A., Boumas, L., Batjes, H., (2002) Emissions of N_2O and NO from fertilized fields, Summary of available measurement data.*Global Biogeochemical Cycles*. 16 : 1-13.

[5] Chibane, A., (1999) Fiche technique, tomate sous serre. Transfert de Technologie en Agriculture. *Bulletin mensuel d'information et de liaison du PNTTA*. Institut Agronomique et Vétérinaire Hassan II, Maroc. 57: 4.

[6] De Neve, S., Gaona, S., Daguilar, B., Sleutel, S., Hofman, G., (2004) Manipulating N mineralization from high N crop residues using on and off farm organic materials.*Soil Biology & Biochemistry*. 36:127-134.

[7] Duhan, B., Devender, K., Singh,J., Kuhad, M., Dahiya, S., (2005) Effect of nitrogen, farmyard manure and metribuzin on nitrogen transformation.*Journal Indian society soil Science*. 53:184–187.

[8] Eigenberg, R., Doran, W., Nienaber, J., Ferguson, R., Woodbury, L., (2002) Electrical conductivity monitoring of soil condition and available N with animal manure and a cover crop. *Agriculture, Ecosystems and Environment*.88:183–193.

[9] Faostat (2016): http:// wwwfao. Org/ contact-us/term/ fr/. accesson 27/01 /2016.

[10] Good, A., Shrawat, K., Muench, G., (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends Plant Science*.9:597–605.

[11] Hendrickson, R., (2005) American Tomato: The Complete Guide to Growing and Using Tomatoes. *Taylor Trade Publishing, and Lanham*.4.

[12] Herrera-Estrella, L., (1999)Transgenic plants for tropical regions: some considerations about their development and their transfer to the small farmer. *Proceedings of the National Academy of Sciences of the United States of America*. 96:5978–5981.

[13] Hodges, M., (2002) Enzyme redundancy and the importance of 2-oxoglutarate in plant ammonium assimilation. *Journal of Experimental Botany*. 53:905–916.

[14] Hongmei, C., Ying, Z., Jinghua, X., Xianghua, X., Xianghua, L., Zhang, Q., Xingming, L., (2009) Overexpressed glutamine synthetase gene modifies nitrogen metabolism and abiotic stress responses in rice. *Plant Cell Rep*. 28:527–537.

[15] Jama, B., Palm, C., Buresh, R., Niang, A., Gachengo, C., Nziguheba, G., Amadalo, B., (2000) *Tithonia diversifolia* as a green manure for soil fertility improvement in western Kenya: *Some review agroforestry systems*. 49 :201-221.

[16] Kaho, M., Yemefack, P., Feujio-Teguefouet, Tchantchaouang, J.,(2011) Effet combiné des feuilles de

Tithonia diversifolia et des engrais inorganiques sur les rendements du maïs et les propriétés d'un sol ferralitique au Centre Cameroun. Tropicultura. 29(1): 39-45.
[17] Khalil, M., Hossain, M., Schmidhalter, U., (2005) Carbon and nitrogen mineralization indifferent upland soils of the subtropics treated with organic materials. *Soil Biologyand Biochemistry*. 11:1507–1518.
[18] Lewandowski, I., Schmidt, U., (2006) Nitrogen, energy and land use efficiencies of *miscanthus*, reed canary grass and triticale as determined by the boundary line approach. *Agriculture, Ecosystems and Environment.*112:335-346.
[19] Masclaux-Daubresse, C., Vedele, F., Dechorgnat, J., Chardon ,F., Gaufichon, L., Suzuki A., (2010)Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Journal of Experimental Botany.* 62:1375-1390.
[20] Mérigout, P.,(2006) Étude du métabolisme de la plante en réponse à l'apport de différents fertilisants et adjuvants culturaux. Influence des phytohormones sur le métabolisme azoté. Thèse de doctorat de l'Institut National Agronomique Paris-Grignon. 229.
[21] Miller, A., Cramer, M., (2005) Root nitrogen acquisition and assimilation. *Plant and Soil.35:*1–36.
[22] Mulvaney, R., Khan, A., Ellsworth, R., (2009) Synthetic nitrogen fertilisers deplete soil nitrogen: a global dilemma for sustainable cereal production. *Journal Environmental Quality*. 38 :2295- 2314.
[23] Moke,N.K.N., Ayingwe, L., Ndiku,L.L., Babelangi, A., (2013) Amendement des sols : Influence des fertilisants pour l'amélioration de la culture de *Vignaunguiculata* (L) Walp. *International Journal of Biochemical and Chemical Sciences.*7:2029-2039.
[24] Nuraini, Y., Sukmawatie, N., (2014) Regulating mineralization rates of *Tithonia diversifolia* and *Lantana camara*prunings to improve phosphorus availability in calcareous soils. *Journal of Degraded and Mining Lands Management*. 2:79-86.
[25] Poeples, M., Ladha, J., Herridge, D., (1995) Enhancing legume N_2 fixation through plants and soil management. Plant Soil. 174:83-101.
[26] Rahman, M., Rafiqul-Islam, M., Jahiruddin, M., Puteh, B., Monjurul M.A., (2013) Influence of organic matter on Nitrogen mineralization pattern in soils under different moisture regimes. *International Journal of Agriculture and Biology.*15:55–61.
[27] Raun, W., Gordon, V., (1999) Improving Nitrogen Use Efficiency for Cereal Production. *Agronomy journal*. 91:363–367.
[28] Roy, S., Kashem, Md.A., (2014) Effects of organic manures in changes of some soil properties at different incubation periods. *Open Journal of Soil Science*. 4:81-86.
[29] Sherlock, R., Goh, K., (1985) Dynamics of ammonia volatilization from simulated urine patches and aqueous urea applied to pasture. Theoretical derivation of a simplified model. *Fertiliser Research*. 6:3-22.
[30] Smil, V., 2001.Enriching the earth: Fritz Haber, Carl Bosch, and the transformation of world food production. The MIT Press, Cambridge, MS, London.
[31] Smith, J., Doran, W., (1996) Measurement and use of pH and electrical conductivity for soil quality analysis. *Methods* for Assessing Soil Quality. *Soil Science Society of America.* Special Publication 49.SSSA, Madison, WI.
[32] Swaim,H.,Abhijita, S., (2013) Nitrogen fixation and its improvement through genetic engineering. *Journal of Global Biosciences*. 5:98-112.
[33] Tanimu, T., Uyovbisere, E., Lyocks, W., Tanimu, Y., (2013) Effects of Cow Dung on the growth and development of Maize crop. *Greener Journal of Agricultural Sciences.* 5 :371-383.
[34] UNIFA, (1998) La fertilisation. 7^eEdition. 150. 1-25. (Cfppah.free.fr/Sol fichiers/Unifa/UNIFA_La Fertilisation. PDF).Access on 03/03/ 2016.
[35] USDA, (2014)Soil Health-Electrical. *Guides for educators conductivity*.8:1-9.
[36] Zaman, M., Nguyen, L., Matheson, F., Blennerhassett, J., Quin, B., (2007)Can soil amendments zeolite or lime shift the balance between nitrous oxide and dinitrogen emissions from pasture and wetland soils receiving urine or Urea-N? *Australian Journal of Soil Research.* 45:543-553.
[37] Zaman, M., Nguyen, M., Blennerhassett, J., Quin, B., (2008) Reducing NH_3, N_2O and NO_3^- losses from a pasture soil with urease or nitrification inhibitors and elemental S-amended nitrogenous fertilisers. *Biology and Fertility of Soils*. 44:693-705.
[38] Zeigler, R., Mohanty, S., (2010) Support for international agricultural research: current status and future challenges. *New Biotechnology*.27:565–572.
[39] Zub,H., Brancourt-Hulmel, M., (2010) Agronomic and physiological performances of different species of *Miscanthus*, a major energy crop. A review. *Agronomy for Sustainable Development*. 30:201-214.
[40] Standard NF ISO 11260 (1995 Revised in 2014): Soil quality - Determination of total nitrogen - Modified Kjeldahl method (Classification index: X31-111).
[41] Standard NF ISO 11265 (1995 Revised in 2014): Soil quality-Determination of specific electrical conductivity (Classification index: X31-113).
[42] Standard NF ISO 10390 (2005 Revised in 2014): Soil quality-Determination of pH (Classification index: X31-117).
[43] Standard NF ISO 14256 (2007 Revised in 2014): Soil quality-Determination of nitrates, nitrites and ammonium in raw soils by extraction with a solution of potassium chloride. (Classification index: X31-423).

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Potential Health Risk Assessment for the Occurrence of Heavy Metals in Rice field Influenced by Landfill Activity in Can Tho City, Vietnam

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Abstract— *The study was conducted to assess potential risk of heavy metals in the soil and rice plants in the ricefield around the landfill in Dong Thang commune, Co Do district, Can Tho city, Vietnam. Four soil samples in which three samples were collected around the landfill and one sample was collected one km away from the landfill for the analysis of heavy metals including Mn, Zn, Cu, Cr, Ni, Pb and Cd. Rice samples were collected during ripening stage (few days before the harvest) at the same locations with the soil sampling, for the same heavy metal species analysis. The findings revealed that six out of seven heavy metals occurred in the soil.The decreasing order of theheavy metals concentrationsin the soil samples was Mn > Zn> Ni> Cr> Cu> Pb. This study found that accumulation of heavy metals in parts of rice at S1-S3 was higher that those at S4 (except for Zn and Pb at rice roots) and decreased in the order Mn> Zn> Cu> Ni> Cr (except in rice grain, Cr> Cu> Ni). Heavy metalsgenerally in the rice partswere in the magnitude order of root > stem-leave> grain. The calculated hazard index (HI) indicated that the accumulation of heavy metals in soil and rice grain is not likely to pose a threat to public health (HI <1), however, potential health and ecological risk may still exist. Measures should be taken to prevent landfill leachate leaching into the agricultural areas to minimize potential environmental and health risks.*

Keywords*— *Landfill; leachate; heavy metals; health risk; ricefield.

I. INTRODUCTION

Vietnam has recently been facing serious environmental pollution from solid wastes as the amounts of generated wasteshave been increasing in both quantity and toxic level. According to the National Environmental Report 2011-2015 **(MONRE, 2015)**, the total amount of urban domestic solid waste generated in the country was 32,000 tons in 2014. The amount of solid waste generated in the Mekong Delta region accounted for 5% of the generation of the whole country. Can Tho city is generatingsolid wastes of approximate 893 tons day^{-1}**(People's Committee of Can Tho City, 2015)**. Solid wastes have been collected and treated at landfills. However, landfills have also been identified as a cause of soil and groundwater pollution**(Fatta et al., 1999)**. According to **MONRE (2015)**, only 203 out of 660 landfills across the country are sanitary landfills, and the remaining were unsanitary. However, the majority of landfills have been overloaded, exacerbating the environmental impacts, which has led to increasingly serious and complex pollution problem in the landfilling areas.

The landfill at Dong Thang Commune, Co Do District, Can Tho City, Vietnam is in a state of serious overload due to receiving a fairly large amount of waste approximate 370 tons per day^{-1} from several districts of Can Tho city. The untreated leachate has significantly affected water quality, soil and rice yield in the land adjacent to the landfill **(Nhien and Giao, 2019)**. Leachate not only contains high levels of organic matter, nitrogen but also significant concentrations of heavy metals, so it may cause pollution of soil and surface water **(Nhien and Giao, 2019)**. Several studies have also shown that heavy metals are often found in high concentrations in and around landfills all over the world **(Alam et al., 2012; Nava-Martinez et al., 2012; Ajah et al., 2015)**. In addition, heavy metals could potentially present in paddy fields due to impurities of chemical fertilizers and pesticides **(Liu et al., 2003; Kingsawat and Roachanakanan, 2011)**. Therefore, heavy metal contamination is always a major focus in several environmental studiessince it could bioaccumulate in microorganisms and then transfer into food chains, for example, from plant to animal and to human being**(Munees and Abdul, 2012; Klinsawathom et al., 2017)**. The former study pointed out that heavy metals could move from soil and water to plants' tissues via uptakingby roots **(Kingsawat and Roachanakanan, 2011)**, posingpotential risks for human health and ecosystems**(Satachon et al., 2019)**.Currently, several studies reported on the quality of water and soil at the landfill and surrounding areas **(Kanmani and Gandhimathi, 2013; Huang et al., 2013; Nhien and Giao, 2019)** but very few studies have been carried out on assessment of potential risk resulting from exposure to heavy metals in rice grains and soil.This study was implemented to examine the potential risk for the presence of heavy metals in soil and rice parts around Dong Thang landfill, Co Do district, Can Tho city, Vietnam. The findings from this study could provide useful information for local authorities for managing risk resulting from heavy metal occurrence.

II. MATERIALS AND METHODS

1) Soil sampling and analysis

Soil samples were collected at the depth of 0-25 cm at 4 locations, of which 3 locations in the rice field surrounding the landfill (namely S1, S2, S3) and 1 locationin the ricefield1km away from the landfill (namely S4) (Figure 1). After the collection, the soil samples were dried at room temperature, pulverized and sieved through mesh with the pore size of 0.5 mm for heavy metal analysis. The pulverized soil sample (0.5g) was digested using a microwave digester (Microwave digester, Milestone, Ethos) using the method of the United State Environmental Protection Agency (EPA3051) by adding 10 ml of 65% nitric acid and operated at 1,000 watts of power, temperature of 175^{o}C for 15 minutes 30 seconds. Heavy metals including Cd, Cr, Cu, Fe, Ni, Mn, Pb and Zn were determined by atomic absorption spectrometry (AAS, Agilent, AA240). All glasswares used in heavy metal analysis were cleaned washed using 0.1 M nitric acid for 24 hours and then rinsed with distilled water. Analysis of heavy metals was performed in triplicates.

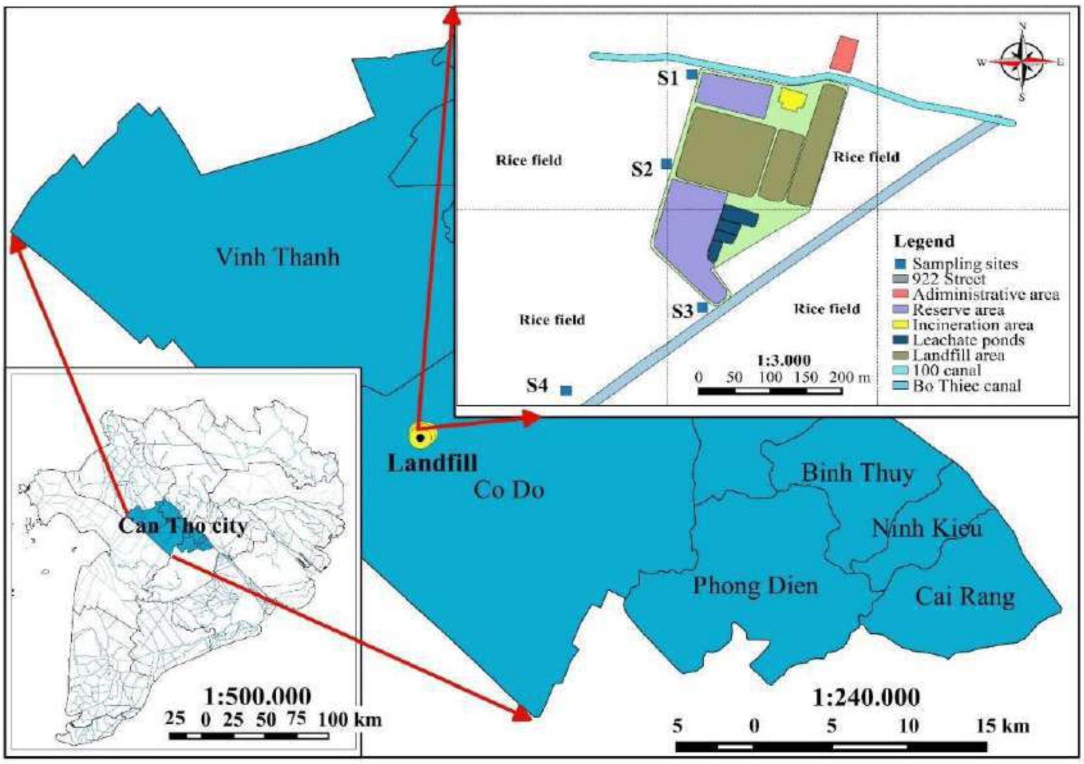

Fig.1: Soil sampling locations.

2) Rice sample collection and analysis for heavy metals

Rice samples were collected during ripening stage (few days before the harvest) at the same locations with the soil sampling (Figure 1). Five whole rice plants were carefully removed from soil at five positions in an area of 1 m^2 for every sampling location. The collected rice plants were divided into three parts including the root, stem and leave, and grain. The separated parts of the rice plants at three locations surrounding landfill (S1, S2, and S3) were pooled to reduce the analysis cost due to limited budget. The heavy metals including Cd, Cr, Cu, Ni, Mn, Pb and Zn were analyzed in the rice parts. The procedure for analyzing heavy metals in rice samples was performed in the similar manner to that for analyzing soil samples.

3) Risk assessment

Hazard index (HI) due to heavy metals in soil and rice (grain) was assessed according to **Hang *et al.* (2009) and Ferreira- Baptista and de Miguel (2005)**. Heavy metal enters the body daily (D) through the three main routes including ingestion (D_{ing}), inhalation (D_{inh}), and dermal contact (D_{der}). Heavy metalsin rice grain only enter the body through ingestion (D_{ing}). Daily consumption levels through different contact routes were calculated based on Equation 2, 3, and 4.

- Direct ingestion rice grain (D_{ing}):

$$D_{ing} = \frac{C_S x IngRxEFxEDxCF}{BWxAT} \qquad \text{(Eq.2)}$$

- Inhalation of suspended particles via mouth and noise (D_{inh}):

$$D_{inh} = \frac{C_S x InhRxEFxED}{BWxATxPEF} \qquad \text{(Eq.3)}$$

- Dermal contact (D_{der}):

$$D_{der} = \frac{C_S x SAxSLxABSxEFxEDxCF}{BWxAT} \text{(Eq.4)}$$

Where: C_s is concentration of pollutants in soil or rice (mg/kg); IngR is the rate of ingestion of pollutants in soil or rice (mg day^{-1}); InhR is inhalation rate of suspended particles in soil ($m^3 day^{-1}$); EF and ED are frequency of exposure ($dayyear^{-1}$) and duration of exposure (years); CF is conversion factor = 1.00E-06 (kg mg^{-1}) and , BW is average body weight (kg); AT is average time of noncarcinogenic (days); PEF is soil-to-air particulate emission factor (m^3 kg^{-1}), SL is soil-to-skin adherence factor ($mgcm^{-2}$); SA is skin surface area available for exposure (cm^2) and ABS is dermal absorption factor. Detail of these factorfor risk assessment wasindicated in Table 1.

The risk assessment for non-carcinogenic was calculated using Equation 5:

$$HI = \sum_{i=1}^{m} \sum_{j=1}^{n} HQ_{ij} = \sum_{i=1}^{m} \sum_{j=1}^{n} \left(\frac{D}{RfD}\right)_{ij}$$
(Eq.5)

where m and n are type and number of pollutants; R_fD is reference dose (mg kg day^{-1}) (Table 1); D is daily uptakedose (mg kg^{-1} day^{-1}); $HQ_{ij}$is risk for the exposure path. HI <1 means there is no possibility of adverse human health effects, whereas HI> 1 means there is likely to have adverse effect on human health.

Table1 Parameters used for risk assessment (Hang et al., 2009)

Parameters	Adult	Children
IngR		
-Soil ($mgday^{-1}$)	100	200
- Rice grain (g day^{-1})	389.2	198.4
InhR ($m^3 day^{-1}$)	12.8	7.63
EF (day)	350	320
ED (year)	24	6
BW (kg)	59.95	23.9
AT (day)	8,760	2,190
SA (cm^2)	2,145	1,150
SL (mg cm^{-2})	0.07	0.20
ABS	0.001	0.001
PEF ($m^3 kg^{-1}$)	1.36E + 09	1.36E + 09

Table.2: Reference doses of some heavy metalsFerreira-Baptista and de Miguel (2005)

Heavy metals	RfD (mg $kg^{-1} day^{-1}$)		
	RfD_{ing}	RfD_{inh}	RfD_{der}
Cd	1.00E - 03		1.00E - 05
Cr	3.00E - 03	2.86E - 05	6.00E - 05
Cu	4.00E - 02	4.02E 02	1.20E - 02
Mn	4.60E - 02	1.43E - 05	1.84E - 03
Ni	2.00E - 02	2.06E - 02	5.40E - 03
Pb	1.40E - 03	3.52E - 05	5.25E - 05
Zn	3.00E - 01	3.00E - 01	6.00E - 02

III. RESULTS AND DISCUSSION

1) Occurrence of heavy metals in soil

Table 3 presented the concentrations of heavy metals in the soil surrounding the landfill. Six out of seven heavy metals occurred in the two soil layers around the landfill with average concentrations ranging from 12.3 ± 2.14 - 291 ± 38.85 mg kg^{-1}. The concentrations of Mn, Zn, Cu, Cr at the locations S1, S2 and S3 were all higher than those at S4 (1 km away from the landfill). Cd was the only metal not detected in all soil samples.

Table.3: Heavy metals concentrations in the soilsurrounding the landfill

Heavy metals	Heavy metal concentration (mg kg^{-1})					QCVN 03-MT:2015/BTNMT	CCME, 2007
	S1	S2	S3	S4	Average (S1, S2, S3)		
Mn	321^a±2	240^b±0	315^a ±2	234^b±8	291± 38.85	-	-
Zn	78.8^b±0	82.7^a± 0.70	65.8^d± 0.35	74.7^c±0	75.8± 7.70	200	200
Cu	20.4^a±0	19.0^b± 0.27	14.7^d± 0.01	17.6^c±0	18.1± 2.66	100	63
Cr	9.66^c± 0.56	27.5^a± 0.70	28.3^a± 0.05	11.1^b± 0.4	21.8± 9.12	150	64
Ni	34.9^b±0	36.3^a± 0.50	30.5^c± 0.25	35.6^b± 0.45	33.9± 2.66	-	50
Pb	14.6^a± 0.03	12.6^c± 0.02	9.66^d± 0.08	13.1^b± 0.50	12.3± 2.14	70	70
Cd	ND	ND	ND	ND	ND	-	3^a

Notes: Data were presented as Mean ± SD, n = 3. Diffrerent letters [a, b, c] indicated statistically significant at significance level 5% ($p<0.05$).[a] **Error! Reference source not found.**, [b]**Error! Reference source not found.**; ND: Not detected.

Most of heavy metal concentrations in soil were in compliance with QCVN 03-MT: 2015/BTNMT, CCME (2007). Concentration of Mn was the metal with the highest concentrations in soil ranging from 240 ± 0 - 321 ± 2 mg kg^{-1} (Table 3). Mn concentrations at S1 and S3 were always higher than that at S4 showing the negative impact of the landfill leachate on soil environment. Similar to Mn, Cr concentration at S4 was lower than those at S2 and S3 and this could be because Cr is not directly affected by the landfill leachate. The presence of Cr in soil is a major threat to plants and humans because under appropriate environmental conditions, Cr (III) is easily converted to Cr (VI) - a form always toxic to plants **(Ba, 2008)**. At locations around the landfill sites (except S3), Ni concentration ranging from 30.5 ± 0.25 - 36.3 ± 0.50 mg kg^{-1}. The results of Zn concentration ranged from 65.8 ± 0.35 - 82.7 ± 0.70 mg kg^{-1}. The distribution of Ni and Zn concentration at the locations and the soil were mainly influenced by the impact of leachate, mobility of the metals and soil properties. Cu and Pb were presented in soil with relatively low concentration at 16.3 ± 2.20 - 18.1 ± 2.66 mg kg^{-1} and 11.2 ± 0.46 - 12.3 ± 2.14 mg kg^{-1}, respectively (Table 3). Pb concentration in the soilat S4 (13.1 ± 0.50 mg kg^{-1}) was higher than those at S1-S3 (9.66 ± 0.08 -12.6 ± 0.02 mg kg^{-1}). Six out of seven heavy metals occurred in the soil samples collected at the surrounding landfill and 1km away from landfill. The presence of heavy metals not only affects the quality of the soil but also threatens the groundwater and rice production.

2) Heavy metals in rice plant

It was found that six out of seven heavy metals occurred in the rice plant parts including root, stem-leave, and rice grain (Table 4). The Cd concentration was below the detection limit, and below the FAO/WHO regulatory standard (0.2 mg kg^{-1}).Heavy metals were found highly accumulated in the rice roots in this study (Table 4). The concentrations of Mn, Zn, Cu, Pb, Ni and Cr in the rice root at S1-S3 were 674 ± 12.53 mg kg^{-1}, 87.6 ± 0.93 mg kg^{-1}, 29.3 ± 0.20 mg kg^{-1}, 11.7 ± 0.07 mg kg^{-1}, 16.9 ± 0.68 mg kg^{-1} and 10.4 ± 0.06 mg kg^{-1}, respectively, while these heavy metals at S4 were 403 ± 6.66 mg kg^{-1}, 104 ± 2.08 mg kg^{-1}, 28.0 ± 1.85 mg kg^{-1}, 14.5 ± 0.80 mg kg^{-1}, 7.95 ± 0.34 mg kg^{-1} and 5.04 ± 0.09 mg kg^{-1}, respectively (Table 4). The results indicated that heavy metal concentrations in rice roots in the area influenced by the landfill leachate were higher than those without influenced by the landfill activity.

The heavy metals including Mn, Zn, Cu, Ni, and Cr at the locations surrounding the landfills (S1-S3) were detected in the stem and leave of the rice plants at the concentrations of 645 ± 8.72 mg kg^{-1}, 47.6 ± 1.08 mg kg^{-1}, 5.42 ± 0.34 mg kg^{-1}, 4.37 ± 0.16 mg kg^{-1}, 2.30 ± 0.05 mg kg^{-1}, respectively. There were only Mn, Zn, Ni and Cu found at S4 at the concentrations of 544 ± 15.87 mg kg^{-1}, 61.5 ± 0.55 mg kg^{-1}, 2.78 ± 0.09 mg kg^{-1} and 1.96 ± 0.82 mg kg^{-1}, respectively (Table 4).

Table.4: Concentrations of heavy metals in rice plants

Sampling sites	**Heavy metals**	**Concentration of heavy metals (mg kg^{-1})**		
		Root	Stem - Leave	Grains
S4	Mn	403^{b}±6.66	544^{a}±15.87	129^{c}±11.59
	Zn	104^{a}±2.08	61.5^{b}±0.55	17.7^{c}±0.82
	Cu	28.0^{a}±1.85	1.96^{b}±0.82	1.45^{b}±0.13
	Cr	5.04±0.09	ND	0.57±0.01
	Ni	7.95^{a} ± 0.34	2.78^{b}±0.09	1.68^{c}±0.30
	Pb	14.5±0.80	ND	ND
	Cd	ND	ND	ND
S1-S3	Mn	674^{a}±12.53	645^{a}±8.72	237^{b}±21.79
	Zn	87.6^{a}±0.93	47.6^{b}±1.08	35.8^{c}±0.17
	Cu	29.3^{a}±0.20	5.42^{b}±0.34	4.27^{c}±0.07
	Cr	10.4^{a}±0.06	2.30^{c}±0.05	5.67^{b}±0.25
	Ni	16.9^{a}±0.68	4.37^{b}±0.16	4.25^{b}±0.13
	Pb	11.7±0.07	ND	ND
	Cd	ND	ND	ND

Notes: Data were presented as Mean ± SD, n = 3. Diffrerent letters $^{a, b, c}$ indicated statistically significant at significance level 5% ($p<0.05$). ND: not detected.

The concentrations of Mn, Zn, Cr, Cu and Ni in the rice grains surrounded the landfill were 237 ± 21.79 mg kg^{-1}, 35.8 ± 0.17 mg kg^{-1}, 5.67 ± 0.25 mg kg^{-1}, 4.27 ± 0.07 mg kg^{-1}, 4.25 ± 0.13 mg kg^{-1}, respectively. At the S4 location, the concentrations of Mn, Zn, Ni, Cu and Cr in the rice grains were 129 ± 11.59 mg kg^{-1}, 17.7 ± 0.82 mg kg^{-1}, 1.68 ± 0.30 mg kg^{-1}, 1.45 ± 0.13 mg kg^{-1} and 0.57 ± 0.01 mg kg^{-1}, respectively. The average concentration of heavy metals in the rice grains at the locations S1-S3 were significantly higher than those at S4 from 1.49 - 2.94 times. The concentration of Cr in rice grainat S1-S3 (near the landfill)was 10 time higher than that at S4 (1km away from the landfill) could indicate serious impact of landfill leachate on the rice production and pose a threat to rice consumption since Cr is considered carcinogenic metal **(Ba, 2008)**.

Among the heavy metals, Mn was highly accumulated in rice plants that could be due to its higher mobility compared to the others **(Prechthai et al., 2008)**. This study found that accumulation of heavy metals in parts of rice at S1-S3 was higher that those at S4 (except for Zn and Pb at rice roots) and decreased in the order Mn> Zn> Cu> Ni> Cr (except in rice grain, Cr> Cu> Ni). Heavy metals accumulated in rice parts with decreasing order root> stem - leave>grain (except for Mn at S4 and Cr at S1 - S3).

3) Health risk assessment

The mean concentration of heavy metals found in soils and rice grainswere used to calculate health risk and the results were showed in Table 5.

Health risk assessment was performed for heavy metals contaminated in soil and rice grains. The result indicated that there is no health risk for children and adults since all the hazard indexes (HI) were less than 1. Children were likely to suffer more risk than adults because the HI values for children were higher than adults (Table 5). Previous studies also indicated that there was no possible risk for human when exposed to soil and rice grains contaminated with heavy metals surrounding the landfill [13, 20]. It was clearly showed that HI values calculated for the heavy metals in the area surrounding the landfill (S1-S3) were higher than those calculated for heavy metals at the location S4 (Table 5). This could mean that higher health risk was expected for the area around the landfill. In the soil sample, the level of health risk (for adult) gradually decreases via HQ_{ing}>HQ_{inh}>HQ_{der} routes; However, the potential health risk for children via ingestion (HQ_{der}) was higher that that via inhalation (HQ_{inh}). Among the heavy metals, Pb was the metal could pose the highest health risk, although this is the metal present with the lowest concentration in the soil. The health risk levels of the examined heavy metals were arranged as decreasing order Pb> Mn> Cr> Ni> Cu > Zn. In the rice grains, the estimated potential health risk of the heavy metals were in the order of Mn> Cr> Ni> Zn> Cu. Comparing the values of HI between soil and rice grains in both child and adult, it could be seen that the risk for rice grain consumption was higher than the human exposed to the soil contaminated heavy metals. This study suggested that the agricultural activity, especially rice cultivation in the area surrounding the landfill is no longer suitable because the soil was contaminated with heavy metals and the heavy metals started to be accumulated in the rice parts. Long-term consumption of agricultural products produced in the study area could lead to potential health risk.

Table.5: Health risk assessment for exposure to heavy metals contaminated soil and rice grain

Heavy metals	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**	**Total**
			For soil sample at S4				
			Adult				
HQ_{ing}	5.92E-03	7.05E-04	8.14E-03	2.84E-03	1.50E-02	3.98E-04	**3.30E-02**
HQ_{inh}	5.84E-05	6.60E-08	2.46E-03	2.60E-07	5.62E-07	3.75E-08	**2.52E-03**
HQ_{der}	4.44E-04	3.53E-06	3.05E-04	1.58E-05	6.01E-04	2.99E-06	**1.37E-03**
HI	6.42E-03	7.09E-04	1.09E-02	2.86E-03	1.56E-02	4.01E-04	**3.69E-02**
			Children				
HQ_{ing}	2.71E-02	3.23E-03	3.73E-02	1.30E-02	6.89E-02	1.83E-03	**1.51E-01**
HQ_{inh}	8.79E-05	9.93E-08	3.70E-03	3.91E-07	8.45E-07	5.64E-08	**3.79E-03**
HQ_{der}	1.56E-03	1.24E-05	1.07E-03	5.56E-05	2.11E-03	1.05E-05	**4.82E-03**
HI	2.88E-02	3.24E-03	4.21E-02	1.31E-02	7.08E-02	1.84E-03	**1.60E-01**
			For soil samples at S1-S3				
			Adult				
HQ_{ing}	1.16E-02	7.22E-04	1.01E-02	2.71E-03	1.40E-02	4.04E-04	**3.96E-02**
HQ_{inh}	1.15E-04	6.76E-08	3.07E-03	2.48E-07	5.25E-07	3.80E-08	**3.19E-03**
HQ_{der}	8.73E-04	3.61E-06	3.81E-04	1.51E-05	5.61E-04	3.03E-06	**1.84E-03**
HI	1.26E-02	7.25E-04	1.36E-02	2.73E-03	1.46E-02	4.07E-04	**4.46E-02**
			Children				
HQ_{ing}	5.33E-02	3.31E-03	4.65E-02	1.24E-02	6.43E-02	1.85E-03	**1.82E-01**
HQ_{inh}	1.73E-04	1.02E-07	4.62E-03	3.72E-07	7.89E-07	5.72E-08	**4.79E-03**
HQ_{der}	3.07E-03	1.27E-05	1.33E-03	5.30E-05	1.98E-03	1.07E-05	**6.45E-03**
HI	5.65E-02	3.33E-03	5.24E-02	1.25E-02	6.64E-02	1.86E-03	**1.93E-01**
			For rice grain at S4				
			Adult				
HI	1.18E-03	2.26E-04	1.75E-02	5.23E-04	-	3.67E-04	**1.98E-01**

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Heavy metals	Cr	Cu	Mn	Ni	Pb	Zn	Total
			Children				
HI	1.38E-03	2.64E-04	2.04E-02	6.11E-04	-	4.29E-04	**2.31E-01**
			For rice grain at S1-S3				
			Adult				
HI	1.18E-02	6.65E-04	3.21E-02	1.32E-03	-	7.43E-04	**4.66E-01**
			Children				
HI	1.38E-02	7.77E-04	3.75E-02	1.55E-03	-	8.68E-04	**5.44E-01**

IV. CONCLUSION

Six out of seven heavy metals including Mn, Zn, Ni, Cr, Cu and Pd were detected and were lower than the permitted limits of QCVN 03-MT: 2015/BTNMT and CCME. The concentration of the detected heavy metals in the topsoil (0-25cm) around the landfill (S1-S3) were higher than those at the location 1 km from the landfill (S4) with the exception for Ni, Pb. The concentration of the heavy metals in the rice parts in the surrounding landfill sites decreased from Mn> Zn> Cu> Ni> Cr (except for the heavy metals in the rice grains with the order of Cr> Cu> Ni). Cd was not detected in the rice and Pb only appeared in the rice roots. The calculation of the hazard index (HI) shows that the health risk due to heavy metals contamination in soil and rice grain for children was higher than for adult, however, all HI values fell into safe level. Health risk for rice consumption was higher than that for exposure to soil contaminating heavy metals. In addition, health risk for due to exposure to heavy metals by all routes in the area surrounding the landfill were higher than that at 1km away from the landfill. Measures should be taken to minimize the leakage of leachate into rice fields.

REFERENCES

[1] Ministry of Natural Resources and Environment. Vietnam state of environment report for the period 2011-2015, 2015.
[2] People's Committee of Can Tho City. Report on state of environment in Can Tho city in the period of 2011 – 2015, 2015.
[3] **Fatta**, D., Papadopoulos, A., Loizidou, M. A study on the landfill leachate and its impact on the groundwater quality of the greater area. Environ Geochem Health, 1999, 21(2), 175-190.
[4] Nhien, H.T.H., Giao, N.T. Environmental Soil, Water, and Sediment Quality of Dong Thang Landfill in Can Tho City, Vietnam. Applied Environmental Research, 2019, 41(2), 73-83.
[5] Alam, S.S., Osman, K.T., Kibria, G. Heavy metal pollution of soil from industrial and municipal wastes in Chittagong, Bangladesh. Archives of Agronomy and Soil Science, 2012, 58(12), 1427-38.
[6] Nava-Martinez, E.C., Flores-Garcia, E., Espinoza-Gomez, H., Wakida, F.T. Heavy metals pollution in the soil of an irregular urban settlement built on a former dumpsite in the city of Tijuana, Mexico. Environmental Earth Sciences, 2012, 66(4), 1239-1245.
[7] Ajah, K.C., Ademiluyi, J., Nnaji, C.C. Spatiality, seasonality and ecological risks of heavy metals in the vicinity of a degenerate municipal central dumpsite in Enugu, Nigeria. Journal of Environmental Health Science and Engineering, 2015,13, 1-14.
[8] Kingsawat, R., Roachanakanan, R. Accumulation and distribution of some heavy metals in water, soil and rice fields along the Pradu and Phi Lok canals, Samut Songkhram province, Thailand. Environment and Natural Resources, 2011, 9(1), 38-48.
[9] Liu, J., Li, K., Xu, J., Zhang, Z., Ma, T. Lead toxicity, uptake and translocation in different rice cultivars. Plant Science, 2003, 165, 793-802.
[10] Munees, A., Abdul, M. Bioaccumulation of heavy metals by Zn resistant bacteria isolated from agricultural soils irrigated with waste water. Bacteriology Journal, 2012, 2(1), 12-21.
[11] Klinsawathom, T., Songsakunrungrueng, B., Pattanamahakul, P. Heavy Metal Concentration and Risk Assessment of Soil and Rice in and around an Open Dumpsite in Thailand. EnvironmentAsia, 2017, 10(2), 53-64.
[12] Satachon, P., Keawmoon, S., Rengsungnoen, P., Thummajitsakul, S., Silprasit, K. Source and Health Risk Assessment of Some Heavy Metals in Non-Certified Organic Rice Farming at Nakhon Nayok Province, Thailand. Applied Environmental Research, 2019, 41(3), 96-106.
[13] Kanmani, S., Gandhimathi, R. Assessment of heavy metal contamination in soil due to leachate migration from an open dumpingsite. Applied Water Science, 2013, 3(1), 193–205.
[14] Huang, Z., Pan, X.D., Wu, P.G., Han, J.L., Chen, Q. Health Risk Assessment of Heavy Metals in Rice to the Population in Zhejiang, China. PLoS ONE, 2013, 8(9), e75007.
[15] Hang, X., Wang, H., Zhou, J., Ma, C., Du, C., Chen, X. Risk assessment of potentially toxic element pollution in soil and

rice (*Oryza sativa*) in a typical area of the Yangtze River delta. Environmental Pollution, 2009,157 (8-9), 2542-2549.

[16] Ferreira-Baptista, L., de Miguel, E. Geochemistry and risk assessment of street dust in Luanda, Angola: a tropical urban environment. Atmospheric Environment, 2005, 39(25), 4501-4512.

[17] Ministry of Natural Resources and Environment. QCVN 03-MT: 2015/BTNMT National technical regulation on the allowable limits of heavy metals in the soil, 2015.

[18] Canadian Council of Ministers of the Environment. Soil quality guidelines for the protection of environmental and human health. CCME, 2007.

[19] Ba, L.H. Fundamental on Environmental Toxicology, third edition. Ho Chi Minh City: Ho Chi Minh City National University Press, 2008, 639 pages.

[20] Prechthai, T., Parkpian, P., Visvanathan, C. Assessment of heavy metal contamination and its mobilization from municipal solid waste open dumping site. Journal of Hazardous Materials, 2008, 156(1-3), 86-94.

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Shoot induction using Benzyl Adenine in three accessions of patchouli plant (*Pogostemon cablin* Benth) from West Pasaman

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Abstract— *Patchouli (*Pogostemon cablin* Benth) is a major essential oil-producing plant in Indonesia. But its productivity is still low that influenced by low genetic quality. In West Pasaman Regency, there are three patchouli local accessions namely Situak, Rimbo Binuang, and Tombang that have potential as a genetic resource in the breeding program. In this study, the tissue culture method was used for plant propagation to produce superior seedling in a relatively short time. This study aims to determine the effective concentration of BA for in vitro callus formation and plantlet regeneration in three patchouli accessions. The method used is Random Design (CRD) with 2 factors, namely the concentration of BA (0.01 and 0.03 ppm) and plant accessions (Situak, Rimbo Binuang, and Tombang), with 6 total treatments using 5 replications. Data were analyzed statistically by the F test at a 5% significance level and followed by the DNMRT at a 5% level. The results showed that the treatment of BA 0.01 ppm in Rimbo Binuang accession produce the highest value in all variables which are live explants percentage, explants forming callus percentage, number of shoots per explant, number of total leaves, and number of leaves per shoot are 92 %, 92 %, 5.30 shoots, 28.80 leaves, and 4.20 leaves respectively. So, it concluded that the 0.01 ppm BA is an optimal concentration as a patchouli in-vitro growth inducer. The best plant accession as a genetic source in plant propagation using BA is the Rimbo Binuang accession.*

Keywords— ***aromatherapy, cytokinin, essential oil, organogenesis, perfume.***

I. INTRODUCTION

Patchouli (*Pogostemon cablin* Benth) is an important plant act as an essential oil-producing plant. Usually, the patchouli essential oil is used as raw material for pharmaceutical, perfume, and aromatherapy industries (Wahyudi and Ermiati, 2012). Patchouli essential oils are produced from the distillation of leaves and stems. This means the production of stem and leaf biomass directly will affect the productivity and quality of essential oils. The quality of patchouli essential oil is largely determined by patchouli alcohol (PA). PA is the main compound in patchouli oil which belongs to the sesquiterpene group (with the molecular formula $C_{15}H_2O_6$). High PA levels indicate the better quality of the oil (Idris *et al.*, 2014).

Indonesia has a role as a supplier of 90% of the world's patchouli oil needs. Initially, the centers of Indonesia's patchouli oil production were in Java and Sumatra. In recent years Sulawesi has dominated, namely 80% of national production. However, based on patchouli alcohol (PA) levels, the minimum standard for quality of Sumatran patchouli oil (30-34%) is higher than Sulawesi (26-30%). Then at the same quality, namely PA level 30%, Sumatra patchouli oil is valued at 56 USD/kg while Sulawesi is only 50 USD/kg (Caiger, 2016).

An important factor influencing the productivity and quality of patchouli essential oil is the variety and genetic quality of the cultivated plant. Patchouli plants are generally not flowering and propagated vegetatively so that the frequency of genetic diversity is naturally low (Nuryani *et al.*, 2003). Therefore, exploration and collection of germplasm from various locations need to be done to assemble new superior varieties. Assembling new high-yielding varieties requires local genotype plants as a source of germplasm because it has genetic diversity that is still natural. Plants that are suitable for use as germplasms are genotypes that have broad and specific adaptability at the local location. Tissue culture was chosen as a method of propagating large numbers of seedlings in a relatively short, uniform, and minimal source of disease (Hadipoentyanti, 2010). The type of explants and plant growth regulators (PGR) are important factors that determine the success of the tissue culture propagation method (Swamy *et al.*, 2010).

Variants of explant types can be obtained from patchouli production centers. In West Sumatera Province, West Pasaman Regency has seven patchouli oil cultivation and refining locations. West Pasaman farmers cultivate various types of local patchouli but their characteristics have not been able to be clearly described (Mayerni *et al.*, 2018). However, the Rimbo Binuang accession has the highest oil yield value, while the Situak and the Tombang accession produce high PA content (Febriyetty, 2018). So that these three accessions serve as sources of explants in this study. Whereas PGR which is used as a direct inducer of organogenesis in this study is synthetic cytokinin type Benzyl Adenine (BA). This research is expected to be able to identify the best local patchouli genotype as a genetic source to develop superior varieties and to determine the best BA concentration that can stimulate in-vitro explants' shoot formation.

II. MATERIALS AND METHODS

2.1. Isolate the explant source

Three accessions of patchouli plants as sources of explants were obtained from three districts in West Pasaman Regency, namely Situak accession (Lembah Melintang District), Rimbo Binuang accession (Pasaman District), and Tombang accession (Tamalau District). Patchouli plant cuttings are maintained at Greenhouse of Balittro Laing Experimental Garden in Solok. In order not to be contaminated with pathogens, plants were treated with a fungicide (300 mg/L Dithane M-45) and bactericide (300 mg/L Agrept 20 WP). Plant young leaves are used as explants because it composed of rapidly differentiating meristem tissues.

2.2. Explant planting for callus induction

Callus induction was arranged in a completely randomized design (CRD) in factorial consisting of two factors. The first factor is BA concentrations and the second factor is patchouli accessions. The first factor consisted of 2 levels of treatment (0.01 ppm and 0.03 ppm) and the second factor consisted of 3 levels of treatment (Situak, Rimbo Binuang, and Tombang). Thus obtained 6 treatments with consisted of 5 replications and each experimental unit consisted of 5 units, so that there were 150 experimental units.

In this study, we used Murashige and Skoog (MS) as culture media. Explants are sterilized by washing the young leaves with running water, detergents, and sterile aqua dest 3-4 times. Then the leaves were soaked in Tween 80 for 5 minutes. Next in Laminar Air Flow Cabinet, the explants were soaked with 70% alcohol for 30 seconds, then rinsed with sterile aqua dest. After that, the explants were immersed in a 50% Clorox solution for 5 minutes and rinsed with sterile distilled water 3-4 times. Then soaked with antibiotics for 30 seconds. After that, explants are cut about 1x1 cm in the sterile Petri dish and air-dried around bunsen' fire for several seconds. The dried-air explant then placed transversely upward (abaxial position) and touch the media on the lower surface. Bottles containing explants were incubated at 23 ° C for 4 weeks. The incubation room is sprayed with 70% alcohol every day, the contaminated bottle is immediately removed so it is not transmitted to other bottles.

2.3 Observation of callus induction

The observed variables were live explants percentage, explants forming callus percentage, number of shoots per explant, number of leaves per shoot, and the total number of leaves. The live explants percentage was observed on the 1st day to the 30th day after planting in the medium. Live explants are characterized by no discoloration (not browning) and are not contaminated by microorganisms. Observation of the percentage of callus formation was carried out in one day after planting until the end of the observation period (4th week). The data were analyzed statistically by the F test at a 5% significance level and followed by the DNMRT at a 5% level.

III. RESULTS AND DISCUSSION

3.1 Live explants percentage

Live explants are usually characterized by fresh green explants, the presence of protuberances caused by cell division, and not browning. The interaction between BA concentration and patchouli accession to live explants percentage can be seen in Table 1.

Table 1. Live explants percentage of three patchouli accessions in different BA concentration

Accession	BA concentration	
	0.01 ppm	0.03 ppm
Situak	80 % bA	68 % bB
Rimbo Binuang	92 % aA	80 % aB
Tombang	72 % bA	40 % cB

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

Table 1 shows that the Rimbo Binuang accession is the best accession that can produce the highest percentage of live explants in the two BA concentration treatments, namely 92% at 0.01 ppm of BA and 80% at 0.03 ppm of BA. While the Situak accession resulted in the second position in both BA treatments followed by the Tombang accession in the lowest percentage. This difference in value between accessions may be influenced by genetic factors that affect the ability of tissues to absorb nutrients in culture media (Mahadi *et al.,* 2016).

The percentage of the live explants of the three accessions in this study was higher in media with 0.01 ppm of BA compared to 0.03 ppm of BA. This means that patchouli explants have a higher chance of survival at lower concentrations. This is similar to the opinion of (Salisbury and Ross, 1995) that the main function of cytokinins is to stimulate cytokinesis or cell division, but if the cytokinin concentration is high it will decrease the percentage of live explants. Other studies using BA type cytokinins with a concentration of 1.5 mg/L combined with 2,4-D 0.3 mg/L were able to stimulate the formation of adventitious roots in Inggu plants (Lestari, 2011). Application of BA and GA as growth regulators able to increase the multiplication of shoots in *B. homonyms* plants (Kumari *et al.*, 2017).

3.2 Explants forming callus percentage

Callus initiation is characterized by the thickening of the leaf bone and discoloration to brownish-yellow in the cut explant area (Astuti and Andayani, 2007). Plants will form a callus that is caused by cell damage and autolysis occurs when injured. The injured cells in the explant will make repairs that begin with swelling due to the influence of turgor pressure (Taiz and Zeiger, 1998; Indah and Erma 2013). Patchouli callus structure formed in this study can be seen in the following figure.

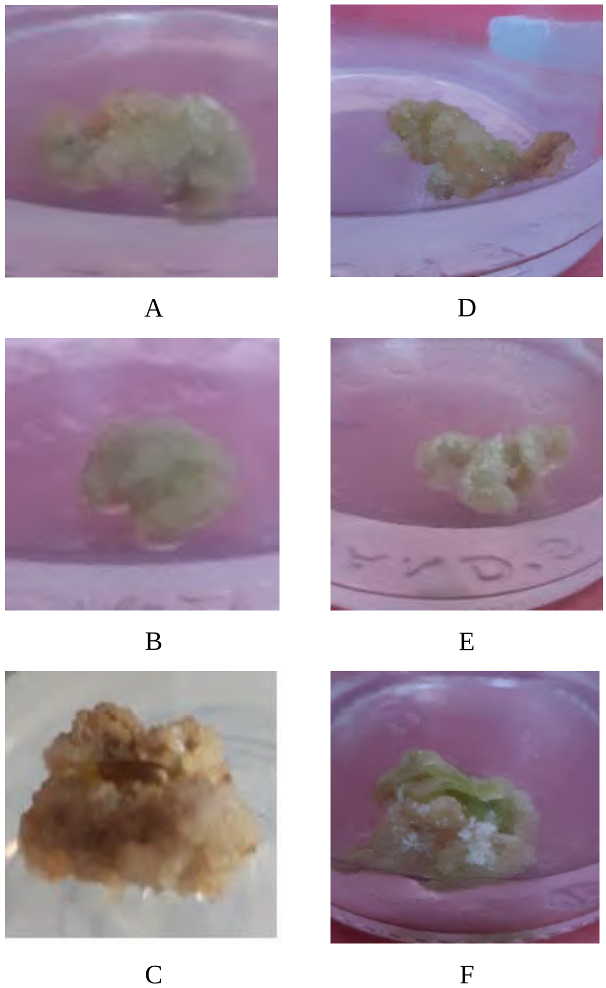


Fig.1: Patchouli Callus. A: 0.01 ppm of BA + Situak; B: 0.01 ppm of BA + Rimbo Binuang; C: 0.01 ppm of BA + Tombang; D: 0.03 ppm of BA + Situak; E: 0.03 ppm of BA + Rimbo Binuang; F: 0.03 ppm of BA + Tombang.

Compact callus texture is a good callus because it can accumulate more secondary metabolites. Cytokinin treatments in callus culture play an important role in triggering cell division and elongation to accelerate the growth and development of callus (Taji *et al.,* 2006).

The percentage of explants forming callus in each plant has a very diverse variation. The results of the analysis of variance showed that the use of BA to patchouli plant accessions had an interaction with the percentage of explants forming callus (Table 2).

Table 2. Explants forming callus percentage of three patchouli accession in different BA concentration

Accession	BA concentration	
	0.01 ppm	0.03 ppm
Situak	80 % bA	68 % bB
Rimbo Binuang	92 % aA	80 % aB
Tombang	72 % bA	40 % cB

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

Rimbo Binuang accession can produce the best percentage of explants forming callus at a concentration of BA 0.01 ppm, namely 92%, followed by Situak accession (80%) and Tombang accession (72%). In the treatment of BA 0.03 ppm, the accession of Rimbo Binuang also had the best explant percentage forming callus (80%). Thereafter followed by accessions Situak (68%) and Tombang (40%). Based on the concentration of BA used, the percentage of callus formation in all three accessions was higher at a concentration of 0.01 ppm compared to 0.03 ppm. This shows that the application of BA growth regulators at lower concentrations can produce a high percentage of explants forming callus. These results differ from studies conducted by Harahap (2015) where BA concentrations of 1 mg/L were faster to induce callus than concentrations of 0.5 mg/L. In the *Angelica keiskei* Koidzumi plant also showed the same thing in the use of 1 mg/L BA can induce callus fastest (Yelnititis and Komar, 2011).

3.3 Shoot numbers per explant

Murashige (1974) states that the induction and proliferation of shoots can be stimulated by only using BA. However, the analysis of variance showed that there was no interaction between the concentration of BA and patchouli accessions used on the number of shoots per explant, but the two single factors had a significant effect (Table 3).

The number of formed shoots per explant per accession was higher at concentrations of BA 0.01 ppm compared to 0.03 ppm of BA. 0.01 ppm of BA resulted in an average number of shoots per explant of 4.73 whereas at 0.03 ppm of BA only resulted in about 3.40. The highest number of shoots formed was obtained from Rimbo Binuang accessions, which were 6 at concentrations of BA 0.01 ppm and 4.6 at concentrations of BA 0.03 ppm, the average shoots formed at these accessions was 5.30. While the average shoots formed in Situak accessions are only 3.60 and 3.30 in Tombang accessions.

Table 3. Shoot numbers per explant of three patchouli accession in different BA concentration

Accession	BA concentration		
	0.01 ppm	0.03 ppm	Mean
Situak	4.4	2.8	3.60 b
Rimbo Binuang	6.0	4.6	5.30 a
Tombang	3.8	2.8	3.30 b
Mean	4.73 A	3.40 B	

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

The high percentage of explants forming callus is not followed by the number of shoots that grow on the explant. This is caused by the callus experiencing browning. Zulkarnain (2011) states that excessive doses of cytokinin or types of cytokinins that are not following the needs of plants can be the cause of epigenetic diversity and adversely affect the next micropropagation stage. Callus forming shoot that observed in this study can be seen in the following figure.

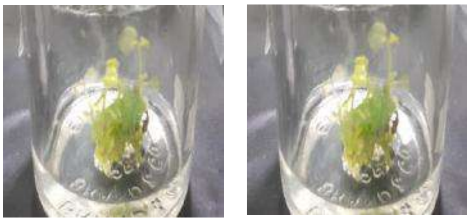
Day after planting (DAP)	BA concentration	
	0.01 ppm	0.03 ppm
Situak Accession		
28 DAP		
35 DAP		

45 DAP

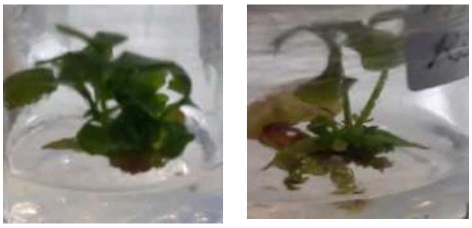
Rimbo Binuang Accession

28 DAP

35 DAP



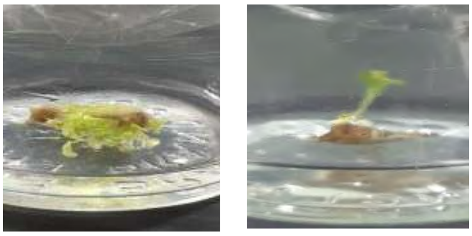
45 DAP



Tombang Accession

28 DAP

35 DAP



45 DAP

3.4 Total number of leaves

The observation results of the total number of leaves can be seen in Table 4. Rimbo Binuang accession is an accession that can produce the highest total number of leaves at a concentration of BA 0.01 ppm, ie 28.80 leaves, and 16.6 leaves at BA 0.03 ppm. The number of leaves formed from the three accessions was higher in the use of BA 0.01 ppm. While in sandalwood explant, BA with a concentration of 2 to 4 mg/L gives the best results for the induction of leaf numbers (Sari *et al.*, 2009).

Table 4 Total number of leaves of three patchouli accession in different BA concentration

Accession	**BA concentration**	
	0.01 ppm	0.03 ppm
Situak	18.50 bA	9.20 bB
Rimbo Binuang	28.80 aA	16.60 aB
Tombang	12.20 cA	7.80 bB

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

3.5 Total number of leaves per explant

The observation results of the total number of leaves per explant can be seen in Table 5.

Table 5. Total numbers of leaves per explant of three patchouli accession in different BA concentration

Accession	**BA concentration**		
	0.01 ppm	0.03 ppm	Mean
Situak	4.2	3.2	3.70 b
Rimbo Binuang	4.8	3.6	4.20 a
Tombang	3.2	2.8	3.00 b
Mean	4.07 A	3.20 B	

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

Table 5 shows that there is no interaction between the concentration of growth regulator BA and the number of leaves per shoots of three patchouli accession. However, the two single factors have a significant effect on the number of leaves per shoot produced. The average number of leaves per shoot which is the highest in the accession of Rimbo Binuang (4.20 leaves). In the second place is occupied by Situak accessions (3.70 leaves) and the lowest is Tombang accessions (3.00 leaves). The average number of leaves per shoot of the three accessions influenced by BA concentration resulted in a higher number in BA 0.01 ppm treatment.

In the multiplication of chrysanthemum varieties of Puspita Asri and Puspita Nusantara, where the use of BA at a concentration of 4.44 μM gives maximum results to the number of leaves, but an increase in BA to a concentration of 6.66 and 8.88 μM, actually decreases the average number of leaves. This shows that the use of cytokinins at optimum concentrations will be able to provide maximum response to plant growth, but increasing the concentration of cytokines beyond its optimum point will inhibit plant growth (Syaifan, 2010).

In the treatment of growth regulators giving BA concentrations of 0.01 ppm is seen in the accessions of Rimbo Binuang, Situak, and Tombang resulting in the best number of leaves per shoot and significantly different from giving BA concentrations of 0.03 ppm and the lowest is found in the accession of Tombang.

Cytokinins are known to play a role in delaying leaf senescence by inhibiting protein breakdown. The greater number of leaves that can be maintained will certainly increase photosynthetic activity which will ultimately increase the production of plant biomass and increase the multiplication of shoots (Haeria, 2012).

IV. CONCLUSION

The best concentration of BA used in the in-vitro culture medium to induce patchouli plant growth is 0.01 ppm. Then, the local West Pasaman patchouli accession that has the highest responses in all observation variables when induced by BA is Rimbo Binuang accession. So it can be concluded that to propagate patchouli seedling via tissue culture, the Rimbo Binuang accession is a good source of explant.

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REFERENCES

[1] Astuti and Andayani. (2007). *Pengaruh Pemberian BAP dan NAA terhadap Pertumbuhan Krisan* (Chrysanthemum morifolium, *Ram.).* Jurnal Kultur Jaringan Biota, X(3): 31-35.

[2] Caiger S. (2016). *Essential Oil and Oleoresins*. Market Insider April 2016 Report. http://www.intracen.org/uploadedFiles/intracenorg/Content/Exporters/Market_Data_and_Information/Market_information/Market_Insider/Essential_Oils/Monthly%20Report%20April%20%202016.pdf.

[3] Febriyetty L. (2018). *Identifikasi Karakteristik Morfologis, Anatomis dan Mutu Minyak Atsiri Tanaman Nilam (*Pogostemon cablin *Benth) di Kabupaten Pasaman Barat* [Tesis]. Padang. Program Pascasarjana Universitas Andalas.

[4] Hadipoentyanti E. (2010). *Perbanyakan Benih Nilam Veritas Unggul Sidikalang (Produksi* Minyak ≥ 300 kg/ha), Sehat dan Murah *Hasil Kultur Jaringan (30 % dari Biaya Standar).* Balai Penelitian Tanaman Obatdan Aromatik. Bogor.

[5] Haeria. (2012). Organogenesis Tanaman Jarak Pagar (Jatrophacurcas *L)* pada Medium MS dengan Penambahan Berbagai Konsentrasi BAP dan NAA. *Skripsi. Fakultas MIPA Universitas Tadulako. Palu.*

[6] Harahap, F. (2005). *Induksi Variasi Genetic Tanaman Manggis* (Garcinia mangostana *L.) dengan Radiasi Sinar Gamma* (Disertasi). Bogor. Sekolah Pascasarja Institut Pertanian Bogor. p:131.

[7] Idris A., M. Ramajura, and I. Said. (2014). *Quality Analysis of Patchouli Oil (*Pogostemon cablin *Bent) Production*. Buol. District. J. Akad. Kim. 3(2): 79-85.

[8] Indah, P. N., and V.D. Erma. (2013). *Induksi Kalus Daun Nyamplung* (Calophyllum inophylum *Linn.) pada Beberapa Kombinasi Konsentrasi* 6- Benzylaminopurine *(BAP) dan* 2,4-Dichlorophrnoxyacetic *(2,4-D).* Jurnal Sains dan Seni Pomits. 2(1), pp. 2337-3520.

[9] Kumari S., P. Baskaran, and J. van Standen. (2017). *In vitro Regeneration of* Begonia homonyms - *a threatened plant.* South African Journal of Botany 109: 174-177.

[10] Lestari E.G. (2011). *Peranan Zat Pengatur Tumbuh Dalam Perbanyakan Tanaman Melalui Kultur Jaringan*. Jurnal AgroBiogen. Vol 7 No 1.

[11] Mahadi I, W. Syafi'I, and Y. Sari (2016). *Induksi Kalus Jeruk Kasturi* (Citrus microcarpa) *Menggunakan Hormon 2,4-D dan BAP dengan Metode In Vitro*. Jurnal Ilmu Pertanian Indonesia 21 (2): 84-89.

[12] Mayerni R., A. Syarif, and Hidayat. (2018). *Potensi dan Pengembangan Tanaman Nilam Sumatera Barat.*

[13] Murashige, T. (1974). *Plant propagation through tissue culture.* Ann. Rev. Plant Physiol.25:135—166.

[14] Nuryani Y., Hobir, and C. Syukur. (2003). *Status Pemuliaan Tanaman Nilam (*Pogostemon cablin *Benth).* Perkembangan Tekhnologi TRO XV, 2: 56-57.

[15] Salisbury, F.B. and C.W. Ross. (1995). *Fisiologi Tumbuhan*. Dialih bahasakan oleh Diah R, Lukman, dan Sumaryono. Disunting oleh Sofia Niksolihin. Penerbit ITB. Bandung. 343 pp.

[16] Sari Y.P., D. Susanto, and F. Irawan. (2009). *Respon Pertumbuhan Tunas Meranti Merah (*Shoreaseminis *(de Vriese) Slooten) dengan Pemberian Zat Pengatur Tumbuh BA (Benzil adenin) secara In Vitro.* Bioprospek Volume 6, Nomor II.
[17] Swamy M.K., S. Balasubramanya, and M. Anuradha. (2010). *In vitro multiplication of* Pogostemon cablin *Benth. Through Direct Regeneration.* African J. Biotech. Vol. 9(14): 2069-2075.
[18] Syaifan U. (2010). *Pengaruh Benzyl Adenine (BA) terhadap Pertumbuhan Eksplan Dua Kultivar Krisan (*Drendanthema grandiflora *Tzelev Syn.) secara In Vitro.* [Skripsi]. Program Studi Agronomi. Institut Pertanian Bogor. Bogor.
[19] Taiz L. and E. Zeiger. (1998) *Plant Physiology*. 2nd Edition. Sinauer Associates Inc. Sunderland.
[20] Taji A.M., W.A. Dodd, and R.R. Williams. (2006). *Teknik Kultur Jaringan Tanaman.* Penerjemah: Zulkarnain. Terjamahan dari: Plant Tissue Culture Practise. 157 pp.
[21] Wahyudi A. and Ermiati. (2012). *Prospek Pengembangan Industri Minyak Nilam di Indonesia.* Bunga Rampai Inovasi Tanaman Atsiri Indonesia. Balai Penelitian Tanaman Rempah dan Obat. Bogor. 1-6 pp.
[22] Yelnititis and T.E. Komar. (2011). *Mikropropagasi Ramin (*Gonistylus bancanus (Miq.) Kurz) *dari Eksplan Batang Satu Buku Secara In Vitro.* Jurnal Permuliaan Tanaman Hutan 5(3):149 157.Zulkarnain H. (2011) *Kultur Jaringan Tanaman: Solusi Perbanyakan Tanaman Shooti Daya.* Bumi Aksara, Jakarta.

Optimization of Milk Performance and Quality in Dairy Farms by using a Quarter individual Milking System *"MultiLactor"*

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Abstract--- *Within the daily work on dairy farms milk harvesting is a crucial factor in optimizing milk performance and quality. The objective of this study was to investigate the influence of optimizing the milking process by using a quarter individual milking system "MultiLactor" (ML) on milk performance and quality. 170 Holstein-Friesian dairy cows were used on a farm in Switzerland. The cows were milked three times a day with an ML milking system. Recording of milk yield and collecting of milk sample were carried out monthly from each cow for one year. After that, the main milk parameters were analyzed by Association of Milk Records Switzerland. The primary milk data was processed with Excel program. Thereafter, analysis was carried out using the Statistical Analysis System (SAS). The average milk yield was 35.84±0.28 kg/cow/day. This corresponds to an annual milk yield of 12000 kg/cow. The mean values of milk composition were 4.36±0.02%, 3.28±0.01%, 4.75±0.01%, 20.65±0.20 mg/dl and 99.63±6.48 x 10^3 cells/ml for fat, protein, lactose, urea and somatic cell count (SCC) respectively. It was also shown that the cows remained healthy in the farm during the study period. In conclusion, the obtained results demonstrated that the cows produced a higher milk yield with good quality, since the milking system adapts the physiological requirements of dairy cows.*

Keywords---Cow, Fat, Lactose, Milk, MultiLactor, Protein, Somatic Cell Count, Urea.

I. INTRODUCTION

Every day, billions of people around the world consume milk and milk products, which play a key role in healthy human nutrition and development throughout life. Currently, dairy cows are the main source of milk production in the world. Milking is a central part in dairy management in order to optimize milk production and quality. Mechanical milk removal is indispensable in today's milk production. In this process, you have to consider that the milking machine is the technology that works directly on the animal (1). The main requirement for every milking system is to achieve the greatest possible milk yield in the shortest possible time, with the least amount of work and without harming the udders of the cows (2). Thus, the key to successful milk removal lies in the optimal realization of all these factors (3). At this point, research and development relating to milking machine plays a crucial role. The interaction between animals, humans and milking technology, in terms of animal welfare and milk letdown and the optimization of the milking workplace are becoming increasingly important (4,5). In addition to many other factors, milking technology has a significant impact on milk performance, improved milk ingredients and lower cell counts (6-9). Nowadays, different milking technologies can be used for the milking process in the world. But milking equipment and routine need to be adjusted according to the animals' physiology mechanism in order to achieve optimal milk removal (10, 11).

For these reasons, ML milking system was developed by Silicon form in Germany and used in the field since 2008. The field results have shown that the using of ML in comparison with the conventional milking systems is more effective in term of positive stimulation effect (12). Furthermore, it was shown that ML milking system had a positive influence on milk yield and composition (10). These results confirm the importance of the type of used milking machine in dairy farms.

The aim of this study was therefore to investigate the influence of the optimization of the milking process by using a quarter individual milking system ML on the milk yield and its composition.

II. MATERIAL AND METHODS

2.1. Research location:

The present study was conducted in the department of research and development of Silicon form in Germany and in a dairy farm in Switzerland, which has organized everything almost perfectly from rearing calves to old cows.

2.2. Animals and husbandry:

170 Holstein-Friesian dairy cows were used (Fig. 1). The cows were kept in loose housing and they were fed ad libitum with a partial mixed ration (grass-and corn-silage, hay) and received concentrate according to the production level. Furthermore, the cows received fresh feed three times a day and each animal had a feeding place. The farm is also one of the first to use sandboxes for lying the cows, which was 1.30 m wide and 2.90 m long. In the dry period, the cows were placed on a deep straw mat with specific feed ration. Research has clearly demonstrated the impact of dry period nutrition and management on postpartum health and performance. The goal for dry cows in the farm include: maintain dry matter intake, optimize comfort, prevent body condition score gain, and address hoof health. After calving, feed intake for lactating cows was optimized and the fresh calves were taken into good place, which was very comfortable.

Fig.1: Herd of the examined field farm.

2.3. Used milking system:

All dairy cows were milked three times a day at 5:00, 13:00 and 21:00 hours in a carousel milking parlor (24 places) with a ML milking system (Fig. 2).

Fig. 2: The new milking technology ML in the carousel milking parlor.

2.4. Characteristics milking system:

ML is the essential instrument of an innovative milking process, which has set new standards in milking technology. ML is a well-handled and animal-friendly semi-automatic milking system that differs technically from conventional milking machine. It is based on a quarter-individual milking system. Features and characteristics of the used milking technology are summarized in Table 1:

Table 1. Features of the used milking technology.

Parameters	Feature
Milking vacuum (kPa)	34
Pulse rate (cycles/min)	60
Pulsation ratio	60:40
Pulsation type	sequential
Air intake	Periodically in the teat cup
Teat cup removal	Fully automatically
Cleaning and intermediate disinfection	After each milked cow and after the milked herd
Dipping	Automatic dipping

2.5. Milk routine:

At the milking time in the investigated farm, the cows came voluntary to the milking parlor. After identification of the cow the ML automatically turns into attachment position directly in front of the udder. Afterwards, the milker has cleaned the teats, pre-milked and visually checked the milk. Then, each teat cup is individually or in pairs pulled out of the magazine and manually attached to the teats. Subsequent to this step, the system is started on the control display and the pre-stimulation begins. The pre-stimulation is structured to be intensively activated with a normal pulse rate (60 cycles/min) and reduces the milking phase (b-phase) of 10% over a period of 50 s. At the same time, intensive movement of the teat cups is regulated as an additional stimulation by an actuator. This is an arm on which the four milk tubes are placed. During the pre-stimulation and the milking time, this arm moves up and down. This movement is transferred to the teat cups and makes the teats erect.

After stimulation the main milk phase begins and the milk flow is observed on the display. When the milk flow has decreased to a certain level, the milking process is automatically stopped by detaching the milking unit and each teat is dipped with a solution containing Chlorexidin. After milking, the teat cups are cleaned and disinfected automatically with water and per acetic acid solution (0.5%).

2.6. Milk recording, sampling and analysis:

Recording of milk yield and collecting of milk sample were carried out monthly from each cow of the experimental farm during the study period. The milk samples have been analyzed for fat, protein, lactose, urea and SCC by Association of Milk Records Switzerland. Electronic fluorescence was used to analyse SCC in the milk samples. The mid-infrared spectroscopy method was used for the determination of gross milk composition (fat, protein, lactose %). Infrared measurement with PLS calibration was used to determine the urea in the milk.

2.7. Statistical Analysis:

All milk parameters measured were analysed by ANOVA, using the SAS Statistical Software Package (SAS Institute, Cary, NC, 1998) (13) and the Least Square Means (LSM) were compared using F-Test. The results were shown as LSM±SE.

III. THE RESULTS

3.1. General mean values of the examined parameters:

The average daily milk yield for the entire investigation period was 35.84±0.28 kg/cow (Tab.2). This corresponds to an annual milk production of 12000 kg/cow. Despite the higher milk yield, the composition of the extracted milk remained high and the mean values were 4.36±0.02 %, 3.28±0.01 % and 4.75±0.01% for fat, protein and lactose respectively. The milk urea content was within the normal range (20.65±0.20 mg/dl) during the study period. This urea level in milk was lead back to the perfect balance between energy and protein in the ration.

It is noteworthy that with high daily milk yield per cow, low SCC have been achieved and the average values remained below 100 x 10^3 cells/ml milk during the study period. The statistical results showed that the average lactation number was at 3.31 in the examined farm.

Table 2. The average (LSM±SE) milk parameters of the examined farm:

Parameters	LSM±SE	Minimal value	Maximal value
Milk yield kg/day	35.84±0.28	7.10	68.60
Fat %	4.36±0.02	2.27	7.74
Protein %	3.28±0.01	2.11	5.02
Lactose %	4.75±0.01	3.59	5.25
Urea mg/dl	20.65±0.20	5.00	46.00
SCC x 10^3 cells/ml	99.63±6.48	6000	300000
Lactation number	3.31±0.04	1.00	8.00

3.2. Influence of the stage of lactation on the examined milk parameters:

Table (3) showed that the daily milk yield remained relatively high until the end of lactation and the values were 41.62±0.39, 36.97±0.39 and 29.66±0.39 kg/day in the first, second and third stage of lactation respectively. Furthermore, the persistence of daily milk production were 88.83% and 71.26% in the second and third stage of lactation.

The change in milk composition with the progress of lactation session have shown a clear ideal tendency. Fat (4.23, 4.28 and 4.56%, $P<0.05$) and protein (3.01, 3.29 and 3.51%, $P<0.05$) content in the milk increased and lactose (4.80, 4.76 and 4.68%, $P<0.05$) content decreased in the first, second and third stage of lactation respectively.

The SCC of milk remained low in the first stage of lactation (82.49±10.42 x 10^3 cells/ml) and then it increased slightly with no significant differences ($P>0.05$) and the values were 107.55±10.42 x10^3 and 108.86±6.98 x 10^3 in the second and third stage of lactation respectively.

Table 3. Average (LSM±SE) milk parameters of the examined farm according to the stage of lactation.

Milk parameter	Stage of lactation		
	<100 days	100-200 days	>200 days
Milk yield (kg/day)	41.62±0.49a	36.97±0.39b	29.66±0.35c
Fat %	4.23±0.04a	4.28±0.04a	4.56±0.04b
Protein %	3.01±0.02a	3.29±0.02b	3.51±0.02c
Lactose %	4.80±0.01a	4.76±0.01b	4.68±0.01c
Urea mg/dl	18.72±0.35a	21.01±0.37b	22.14±0.30c
SCC x 10^3 cells/ml	82.49±10.42a	107.55±16.08a	108.86±6.98a

3.3. Cell number classes in the examined farm during investigation period:

The following figure (4) give us information about the percentage of cell number classes in the examined milk samples. 79% of the milk samples have less than 100 x 10^3 cells/ml milk. Only 11% of the total milk samples have more than 200 x 10^3 SCC cells /ml milk in the examined farm.

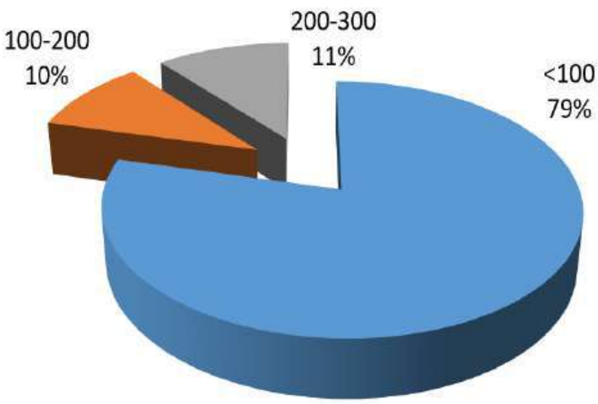

Fig. 4: Cell number classes x 10^3/ cells/ml milk from the examined farm during investigation period

3.4. Herd structure in the examined farm during investigation period:

The Figure (5) provided information about the herd structure of the investigation farm in the study year. The herd structure was typical in the investigation farm, which has optimal management. As shown in the figure that 22% of dairy cows were in the first lactation. However, it should be noted that half of the dairy cows were in the third to fifth lactation.

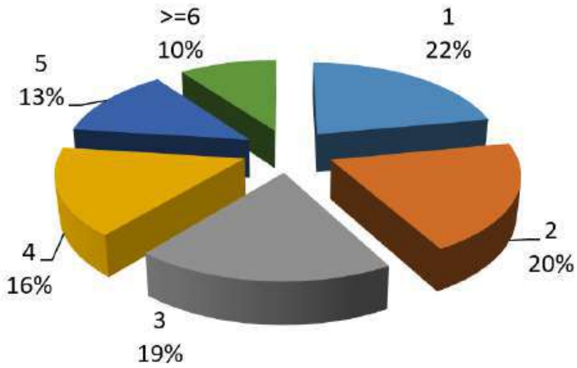

Fig. 5: Herd structure in the examined farm according to lactation number.

3.5. Influence of the number of lactation on the examined milk parameters

The daily milk yield increased to 43.59±0.97 kg/day by the fourth lactation. After that, it decreased slightly until the sixth and more lactations (Fig.3). This change of daily milk yield in term of lactation number is typical of the Holstein Friesian breed and it achieved via the optimal management in the farm. Fat and protein content are negatively correlated with the daily milk yield based on the number of lactation and the values were (-0.36, P <0.001) and (-0.57, P <0.001) respectively. Regarding the lactose content of the milk, the values were normal and ranged between 4.49 and 4.85%. The milk urea content remained almost stable during lactations and the values varied between 19 and 21 mg/dl with no significant differences (P>0.05), since the feeding situation was optimal in the farm. SCC remained also lower than 100 x 10^3 cells/ml until the third lactation. Then, SCC in milk increased from the fourth to sixth lactation slightly but the values remained below 215 x 10^3 cells/ml.

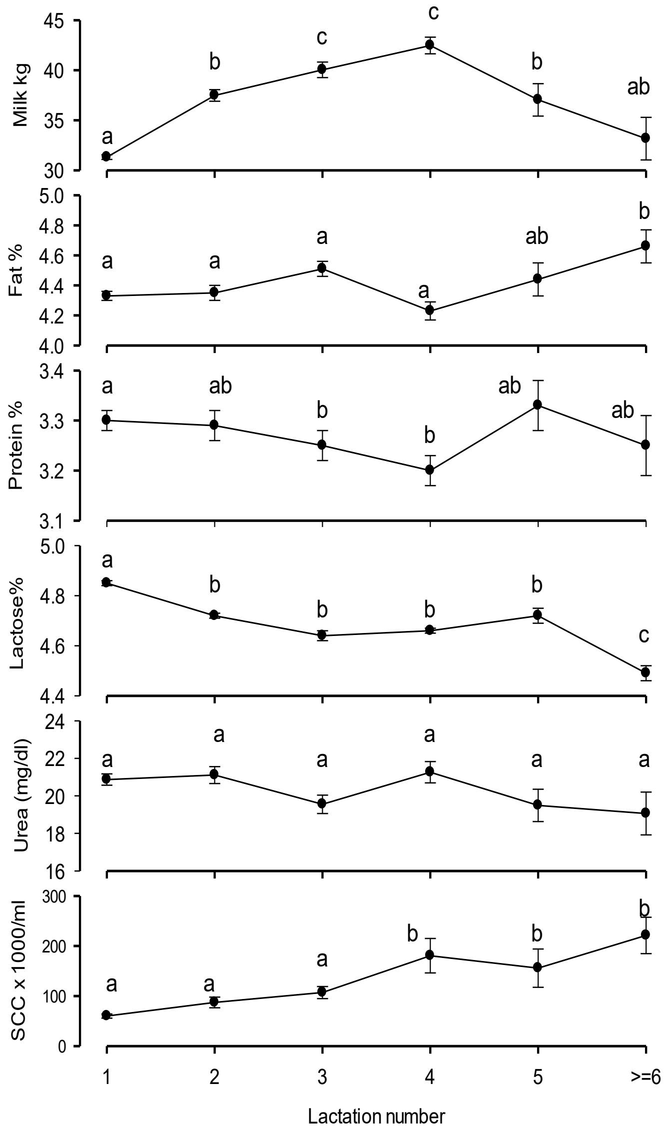

Fig. 3: Average milk parameters of the examined farm according to lactation numbers

IV. DISCUSSION

4.1. Milk performance:

The influence of the milking machine on the high milk performance and quality primarily depends on the type and function of the milking machine. Is it compatible with the mother's calf sucking mechanism or not? Observations on suckling calves clearly showed that a calf is able to obtain the total amount of milk from their dam (including that from the alveoli) (9, 14). It is known that the well-being of the animals is increased by an optimal milking parlor and milking parlor design. Therefore, milking technology should ensure gentle, quick and complete extracted milk. The prerequisite for this is that the cows are able to milk. This can be improved by intensive udder preparation and an optimally set milking technique. The results of the present study showed that the milking process ran well with ML milking system. Various studies have shown similar results and the daily milk yield increased by application of quarter individual milking system ML and the milk composition improved (8-10).

It is known that many factors affect milk performance and milk composition (15-19), but the milking system and milking routine play a crucial role (6, 7). However, milking routine and hygiene during the course of milk removal in this study have to be ideal. It is possible to have a suitable milking technique in the farm and at the same time the lactating cows are poorly milked. Kanswohl et al., (20) reported that milk removal is optimal during the milking time, when the used milking machine is suitable for the requirement of the lactating cows and the cows are well prepared before attaching the teat cups.

Moreover, usually the lactating cows reach their high milk yield shortly after calving (4-6 weeks) and then the daily milk yield steadily declines. Our results clearly showed that the daily milk yield remained quite high until the end of lactation session. That means that the cows have had the ability to continue their milk production at a high level after reaching the peak yield. Several reports indicated that the persistence with optimal management was over 85% in the second stage of lactation (21). Through breeding measures attempted to counteract this decline by selecting for the highest possible persistence of the performance, but no success has been shown (22-24). In this study, an explanation for maintaining the high milk yield until the end of lactation is associated with good husbandry, especially in the milking process and good feeding. As we know, milk is being produced during lactation session, the speed of production depends upon how empty the udder is. At this point, the frequency (three times) and completeness of emptying the udder during milking with ML have shown these results. Milking three times or more per day in relation to once or two times has been shown to increase milk yield by 6 to 50% (18, 25-28).

On the other hand, an important requirement for milking technology in dairy cows is to complete extracting milk from the udder, since the remaining milk after milking inhibits the new milk synthesis (29). That means that there is a switch to the autocrine (or local) control system. In the course of lactation, milk removal is the primary control mechanism for milk production. It is therefore important to completely empty the udder as much as possible during milking. Bruckmaier, (22) reported that the frequency and completeness of emptying the udder during milking determine the activity of the existing lactocytes (epithelial cells in the alveoli), which is responsible for the increase in performance through increased milk synthesis and secretion and a slowed decline in performance over the course of lactation (persistence) is possible due to reduced apoptosis of the lactocytes. However, Alex et al., (18) found that changes in milk production in response to extreme differences in milking frequency may be related to alterations in mitochondrial number and lactose synthesis, but not apoptosis. In addition, many scientists have dealt with the dynamics of udder emptying during milking (30-37).

Furthermore, many studies have shown that udder preparation before attaching the teat cups has a positive effect on the course of milk removal (38-40). That means, it is important to prepare the animals optimally before milking. In this study excellent stimulation system with ML was used, as mentioned in the material and method. Therefore the udder has been completely emptied. Many scientists emphasize that the sufficient stimulation of the teats before milking is the cornerstone for the release of the hormone oxytocin and for a high milk flow and complete milking of the cows (5, 9, 39, 40).

4.2. Milk composition:

Milk composition can be affected by genetic and non-genetic factors for example, breed, stage of lactation, lactation number, milking process, husbandry, feeding, welfare and management (39, 41-44). In our study, only Holstein Friesian dairy cows were kept on the farm which normally produce low milk ingredients. But, our results show completely different results because the keeping conditions, especially the milking process with ML, have been very suitable for the cows. This was proven in the fact that the cows came voluntary to the milking parlor during the milking time.

Usually, the amount of obtained milk has a significant influence on the fat and protein contents in milk. That means, as the amount of milk increases, the fat and Protein content of the milk deceases because the amount of fat and protein in milk per day are limited and the percentage in one liter of milk are diluted due to a lot of milk (45). It is also interesting that the lowest levels of fat and protein in milk were shown in the fourth lactation session (Fig. 4). These change in the fat and protein content in milk by the stage or number of lactation were normal. However, the most important influence on the milk protein content is the energy supply and usable crude protein. It must also be noted that the ration and management control in the examined farm was optimal, since no significant mistakes were made in feeding or milking. Piccioli-Cappelli et al., (46) reported that the feed ration (amount and composition) must be changed in the course of lactation since endocrine-metabolic interactions can influence diet parameters, and so nutrient availability for the mammary gland can significantly vary and affect milk yield and its composition.

Furthermore, the obtained results clearly showed that the urea content of milk fluctuated between 19 and 21 mg/dl in the course of lactation. Several reports have shown that the milk urea content may serve as an on-farm indicator to guide nutritional strategies (47-51). However, the limit values of urea for a normal raw protein supply vary between 15 to 20 mg urea/dl milk. Milking intervals also affects the urea concentration in the obtained milk. That means, dairy cows which are milked three times a day, usually have higher urea in milk than those milked two times a day (52, 53). This statement explains that the urea concentrations in the milk were in the upper limit in our studies. It is noteworthy that the urea concentration in milk has increased continuously with the progress of lactation season (Tab.3). It was lowest during the first 100 d of lactation with (18.72±0.35 mg/dl), higher between 100 and 200 d in milk with (21.01±0.37 mg/dl) and the highest after 200 d in milk with (22.14±0.30 mg/dl). Similar results have been shown by Godden et. al., (54). Furthermore, it should be noted that lactation number in this study has no significant influence on the urea concentration in milk similar to those reportedly Henao-Velasquez et al., (55).

It is known that many factors affect SCC in milk. Although the lactating cows produced high milk yield, the SCC in milk was low during the study period, as shown in Table (2). These results differ from the prevailing opinion that with high milk production the animals are more stressed and the result is an increased SCC in the produced milk (56). As shown in Table (3), the stage of lactation had no significant ($P>0.05$) effect on the SCC in obtained milk. But the lowest level of SCC was in the first stage of lactation and then increased slowly throughout the second and third stages of lactation. These results were also in agreement with Kennedy et al., (57) and Sheldrake et al., (58). The significant influence of parity on SCC in milk was clearly shown in Figure (2). That is, the SCC remained below 100 x 10^3 cells/ml without significant differences ($P>0.05$) up to third lactation; however, it increased significantly ($P<0.05$) in cows having more than four parity. This corresponds to the results of some researchers (59-61).

On the other hand, SCC and milk quality depend mainly on milking technology and milking routine (6). However, the milk of healthy udders contains less than 100 x 10^3 cells/ml. With the irritation and inflammation of the udder quarters, the SCC can rise sharply and up to 20-50 million cells/ml can be achieved. Therefore, the pathogen detection must be carried out in the udder quarter if the cell count in the milk is determined to be more than 100 x 10^3 cells/ml.

Stress during milking is one of the significant factors, which has a negative effect on the SCC in the milk as found in automatic milking systems compared to conventional milking parlors (62). Similarly, Castro et al., (63) found that the SCC values were significantly higher during the 12-month post-installation of the automatic milking system. During the milking course, timing of oxytocin release and milk ejection before the start of milk removal is very important for subsequent milking performance (64) and any interruption of the milk ejection process can disturb milk removal. The result is inflammation in the udder (7). The indicator of inflammation in the udder is an increase in SCC in the removed milk (5). Therefore, ergonomic milking system and responsible milkers are very important for the welfare of dairy cows (65). During the use of the ML milking system in this study, it became clear that there was no stress during milking, as it had a special stimulation system before and during milking that played a crucial role in improving SCC in milk. Therefore, the SCC remained below 100 x 10^3cells/ml during the investigation period. Previous study reported that the ML milking system showed significantly better results in terms of teat color after milking compared to the conventional milking system (66). It is noteworthy that the remaining rest milk in the udder after milking not only adversely affects milk performance, but also affects udder health. Our results clearly showed that the ML milking system had completely emptied the udder, since the obtained milk was high and the SCC remained low. Moreover, Alhussien and Dank (67) emphasized that milk with low SCC means better milk products with a longer shelf life.

4.3. Lactation number and useful life:

Increasing milk yield should not only be achieved through genetic progress (43, 68), but should also be increased through the life performance of dairy cows. Therefore, an increase in life efficiency is possible by lowering the first calving age (69), increasing the milk yield per lactation and extending the useful life at a high performance level.

Our obtained results demonstrated that the udder remained healthy during milking and the cows remained longer in the farm as shown in the figure (5). The average lactation season in the trial year was 3.31 in the farm. Currently, there is an average useful life of approximately 2.5 lactations in Germany (70). However, the highest performance of the lactating cows could be achieved in the 4th or 5th lactation (15, 71). Previous studies have reported that the dairy farms with the ML milking systems have clearly shown that the useful life of the dairy cows is extended because the cows live longer and their performance potential is fully exhausted (10). So that in terms of cow's milk yield, a long useful life with a good performance effect the utilization of the age related performance maximum. As the efficiency of feed conversion increases and the rearing costs per kg milk decrease over a longer useful life.

V. CONCLUSION

A complete milking routine with milking machine can achieve good results by completely emptying the udder and significantly reducing the risk of blind milking. That is why by using new technology MultiLactor, a high milk yield and a better milk quality can be achieved, since it is adapted to the physiology of the dairy cows. The following observation were also observed during the milking with MultiLactor:

- The cows came voluntarily to the milking parlor.
- The cows were quiet during milking.
- The teat cups were never knocked off.
- Very small residual milk remained in the udder after milking.

Beyond, it was observed that ML milking system has positive influenced on SCC content in milk and the udders remained healthy during the investigation period.

REFERENCES

[1] Jungbluth, T., Büscher, W. & Krause, M. (2005). Technik-Tierhaltung. 1. Aufl. Stuttgart: UTB Ulmer.

[2] Becker, N. (2016). Entwicklung der Milchleistungsparameter bei Milchkühen unterschiedlicher Milchviehbetriebe nach der Umstellung auf das viertelindividuelle Melksystem MultiLactor. Master, Technische Universität München, Agrarsystemtechnik.

[3] Ströbel, U., Rose-Meierhöfer, S., Öz, H., Entorf, A.-C., Popp, L. & Brunsch, R. (2011). Analysis and evaluation of the teat-end vacuum condition in different automatic milking

systems. Irish Journal of Agricultural and Food Research 50(2): 209-221.

[4] Jungbluth, T. & Grimm, H. (2009). Melken und Melktechnik- zukünftiger Forschungsbedarf. 2.Tänikoner Melktechniktagung, 25-26 März 2009, Forschungsanstalt Agroscope Tänikon, Ettenhausen, Switzerland.

[5] Bruckmaier, R.M. (2001). Milk ejection during machine milking in dairy cows. Livestock Production Science 70: 121-124.

[6] Dang, A. K. & Anand, S. K. (2007). Effect of milking systems on the milk somatic cell counts and composition. Livest. Res. Rural Dev. 19(6): 1-8.

[7] Petrovska, S. & Jonkus, D. (2014). Milking Technology Influence on Dairy Cow Milk Productivity and Quality. 13th International Scientific Conference, Engineering for Rural Development. Proceedings 13 May 29-30, 2014 Jelgava, Latvia, Ukraine.

[8] Kaskous, S. & Fadlelmoula, A. (2015). Adaptability of dairy cows to individual quarter milking system after changing from tying to loose housing system. Advances in Animal and Veterinary Sciences 3(4): 225-232.

[9] Kaskous, S. (2016). Alte Kühe dank neuer Melktechnik. Hessens Zukunft. 16(3): 36-39.

[10] Kaskous, S. (2018a). The effect of using quarter individual milking system “MultiLactor” on improvement of milk performance and milk quality of different dairy cows breeds in different farms. Emirate Journal of Food and Agriculture 30(1): 57-64.

[11] Kaskous, S. (2018b). Optimization of the pulsation ratio during the course of milk removal after using a quarter individual milking system “MultiLactor”. International Journal of Agriculture Innovations and Research 6 (6): 284-289.

[12] Müller, A.B., Rose-Meierhöfer, S., Ammon, C. & Brunsch, R. (2011). Comparison of the effects of quarter-individual and conventional milking system on milk ability traits. Archiv Tierzucht 54(4): 360-373 .

[13] SAS Institute, Version 8, Cary NC, USA, 1998.

[14] Kälber, T. & Barth, K. (2014). Practical implications of suckling systems for dairy calves in organic production systems-a Review. Appl. Agric Forestry Res. 64:45-58.

[15] Fahr, R. D. & Lengerken, G. (2003). Milcherzeugung, Grundlage Prozesse- Qualitätssicherung. Deutscher Fachverlag. Germany.

[16] Dufour, S., Frechette A., Barkema, H. W., Mussell, A. & Scholl, D. T. (2011). Invited review: Effect of udder health management practices on herd somatic cell count. J. Dairy Science 94: 563-579.

[17] Assan, N. (2014). Significance of litter size, duration of dry period and stage of pregnancy on milk yield and composition in dairy animals. Science Journal of Rev. 3(12): 993-1003.

[18] Alex, A.P., Collier, J. L., Hadsell, D. L. & Collier, R. J. (2015). Milk yield differences between 1x and 4x milking are associated with changes in mammary mitochondrial number and milk protein gene expression, but not mammary cell apoptosis or SOCS gene expression. Journal of Dairy Science 98(7): 4439-4448.

[19] Cinar, M., Serbester, U., Ceyhan, A. & Gorgulu, M. (2015). Effect of somatic cell count on milk yield and composition of first and second lactation Dairy cows. Ital. J. Anim. Sci. 14(1): 105-108.

[20] Kanswohl, N., Schlegel, M., Burgstaler, J. & Wiedow D. (2012). Zügig ans schonend melken. Bauernblatt. 40: 39-41.

[21] Römer, A. (2012). Managementhilfen im Milchviehstall-Möglichkeiten und Grenzen. 1. Auflage Bayerische Landesanstalt für Landwirtschaft, Germany: 21-48.

[22] Bruckmaier, R. M. (2007). Physiology of lactation In: Kroemker V. Milk science and milk Hygiene. Parey. 2007:6-21.

[23] Mandokhial, K.-U., Kaleri, H. A., Kaleri, R. R., Kaleri, A., Safi, A. S., Kaleri, A.H. & Safi M. A. (2017). Study on persistency of lactation in Holstein Friesian cattle. Pure and Applied Biology 6 (1): 159-163.

[24] Koloi, S., Pathak, K., Behera, R., Mandal, D. K., Karunakaran, M., Dutta, T. K. & Mandal, A. (2018). Factors affecting the persistency of milk production in Jersey crossbred cattle. Journal Dairy, Veterinary and Animal Research 7(6): 268-271.

[25] Klei, L. R., Lynch, J. M., Barbano, D. M., Oltenacu, P. A., Lednor, A. J. & Bandler, D. K. (1997). Influence of milking three times a day on milk quality. Journal dairy Science 80: 427-436.

[26] Collier, R. J., Romagnolo, D. & Baumgrad, L. H. (2010). Lactation (f) galactopoiesis/seasonal effects. In: Roginski H., Fuquay J.W. and Fox P.F. Encyclopedia of Dairy Science. Ed Academic Press, New York, NY: 35-46.

[27] Atashi, H. (2015). Effect of milking frequency on the lactation performance and lactation curve of Holstein dairy cows in Iran. Iranian Journal of Applied Animal Science 5(2): 273- 278.

[28] Boujename, I. (2019). Effect of milking frequency on milk production and composition of Holstein cows during their first three lactations. Iranian Journal of Applied Animal Science 9(1): 25-29.

[29] Wilde, C. J. & Peaker, M. (1990). Autocrine control in milk secretion. J. Agric. Sci. 114: 235-238.

[30] Capuco, A. V., Wood, D. L., Baldwin, R., Mcleod, K. & Paape, M. J. (2001). Mammary cell number, proliferation, and apoptosis during a bovine lactation: relation to milk production and effect of bST. Journal of Dairy Science 84: 2177–2187.

[31] Nørgaard, J., Sørensen, A., Sørensen, M. T., Andersen, J. B. & Sejrsen, K. (2005). Mammary cell turnover and enzyme activity in dairy cows: Effects of milking frequency and diet energy density. J. Dairy Science 88: 975-982.

[32] Fitzgerald, A. C., Annen-Dawson, E. L., Baumgard, L. H. & Collier, R. J. (2007). Evaluation of continuous lactation and increased milking frequency on milk production and mammary cell turnover in primiparous Holstein cows. Journal of Dairy Science 90: 5483-5489.

[33] Hickson, R. E., Lopez-Villalobos, N., Dalley, D. E., Clark, D. A. & Holmes, C. W. (2006). Yields and persistency of lactation in Friesian and Jersey cows milked once daily. Journal of Dairy Science 89, 2017–2024.

[34] Caja, G., Ayadi, M. & Knight, C. H. (2004). Changes in cisternal compartment based onstage of lactation and time since milk ejection in the udder of dairy cows. Journal of Dairy Science 87: 2409–2415.

[35] Berry, D. P., Coughlan, B., Enright, B., Coughlan, S. & Burke, M. (2013). Factors associated with milking characteristics in dairy cows. Journal of Dairy Science 96: 5943–5953.

[36] Penry, J. F., Endres, E. L., DE Bruijn, B., Kleinhans, A., Crump, P. M., Reinemann, D. J. & Hernandez, L.L. (2017). Effect of incomplete milking on milk production rate and composition with 2 daily milkings. Journal of Dairy Science 100 (2): 1535-1540.

[37] Gasqui, P. & Trommenschlager, J.-M. (2017). A new standard model for milk yield in dairy cows based on udder physiology at the milking-session level. Scientific Reports 7(1): 1-11.

[38] Weiss, D. & Bruckmaier, R.M. (2005). Optimization of individual pre-stimulation in dairy cows. J. Dairy Science. 88: 137-147.

[39] Tancin, V., Uhrincat, M., Macuhova, J. & Bruckmaier, R. M. (2007). Effect of pre- stimulation on milk flow pattern and distribution of milk constituents at a quarter level. Czech J. Anim. Sci. 52(5): 117-121.

[40] Kaskous, S. & Bruckmaier, R. M. (2011). Best combination of pre-stimulation and latency period duration before cluster attachment for efficient oxytocin release and milk ejection in cows with low to high udder-filling levels. Journal of Dairy Research 78:97-104

[41] Auldist, M.J., Coats, S., Rogers, G. L. & McDowell, G. H. (1995). Changes in the composition of milk from healthy and mastitic dairy cows during the lactation cycle. Australian Journal of Experimental Agriculture 35(4): 427 - 436

[42] Hagen, K., Langbein, J., Schmied, C., Lexer, D. & Waiblinger, S. (2005). Heart rate variability in dairy cows-influences of breed and milking system. Physiology and Behavior 85 (2): 195-204.

[43] Oltenacu, P. A. & Broom, D. M. (2010). The impact of genetic selection for increased milk yield on the welfare of dairy cows. Animal Welfare 19(1): 39-49.

[44] Mullen, M.P., Lynch, C. O., Waters, S. M. (2011). Single nucleotide polymorphisms in the growth hormone and insulin-like growth factor-1 genes are associated with milk production, body condition score and fertility traits in dairy cows. Genetics and Molecular Research 10: 1819-1830.

[45] Richardt, W. (2004). Milchinhaltsstoffe als Indikatoren für Fütterung und Gesundheit von Milchkühen. Fachtagung 2003/2004, Deutsche Vilomix Tierernährung; Sächsischer Landeskontrollverband e.V., Lichtenwalde.

[46] Piccioli-Cappelli, F., Loor, J. J., Seal, C. J., Minuti, A. & Trevisi, E. (2014). Effect of dietary starch level and high rumen-undegradable protein on endocrine-metabolic status, milk yield, and milk composition in dairy cows during early and late lactation. Journal Dairy Science 97(12): 7788-7803.

[47] Eicher, R., Bouchard, E. & Bigras-Poulin, M. (1999). Factors affecting milk urea nitrogen and protein concentration in Quebec dairy cows. Preventive Veterinary Medicine 39(1): 53- 63.

[48] Olmos Colmenero, J. J. O. & Broderick, G. A. (2006). Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. Journal of Dairy Science 89 (5):1704-1712.

[49] Borucki Castro, S. I., Phillip, L. E., Lapierre, H., Jardon, P. W. & Berthiaume, R. (2008). The relative merit of ruminal undegradable protein from soybean meal or soluble fiber from beet pulp to improve nitrogen utilization in dairy cows. Journal of Dairy Science 91: 3947-3957.

[50] Spek, J. W., Dijkstra, J., Van Duinkerken, G. & Bannink A. (2013). A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. Journal of Agricultural Science 151: 407-423.

[51] Siachos, N., Panousis, N., Arsenos, N. & Valergakis, G. (2017). Investigation of milk urea nitrogen concentration and factors affecting its variation in Greek Holstein herds. Journal of the Hellenic Veterinary Medical Society 68(3): 423-432.

[52] Nielsen, N.L., Larsen, T., Bjerring, M. & Ingvartsen, K.L. (2005). Quarter health, milking interval, and sampling time during milking affect the concentration of milk constituents. Journal of Dairy Science 88:3186-3200.

[53] Friggens, N.C. & Rasmussen, M. D. (2001). Milk quality assessment in automatic milking systems: accounting for the effects of variable intervals between milking's on milk composition. Livestock Production Science 73: 45-54.

[54] Godden, S. M., Lissemore, K.D., Kelton, D. F., Leslie, K. E., Walton, J. S. & Lumsden, J. H. (2001). Factors associated with milk urea concentrations in Ontario dairy cows. Journal Dairy Science 84(1): 107-114.

[55] Henao-Velasquez, A. F., Munera-Bedoya, O. D., Herrera, A. C., Agudelo-Trujillo, J. H. & Ceron-Munoz, M. F. (2014). Lactose and milk urea nitrogen: fluctuations during lactation in Holstein cows. Revista Brasileira de Zootecnia 43(9): 479-484.

[56] Mukherjee, J. and Dank, A. K. (2011). Immune activity of milk leukocytes during early lactation period in high and low yielding crossbred cows. Milchwissenschaft 66: 384-388.

[57] Kennedy, B. W., Sethar, M. S., Tong, A. K.W., Moxley, J. E. & Downey, B. R. (1982). Environmental factors influencing test-day somatic cell counts in Holsteins. Journal dairy Science 65: 275-280.

[58] Sheldrake, R. F., Hoare, R. J. T. & McGregor, G. D. (1983). Lactation stage, parity, and infection affecting somatic cells, electrical conductivity, and serum albumin in milk. Journal Dairy science 66: 542-547.

[59] Geneurova, V., Hanns, O., Gabriel, B. & Zvackova, I. (1993). Somatic cell counts of milk in relation to production factors. Zivocisna Vyroba 38: 359-367.

[60] Saravanan, R., Das, D. N., De, S. & Panneerselvam, S. (2015). Effect of season and parity on somatic cell count across zebu and crossbred cattle population. Indian Journal Animal Research 49: 383-387.

[61] Goncalves, J. L., Cue, R. L., Botaro, B. G., Horst, J. A., Valloto, A. A. & Santos, M. V. (2018). Milk losses associated with somatic cell counts by parity and stage of lactation. Journal Dairy Science 101 (5): 4357-4366.

[62] Gygas, L., Neuffer, I., Kaufmann, C., Hauser, R. & Wechsler, B. (2008). Restlessness behavior, heart rate and heart-rate variability of dairy cows milked in two types of automatic milking systems and auto-tandem milking parlours. Applied Animal Behavior Science 109 (2): 167-179.

[63] Castro, A., Pereira, J. M., Amiama, C. & Bueno, J. (2015). Typologies of dairy farms with automatic milking system in northwest Spain and farmer's satisfaction. Italian Journal of Animal Science 14 (2): 207-219.

[64] Bruckmaier, R. M., Macuhova, J. & Meyer, H. H. D. (2001). Specific aspects of milk ejection in robotic milking: A review. Livestock Production Science 72: 169-176.

[65] Rushen, J., Munksgaard, L., Marnet, P. G., & De Passille, A. M. (2001). Human contact and the effects to acute stress on cows at milking. Applied Animal Behavior Science 73 (1): 1-14.

[66] Rose-Meierhöfer, S., Müller A. B., Mittmann, L., Demba, S., Entorf, A. C. H., Hoffmann, G., Ammon, C., Rudovesky, H. J. & Brunsch, R. (2014). Effects of quarter individual and conventional milking systems on teat condition. Prev. Vet. Med. 113: 556-564.

[67] Alhussien, M. N. & Dang, A. K. (2018). Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. Veterinary World.11 (5): 562-577.

[68] Stranden, I., Kantanen, J., Russo, I.-R. M., Wengel, P. O-T., Bruford, M. W. & Consortium, C. (2019). Genomic selection strategies for breeding adaptation and production in dairy cattle under climate change. Heredity 123: 307-317.

[69] Freuen, S. & Hoy, S. (2020). So wirkt sich das Erstkalbealter auf die Lebensleistung aus. Milchpraxis 54(2): 12-14.

[70] Hanauska, A. (2019). Analyse der Entwicklung der Milchleistung in den ersten fünf Laktationen bei Kühen der Rasse Deutsche Holstein unter Beachtung ausgewählter Einflussfaktoren in einem Betrieb in Baden-Württemberg. Bachelorarbeit 72 Seiten, Fachbereich Landwirtschaft, Hochschule Anhalt Bernburg

[71] Mellado, M., Antonio-Chirino, E., Meza-Herrera, C., Veliz, F. G., Arevalo, J. R., Mellado, J. & De Santiago, A. (2011). Effect of lactation number, year, and season of initiation of lactation and treated with recombinant bovine somatotropin. J. Dairy Sci. 94 (9): 4524- 4530.

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Water intake and excretion of growing she-camels in relation to the type of roughage fed and concentrate

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Abstract**—***The objectives of this study were to evaluate the effect of three roughages (Atriplex, clover hay or rice straw) were fed ad lib with two levels of concentrate (95% and 50% ad lib) on feed intake, water utilization,(water intake and excretion)of growing she-camels. Nine healthy growing she-camels (28-30 months old and 376.3 kg body weight, BW) were housed individually in metabolic cages and randomly allotted to three treatments, three camels each. The experiment lasted for 60 days. Results indicated that limiting concentrate to 50% decreased (P <0.05) ME intake, DCP intake and metabolic water as well as average daily gain, while increased (P <0.05) roughage feed intake, free water intake and total water intake as well as non-excreted water. On the other hand, the effect of roughage type indicated that the Atriplex-fed camels had higher (P <0.05)daily feed intake, water intake and water excretion. The camels fed hay were significantly higher in the metabolic water than those fed Atriplex or rice straw(15.23, 11.06 and 11.95 ml/kg0.85, respectively). The camels fed Atriplex recorded significantly (P<0.001) higher free water intake, feed water, total water intake, faecal water output, urinary water excretion and non-excreted water than camels fed hay or straw. The roughage-concentrate interaction was significant (P <0.05) for feed water, faecal water output, urinary water excretion and metabolic water than the other groups. The results indicated that camels fed high concentrate level 95% with clover hay showed the best results concerning by energy intake, body weight gain and feed efficiency. While camels fed Atriplex revealed the highest feed and water intake. The limited concentrate and ad lib roughages offered in camels fed, had significant (P <0.05) increasing free water intake, total water intake, and non-excreted water but decreased metabolic water. There was no observed effect on the amount of faecal water and urinary water excretion.*

Keywords**—growing she-camels, diet selection, feed utilization, growth, water utilization.**

I. INTRODUCTION

Water is an essential nutrient for all animals and total body water is the key component of living ruminants [1]. It is a major constituent of muscles and of meat and the proportion diminishes as the camel grows and gets older. Camel's water requirements depend on age, bodyweight, and disease status, level of exercise, lactation status, temperature, humidity, and dry matter content of the feed consumed. Average size camels require 30–40 liters each per day. Camels on natural range browse and graze derive all, or most of their water, from the plants they eat.

Camels consume less water than sheep and goats. Moreover, they tolerate water deprivation for a long period without any adverse effects on their performance [2,3]. Certain types of desert sheep and goats also, possess the ability to abstain from water for some time, but their resistance is nowhere near that of camels. Urinary water excretion was less in camels than in sheep [4].Water deprived camels excreted leusrsi ne than those watered daily [5].

Dromedary camels (*Camels dramedarius*) are distributed in arid and semi-arid areas of North Africa, south- East Asia and India [6]; in areas characterized by sparse and unpredictable food and water supplies. They are particularly well adapted to deserts and are better suited to these areas than are cattle, sheep and goats [7, 8]. Their food and water requirements are relatively lower than those of other species and they can extract more energy from the food they consume [9-11]. Their low water requirements allow them to range great distance from water sources in search of food [2].Herbivores grazing arid rangelands are seasonally challenged with feed and water deficiencies and supplementary feeding is a common practice. To be effective, production wise and economically, the supplement should complement what the animal gets from the pasture to presumably fulfill its nutritional requirements.

Farid[12]conducted a feeding trials to investigate diet selection, feed intake capacity (FIC) and animal performance when concentrates (corn grains and commercial concentrates mixture) and roughages (Atriplex, clover hay or rice straw) were fed *ad lib* free-choice in a cafeteria feeding system, and also the effect of restricting concentrates offered. The roughages were selected to represent different grazing conditions prevailing in arid rangelands. They indicated that growing camels having free choice to select their diets from both concentrates and roughages were capable of regulating their voluntary food intake predominantly through physiological mechanisms to satisfy energy requirements. This was true for the Atriplex and hay groups but not for the straw group or when concentrates offered was limited

In this regard, the aim of the present experiment was to study the effect of feeding three types of roughages (clover hay, Atriplex and rice straw) with two concentrate levels on the growing she-camel performance and water utilization.

II. MATERIALS AND METHODS

2.1. Animal management and treatments

Nine healthy growing she-camels were used in the present experiment to study feed intake, digestion and utilization under simulated arid grazing conditions with supplementary feeding. They were 28-30 months old and their live body weight averaged 376.3 kg (range: 356-389 kg).Standard management and health care procedures approved by the Desert Research Centre were followed. Animals were randomly allotted to three *ad lib* feeding treatments of three roughage types species(three she-camels in each)to represent the different grazing conditions prevailing in arid rangelands. Egyptian clover hay to represent optimum grazing conditions, rice straw to represent dry season grazing and *Atriplex halimus* to represent arid rangelands dominated by halophytes were the roughages that offered to animals. In addition the concentrates used were corn grains and cottonseed meal at two levels 95 and 50% of *ad lib* as recommended by Farid[12].Proximate composition of the feed ingredients is presented in Table (1).

Table 1. Dry matter, total protein and crude fibres intakes (g/day/kg0.73) at 100% and 50% ad lib concentrates used to calculate dry matter allowances for the present experiment [12].

Feed ingredients	Dry matter		Total protein		Crude fibres	
	100%	50%	100%	50%	100%	50%
Hay group						
Clover hay	22.45	38.11	3.20	5.43	7.68	13.05
Corn grains	43.17	21.78	4.64	2.34	1.63	0.82
Cottonseed meal	18.19	9.05	2.88	1.43	3.51	1.75
Total	83.81	68.94	10.72	9.20	12.82	15.62
Atriplex group						
Atriplexhalimus	37.16	72.64	4.35	8.50	10.64	20.79
Corn grains	45.02	23.90	4.84	2.57	1.70	0.90
Cottonseed meal	11.03	5.66	1.75	0.90	2.13	1.09
Total	93.21	102.20	10.94	11.97	14.47	22.78
Straw group						
Rice straw	14.97	27.30	0.68	1.24	4.32	7.88
Corn grains	44.30	21.85	4.77	2.35	1.67	0.82
Cottonseed meal	13.88	6.85	2.20	1.08	2.68	1.32
Total	73.15	56.00	7.65	4.67	8.67	10.02

The camels were housed individually in metabolism cages (1.3 x 3.0 m), designed for the separate collection of faeces and urine, for the duration of the experiment which lasted 60 days in two periods, 30 days each. The two consecutive experimental periods represented feeding levels of concentrate 95% and 50%, respectively. Those were, respectively, of average ad lib roughage and concentrate intakes calculated, per unit metabolic weight ($kg^{0.73}$), from a previous experiment [12] and summarized in Table (2).

Dry matter intake reported in Table (1) or the present experiment differs because of occasional incomplete consumption of offered rations.

Feeds were offered twice daily at 8:00 and 16:00 hours. Refusals, if any, were weighed the following morning and intake was recorded for each animal. Water was made available free choice once daily at the morning feeding time and water intake was recorded daily for each animal. Animals were weighted periodically after overnight fast and on two consecutive days.

2.2. Digestion trials

Each of the two consecutive digestion and nitrogen balance trials lasted 30 days. Animals were introduced to the respective diets for 15 days, followed by an 8-day preliminary period and a 7-day collection period. During the collection period, food refusals (if any), feces and urine were quantitatively collected once daily before feeding. Composite dried samples of feces were ground for laboratory analysis. Urine was collected in containers containing 50 ml of 50% H_2SO_4 and samples were taken daily for each animal and frozen until later chemical analysis.

2.3. Analytical procedures

Chemical composition of the experimental rations, feed refusals, feces and urinary nitrogen were determined according to procedures of [13].

2.4. Statistical analysis

Main effects and interactions were evaluated using the GLM repeated-measures analysis of variance procedures of the NCSS statistical package [14]. The type of roughage and concentrate levels were the independent variables, and concentrate levels were repeated within roughages. Newman-Keuls multiple comparison test was applied to the means of the main effects, i.e. type of roughage, R-means, and level of concentrates, C-means.

III. RESULTS AND DISCUSSIONS

3. 1. Chemical composition of feed ingredients

Proximate analysis of the three roughages are presented in Table (2) showed that Clover hay was higher in organic matter, crude protein, crude fiber, ether extract and Atriplex was higher in ash, while the straw was the least in these components. These proximate analysis values of feed ingredients used in this experiment were in agreement to previous investigations [12, 15].

Table 2: Proximate composition of feed ingredients, % DM basis

Proximate constituents	Egyptian Clover hay	Rice Straw	Atriplexh alimus[2]	Corn grains	Cottonseed meal[1]
Dry matter	86.08	87.43	34.98	86.65	90.88
Ash	13.35	21.68	25.37	1.71	24.73
Organic matter	86.65	78.32	74.63	98.29	75.27
Total (crude) protein	14.26	4.55	11.70	10.76	15.84
Crude fibers	34.23	28.86	28.62	3.77	19.30
Ether extract	4.40	2.52	2.94	3.92	10.86
N-free extract	33.76	42.39	31.37	79.84	29.27

[1] Un-decorticated, heat treated and mechanically pressed CSM, produced in a traditional mill,

[2]Leaves and succulent branches typically consumed by grazing animals,

3.2. Feed intake

Results indicated that decreasing the level of concentrate from 95% to 50% of *ad lib* intake decreased ($P<0.0001$) total dry matter intake (DMI). However, the intake of the three types of roughages (hay, Atriplex and straw) as a percentage of DMI increased ($p<0.001$). At the same trend, Jakhmola and Roy [16] reported that when growing camels were fed *ad lib* on a local Indian roughage (moth chara) with three levels of protein concentrate supplementation, (LPN), 18 (MPN) and 28 g DM/d/$Kg^{0.75}$

(HPN), their roughage intake decreased significantly by 22% and 12% for camel groups fed high (HPN) and medium (MPN) levels of protein concentrate, respectively and compared less than their mates fed low level (LPN) of concentrate. They indicated that this decrease may be due to changes in the rumen fermentation pattern. Whereas feeding low levels of concentrates stimulated cellulolytic fermentation in the rumen, high levels of concentrates tend to change fermentation pattern from typical cellulolytic to amylolytic. Thus, rumen retention time of roughage might have been increased, thus, reducing the intake of roughages. Also, the present results of the linear relationship between increasing the level of concentrate and total DMI were in agreement with that trends of total DM intake were reported by other investigations [17-20].However, concentrate supplementation reduced roughage intake even though total DMI increased [21, 22].Also, Farid [12] reported that increasing concentrate levels caused a decline in roughage intake and increased total DMI in camels fed hay or straw, but when concentrate level were reduced to 50% of *ad libitum* intake, roughage intake increased while total DMI decreased.

The type of roughage significantly ($P<0.001$) affected DMI. Growing she-camels fed Atriplex recorded greater($P<0.05$) DMI and roughage as a % of DMI (Table 3)than strawfed camels group which had the least DMI value and those fed hay were intermediate. The roughage-concentrate interaction was not significant.

On the other hand, it was noticeable that when the level of concentrate decreased to 50% of *ad lib*, growing she-camels group fed Atriplex recorded higher ($P<0.05$) total DMI and roughage (% of DMI)than their mates fed clover hay or rice straw, respectively. This may be due to the positive response of camels to Atriplex feeding which is attributed to two principal factors [23]. First, camels appear to need more salt, probably more than other herbivores, which is in higher proportion in this plant. This fact was demonstrated previously by Chamberlain [24]who demonstrated that camels require six to eight times the amount of salt required by other livestock, and camels without regular access to salty feed require about 140 g of salt per day. So, these finding explain the higher ($P<0.05$) intake of DM, when camels fed Ariplex in comparison to their mates fed either hay or straw. Second, in comparison to bovines, camel saliva contains a varying content of high molecular weight mucin-glycoprotein (MGP) that confers protection to the mucosa of the digestive tract from mechanical injuries and fixes the plant tannins preventing their negative effects on protein metabolism in the rumen [25]. In addition, Atriplex being a lush green plant was more palatable and preferred by camels in comparison to the dry long clover hay and rice straw. In the straw fed group, the poor digestibility and low nutritive value did limit the VFI and adversely affected animal performance [26].

Data of metabolize energy (ME, kcal/day/kg$^{0.73}$)intake show almost the same significant trend of the impact of the type of roughage and concentrate supplementation level on dry matter intake (Table 3).On the other hand, data of digested crude protein intake (DCP, g/day/kg$^{0.73}$)was affected ($P<0.001$) by changing the type of roughage. Whereas, growing she-camel fed Atriplex recorded comparable DCP intake value to their mates fed clover hay and significantly higher about twice times that of those fed rice straw. This was mainly due to the higher CP% content of clover hay and Atriplex (Table 1)[27].

3.3. She-camel live body weight, daily gain and feed efficiency

Live body weights of growing she-camels were not significantly affected by changing the level of concentrate supplementation or the type of roughage (Table 3). Average daily gains (g/day)of camels fed hay and Atriplex were higher ($P< 0.001$) than those of their mates fed rice straw with limiting concentrate offered to 50%.

These results indicated that ADG was affected ($P<0.001$) by the type of roughages. In this respect, Kamoun [28] found that the ADG ranged from 326 to 525 g/d in one-year old camels fed on *ad libitum* hay and hay plus concentrate (80% wheat bran). While Etman [29] found that the ADG was 412 g for camels fed on berseem or hay plus concentrate, and it was 386 g/d for camels fed on wheat straw plus concentrate. However, Faye [18] indicated that ADG was 550 g/d for concentrate-supplemented camels and 570 g/d for concentrate plus mineral supplemented camels. Kamoun [30, 31] demonstrated that the daily gain of the growing camels at the period from five to ten months of age was 605 g/d and gust 280 g with the diet having 22% protein. Khanna [32] reported that values of daily gain of Kutchi and Bikaneri camels were 800 and 749 g, respectively, up to three months of age.

Table 3: Nutritional and performance parameters of growing she-camels.

Concentrate level [C]	Roughage, *ad lib* [R]			C-means	Repeated-measures ANOVA *(p value)*		
	Hay	Atriplex	Straw		R	C	RxC
Live body weight, kg							
95% ad lib	389.17	355.83	383.83	376.28	0.2245	0.0000	0.0283
50% ad lib	424.83	396.33	402.67	407.94			
R-means	407.00	367.08	393.25				
± SEM		11.132		1.775			
Average daily gain, g/day							
95% ad lib	911.46	760.42	671.88	781.25^{b}	0.0018	0.0006	0.3437
50% ad lib	473.12	355.56	-10.76	272.74^{a}			
R-means	692.29^{b}	557.99^{b}	330.56^{a}				
± SEM		39.483		54.655			
Dry matter intake (DMI), g/day/kg$^{0.73}$							
95% ad lib	77.14	81.11	71.73	76.66^{b}	0.0192	0.0001	0.4289
50% ad lib	62.71	70.10	55.45	62.75^{a}			
R-means	69.92^{ab}	75.60^{b}	63.59^{a}				
± SEM		2.098		1.103			
Roughage, %DMI							
95% ad lib	32.22	39.44	27.92	33.19^{a}	0.0009	0.0000	0.3712
50% ad lib	56.89	60.47	51.73	56.36^{b}			
R-means	44.55^{b}	49.95^{c}	39.83^{a}				
± SEM		0.961		0.715			
ME intake, kcal/day/kg$^{0.73}$							
95% ad lib	218.3	168.4	178.2	188.3^{b}	0.0015	0.0000	0.9184
50% ad lib	148.0	90.9	104.3	114.4^{a}			
R-means	183.2^{b}	129.6^{a}	141.4^{a}				
± SEM		5.837		4.982			
DCP intake, g/day/kg$^{0.73}$							
95% ad lib	6.873	6.857	4.986	6.239^{b}	0.0002	0.0003	0.0206
50% ad lib	5.519	6.182	2.364	4.688^{a}			
R-means	6.196^{b}	6.519^{b}	3.675^{a}				
± SEM		0.2187		0.1432			
Efficiency of ME utilization for gain, kcal/g							
95% ad lib	19.88	17.66	21.66	19.37	0.3377	0.5161	0.3225
50% ad lib	27.33	34.51	-55.24	2.20			
R-means	23.61	26.08	-16.79				
± SEM		21.045		17.976			
Efficiency of DCP utilization for gain, g/g							
95% ad lib	0.630	0.719	0.609	0.653	0.1497	0.9497	0.2091
50% ad lib	1.019	2.406	-1.322	0.701			
R-means	0.825	1.653	-0.356				
± SEM		0.5946		0.5221			

$^{a-b-c}$ means within a main effect, C-means or R-means, not sharing a superscript were significantly (P<0.05) different according to Newman-Keuls multiple range test.

Decreasing the level of concentrate supplementation from 95% to 50% resulted in decreasing (P<0.001) the ADG by 65% (range from 781.25 to 272.74g/day). This result is in agreement with that reported by Farid [12]. They decreased concentrate supplementation from *ad lib* to 50% and found ADG to decrease by 73%. The same trend was reported by Jakhmola and Roy [16], for growing camels. It is noticeable that Atriplex and hay feeding with *ad lib* concentrate supplementation or when limited to 50% of *ad lib*, recorded the highest DMI in comparison to straw-feeding.

Irrespective of the level of concentrate offered, hay and Atriplex feeding promoted better intake, ME and DCP than straw resulting in the highest observed ADG. Similar results were reported earlier by Shawket [23] who indicated that growing camels fed Atriplex supplemented with crushed barley grains (100% of their growth energy requirements.

Efficiency utilization of ME for gain (kcal/g) or DCP utilization for gain (g/g) was not affected by changing the type of roughage or the level of concentrate. The roughage-concentrate interaction also showed no effect.

3.4. Water intake and excretion

Data of average water intake and excretion (ml/day/kg$^{0.82}$) are present in Table (4)and Figure (1).

Table 4. Water intake and excretion, ml/day/kg$^{0.82}$.

Concentrate level [C]	Roughage, *ad lib* [R]			C-means	Repeated-measures ANOVA *(p value)*		
	Hay	Atriplex	Straw		R	C	RxC
Free water intake							
95% ad lib	111.17	144.02	113.39	122.86^{a}	0.0014	0.0000	0.1285
50% ad lib	199.16	247.08	217.86	221.37^{b}			
R-means	155.17^{a}	195.55^{b}	165.63^{a}				
± SEM		4.297		2.173			
Feed water							
95% ad lib	6.74	40.05	6.04	17.61	0.0000	0.0618	0.0089
50% ad lib	5.57	50.64	4.65	20.29			
R-means	6.15^{a}	45.35^{b}	5.34^{a}				
± SEM		1.254		0.827			
Metabolic water							
95% ad lib	18.06	14.05	14.48	15.65^{b}	0.0013	0.0000	0.9733
50% ad lib	12.40	8.07	9.06	9.84^{a}			
R-means	15.23^{b}	11.06^{a}	11.95^{a}				
± SEM		0.444		0.394			
Total water intake							
95% ad lib	135.96	198.12	134.27	156.12^{a}	0.0000	0.0000	0.1259
50% ad lib	217.13	305.80	231.58	251.50^{b}			
R-means	176.55^{a}	251.96^{b}	182.92^{a}				
± SEM		4.607		3.157			
Faecal water output							
95% ad lib	16.26	28.49	20.62	21.79	0.0099	0.5236	0.0142
50% ad lib	13.05	37.04	17.83	22.64			
R-means	14.65^{a}	32.76^{b}	19.22^{a}				
± SEM		2.844		0.889			
Urinary water excretion							
95% ad lib	43.91	33.63	15.49	31.01	0.0084	0.9156	0.0411
50% ad lib	24.06	50.48	19.99	31.05			
R-means	33.99^{ab}	42.05^{b}	17.74^{a}				
± SEM		3.611		3.194			
Non-excreted water							
95% ad lib	75.79	136.00	98.16	103.32^{a}	0.0052	0.0000	0.4814
50% ad lib	180.02	218.28	193.75	197.35^{b}			
R-means	127.91^{a}	177.14^{b}	145.96^{a}				
± SEM		6.575		4.960			

^{a,b,c} means within a main effect, C-means or R-means, not sharing a superscript were significantly (P<0.05) different according to Newman-Keuls multiple range test.

At reducing level of concentrates from 95% to 50% of respective *ad lib* intake, the free water intake, total water intake and non-excreted water increased (P <0.001), and the metabolic water intake decreased (P <0.001), Meanwhile, the feed water, fecal water output and urinary water execration were not significantly affected. On the other hand, the roughage type significantly affected the water intake and excretion. Camels fed *ad lib* Atriplex recorded the highest (P <0.001) free water intake, feed water, total water intake, fecal water output, urinary water execration, and non-excreted water than their mates fed *ad lib* clover hay or rice straw which showed comparable values. She-camels fed Atriplex or rice straw recorded the lower (*P* <0.001) metabolic water values than their mates fed the hay. The roughage-concentrate interaction significantly (*P* <0.05) affect feed water, fecal water output and urinary water excretion, while free water intake, metabolic water, total water intake and had no significant effect on non-excreted water.

Camels fed Atriplex with its high content of sodium chloride and soluble proteins might have resulted in increased rumen osmolality and ammonia concentration, both known to negatively affect voluntary food intake (VFI) [33, 34]. However, since fresh drinking water was freely available, along with that available from the lush plant, it seems that the anticipated negative effect on VFI was counter-balanced. Similar results were reported in previous investigations [35, 36].

These results were in agreement with the findings of other investigators [37, 38] who indicated that increasing salinity of drinking water increased water excretion in urine than in faeces. Ahmed [39]pointed out that increasing water excretion through the urinary pathway is believed to be an adaptive mechanism assisting the animal in getting rid of excess salts and maintain osmolality of food and other body fluids. She-camel group fed Atriplex recorded higher (P <0.05) non-excreted water (ml/day/kg $w^{0.82}$) values than those of camels fed on straw or hay. These results indicated that camels' kidneys seem to be better adapted to handling salt load especially when they fed on halophytic plants [40]. The roughage-concentrate interaction was significant (P <0.05) in affecting feed water, faecal water output and urinary water excretion, while no significant effect on other water parameter was detected.

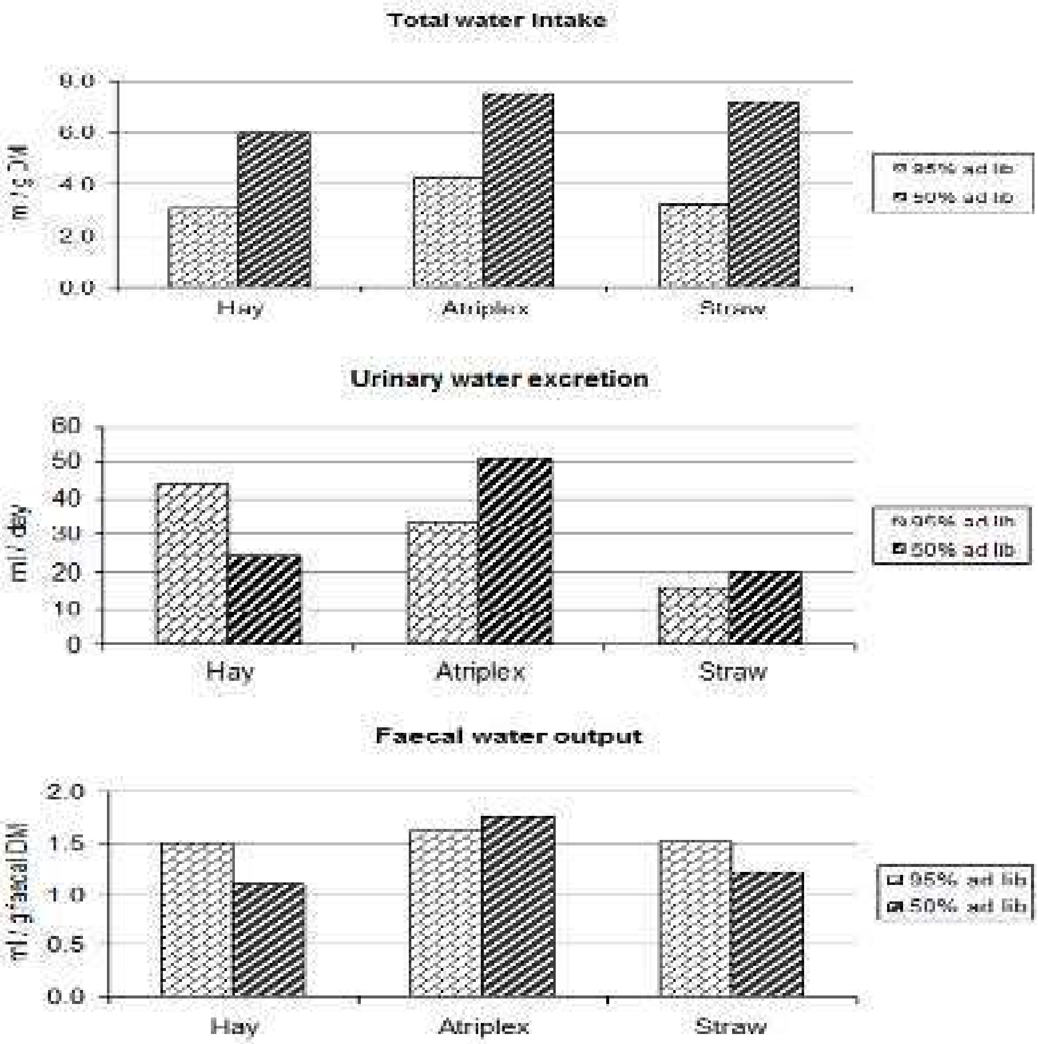

Fig.1: Total water intake and faecal water output as a function of dry matter intake and excretion.

Limiting the concentrate level from 95 to 50% significantly decreased feed water, metabolic water, fecal water and urinary water in relation to total water intake (TWI).On other hand, free water intake and non-excreted water in relation to TWI increased (Table 5). This may be due to decreasing the concentrate level, increased roughage percentage of DMI which need to increase water intake (Table 4) for its rumen fermentation and digestion and this reflect on non-excreted water values.

Table 5. Water intake and excretion in relation to total water intake (TWI), %.

Concentrate level [C]	Roughage, *ad lib* [R]			C-means	Repeated-measures ANOVA *(p value)*		
	Hay	Atriplex	Straw		R	C	RxC
Free water intake, %TWI							
95% ad lib	81.73	72.80	84.83	79.66[a]	0.0000	0.0000	0.2247
50% ad lib	91.71	80.80	94.07	88.86[b]			
R-means	86.72[b]	76.80[a]	89.25[c]				
± SEM		0.568		0.312			
Feed water, %TWI							
95% ad lib	4.96	20.14	4.50	9.86[b]	0.0000	0.0001	0.2509
50% ad lib	2.56	16.56	2.01	7.05[a]			
R-means	3.76[a]	18.35[b]	3.26[a]				
± SEM		0.435		0.203			
Metabolic water, %TWI							
95% ad lib	13.31	7.06	11.07	10.48[b]	0.0001	0.0000	0.0019
50% ad lib	5.72	2.64	3.92	4.09[a]			
R-means	9.52[c]	4.85[a]	7.49[b]				
± SEM		0.274		0.152			
Faecal water, %TWI							
95% ad lib	11.82	14.51	15.35	13.90[b]	0.1611	0.0017	0.1703
50% ad lib	5.97	12.11	7.75	8.61[a]			
R-means	8.90[a]	13.31[a]	11.55[a]				
± SEM		1.401		0.695			
Urinary water, %TWI							
95% ad lib	33.03	17.02	11.51	20.52[a]	0.0828	0.545	0.0909
50% ad lib	11.12	16.52	8.64	12.09[a]			
R-means	22.07[a]	16.77[a]	10.07[a]				
± SEM		3.051		2.500			
Non-excreted water, %TWI							
95% ad lib	55.15	68.46	73.14	65.58[a]	0.1177	0.0089	0.0733
50% ad lib	82.90	71.38	83.62	79.30[b]			
R-means	69.03[a]	69.92[a]	78.38[a]				
± SEM		2.920		2.547			

[a,b,c] means within a main effect, C-means or R-means, not sharing a superscript were significantly ($P<0.05$) different according to Newman-Keuls multiple range test.

The type of roughage affected ($P<0.001$) free water intake as % of TWI. Straw-fed camels recorded the higher value followed by the hay-fed camels and then the Atriplex-fed camels had the least. This result may be due to Atriplex fed camel group which drank greater ($P<0.05$) amount of water than their mates fed straw or hay (Table 4). Also, Atriplex-fed camels' recorded significantly higher feed water % TWI than those fed hay and straw-fed due to thatAtriplex had higher moisture content about 65% (Table 2). Camels group fed hay had higher ($P<0.001$) value of metabolic water as % of TWI, while the Atriplex-fed camels were the least and those fed straw were intermediate. As mentioned earlier, camels fed hay had higher ($P<0.001$) metabolic water ($ml/day/kg^{0.82}$) and recorded the lowest ($P<0.001$) value of TWI compared to their mates fed Atriplex which recorded the highest value of TWI. Fecal water, urinary water and non-excreted water in relation to TWI were not affected significantly by the type of roughage.

3.5. Water intake and non-excreted water in relation to gross energy intake

Data of free water intake, total water intake and non-excreted water (ml/kcal GE intake) in relation to gross energy intake in Table (6) and Figure(2)

Table 6. Water intake and non-excreted water in relation to gross energy intake

Concentrate level [C]	Roughage, *ad lib* [R]			C-means	Repeated-measures ANOVA (*p value*)		
	Hay	Atriplex	Straw		R	C	RxC
Free water intake, ml/kcal GE intake							
95% ad lib	0.570	0.752	0.650	0.657[a]	0.0038	0.0000	0.0014
50% ad lib	1.290	1.556	1.697	1.514[b]			
R-means	0.930[a]	1.154[b]	1.173[b]				
± SEM		0.0336		0.0142			
Total water intake, ml/kcal GE intake							
95% ad lib	0.697	1.032	0.769	0.832[a]	0.0003	0.0000	0.0033
50% ad lib	1.406	1.925	1.804	1.712[b]			
R-means	1.052[a]	1.478[c]	1.286[b]				
± SEM		0.326		0.0161			
Non-excreted water, ml/kcal GE intake							
95% ad lib	0.386	0.708	0.562	0.552[a]	0.0025	0.0000	0.0814
50% ad lib	1.166	1.374	1.509	1.360[b]			
R-means	0.776[a]	1.041[b]	1.036[b]				
± SEM		0.0349		0.029			

[a,b,c] means within a main effect, C-means or R-means, not sharing a superscript were significantly (P<0.05) different according to Newman-Keuls multiple range test.

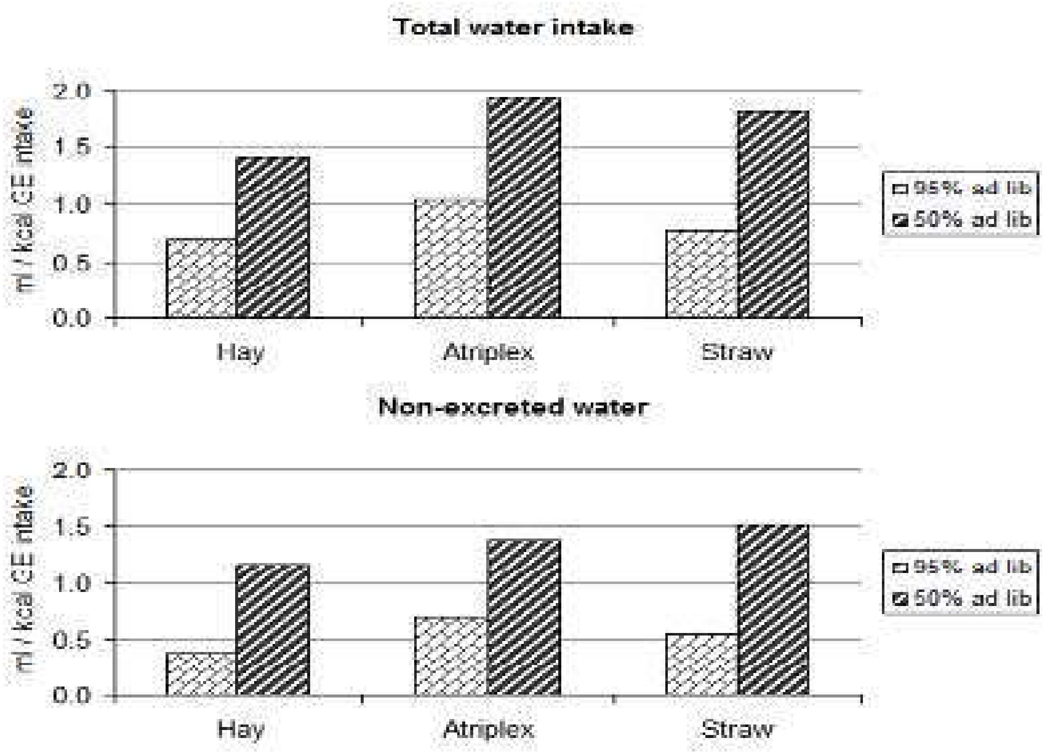

Fig. 2: Total water intake and non-excreted water as a function of gross energy intake.

3.6. Water intake and faecal water excretion in relation to dry matter, ml/g DM

The data in Table (7) indicated that limiting feed concentrate level from 95 to 50%increased (P <0.05)free water intake, and total water intake relation to dry matter intake (ml/g DM).This may be due to that limiting diet concentrate level increased the amount of roughage consumption as a percentage of total dry matter intakes (Table 3). Whereas previously, Greg [41] indicated that feeds high in crude fiber, such as roughages will require more water for ingestion than feeds low in crude fiber such as barley and corn. Type of roughage also significantly affected free water intake, and total water intake in relation to dry matter intake (ml/g DM) which in agreement with results of goats fed different types of roughages [42]. Growing camels group fed hay recorded the lowest ($P<0.05$) values and their mates group fed Atriplex had higher values than those fed straw. This result is due to the higher values of free water intake and total water intake of growing camel group fed Atriplex than their mate groups fed hay and straw by about 25, 18, 43 and 38%, respectively. In this context, Greg [41] reported that increasing the salt concentration level of the diet stimulated water intake in all species because of the increase in urine volume necessary for excretion of salt.

Limiting the diet concentrate level decreased fecal water in relation to fecaldry matter (ml/g fecal DM**)**. This result made clear that the fresh fecal mass weight was higher with its moisture content which resulting in low fecal dry matter due to that high concentrate diets content result in more digestible and nutritious.

The type of roughages had a significant effect on fecal water in relation to its dry matter. The atriplex-fed camels had the highest values in total water intake and fecal water in relation to dry matter faecal, while the hay-fed camels had the lowest values and those fed on straw were intermediate. The roughage-concentrate interaction was significant.

Table 7.Water intake and faecal water excretion in relation to dry matter, ml/g DM

Concentrate level [C]	Roughage, *ad lib*[R]			C-means	Repeated-measures ANOVA (*p value*)		
	Hay	Atriplex	Straw		R	C	RxC
Free water intake, ml/g DMI							
95% ad lib	2.479	3.062	2.714	2.752^{a}	0.0162	0.0000	0.0019
50% ad lib	5.490	6.056	6.741	6.096^{b}			
R-means	3.984^{a}	4.559^{b}	4.728^{b}				
± SEM		0.1311		0.0525			
Total water intake, ml/g DMI							
95% ad lib	3.032	4.200	3.214	3.482^{a}	0.0009	0.0000	0.0072
50% ad lib	5.986	7.495	7.165	6.882^{b}			
R-means	4.509^{a}	5.848^{c}	5.190^{b}				
± SEM		0.1265		0.0585			
Faecal water, ml/g faecal DM							
95% ad lib	1.495	1.619	1.510	1.541^{b}	0.0394	0.0014	0.0015
50% ad lib	1.086	1.753	1.198	1.346^{a}			
R-means	1.290^{a}	1.686^{b}	1.354^{a}				
± SEM		0.0880		0.0246			

[a,b,c] means within a main effect, C-means or R-means, not sharing a superscript were significantly ($P<0.05$) different according to Newman-Keuls multiple range test.

IV. CONCLUSION

The results indicated that camels fed high concentrate level 95% with clover hay showed the best results with respect to energy intake, body weight gain and feed efficiency, while those fed Atriplex revealed the highest feed and water intake. Feeding limited concentrate and *ad lib* roughages resulted in significant ($P <0.05$) increased free water intake, total water intake, and non-excreted water but decreased metabolic water. There was no effect on the amount of faecal water and urinary water excretion. Therefore, camels can be successfully adapted to feeding *Atriplex Halimus* (saltbush plant) with no negative impact on their performance.

V. REFERENCES

[1] Panaretto, B. A. and A. R. Till, 1963. Body composition in vivo. The composition of mature goats and its relationship to the antipyrine, tritiated water and N- acetyl-4 amino-antipyrine spaces. Australian Journal of Agricultural Research, 14: 926-943.
[2] Schmidt-Nielsen, K. (1964): The Camel In: Desert Animals: physiological problems of heat and water. Clarendon Press, Oxford. P. 277.
[3] Farid, M.F.A., Sooud, A. E. O., and Shawket, S.M. (1985): Water intake and excretion of camels and sheep in relation to diet characteristics and water deprivation. 3rd. AAP. Anim. Sci. Cong. May, Seoul, Korea. PP. 799-857.
[4] Macfarlane, W.V., Morris, R. J. H., and Howard, B. (1963): Turnover and distribution of water in desert camels, sheep, cattle and kangaroos, Nature, London. 197: 270.
[5] Kandil, H.M. (1984). Studies on camel's nutrition. Ph. D. Thesis, Fac. of Agric., Ain Shams Univ., Egypt.
[6] Mason, T.L. (1979). The camel in Australia. Melbourne University press. Melbourne Australia.
[7] McKnight, T.L. (1969). The camel in Australia, 154 pp. (Melbourne University Press, Melbourne Australia (154p).
[8] Wilson, R.T. (1984). The camel. Harlow, Essex, Longman Group Ltd., UK.
[9] Macfarlane, W.V., B. Howard, G. M. O Maloly, and D. Hop craft. (1972). Tritiated water in field studies of ruminant metabolism in Africa. In isotope studies on the physiology of domestic animals. Pp. 83-94. International Atomic Energy Authority. Vienna.
[10] Farid, M.F.A., S.M. Shawket, and M.H.A. Abdel-Rahman,(1979). Observations on the nutrition of camels and sheep under stress. In: Camels. IFS Symp., Sudan, 125-170.
[11] Yagil, R.T. (1981). Camel milk and camel products. Report to the Food and Agriculture Organization. Rome.
[12] Farid, M.F.A., A.M. Abdel-Wahed, Safinaz M. Shawket and N.I, Hassan. (2010). Diet Selection, feed Intake capacity and performance of growing female camels: Effects of type of roughage and level of concentrates offered. J. American Sci., 6: 317-326.
[13] AOAC – Association of Official Agricultural Chemists. (1990). Official Methods of Analysis, 15th edition. AOAC, Washington DC.
[14] Hintze, (2007). NCSS. Kaysville, Utah, U.S.A.
[15] Abdel-Wahed, A. M. (2014). Effect of type of roughage of concentrate supplements on feed digestion and utilization in growing female dromedary camels. Journal of American Science 2014;10 (6): 198-206.
[16] Jakhmola, R.C. and A.K. Roy. (1992). Effect of supplementation of concentrate on body weight gain and serum constituents in camel. Indian, J. Anim. Sci. 62:8, 782-784.
[17] Tan, P.V. and M.J. Bryant. (1991). The effects of dietary supplements of fish meal on the voluntary food intake of store lambs. Anim. Prod., 52: 271.
[18] Faye, B., G. Saint-Martin, R. Cherrier, and A. Ruffa. (1992.) The influence of high dietary protein, energy and mineral intake on deficient young Camel (Camelus dromedarius).Changes in metabolic profiles and growth performance. Comp. Bioch. and Physiol. A Comp. Physiol., 102:2, 409 – 416.
[19] Fahmy, A.A. (1993). Some factors affecting the nutritional performance of camels under desert conditions. M.Sc. Thesis, Al-Azhar Univ. Fac. Agric., Cairo, Egypt.
[20] Migwi, P.K., I. Godwin and J.V. Nolan. (2006). The effect of nitrogen, protein and energy supplementation on voluntary intake in sheep fed low quality roughage. Proceedings of the 10th KARI biennial scientific conference, Vol 1, 12-17 November 2006, Nairobi, Kenya.
[21] Jabbar, M.A. and M.I. Anjum. (2008). Effect of diets with different forage to concentrate ratio for fattening of Lohi lambs. Pak. Vet. J., 28: 150-152.
[22] Shawket, S. M., M. H. Ahmed, H.S.Ziwal, A. M. Nour and A.M. Abd EL-Wahed. (2007). Camel's growth performance response to dietary protein levels. Egyptian Journal of Nutrition and Feeds, 10: 477- 489.
[23] Shawket, S. M., K.M. Youssef and M. H. Ahmed. (2010). Comparative evaluation of Egyptian clover and Atriplex halimus diets for growth and milk production in camel. Animal Science Reporter, vol. 4: 9-21.
[24] Chamberlain, A. (1989). Milk production in the tropics. Intermediate Tropical Agricultural Series 13, Camels, 202-210.
[25] Schmidt-Witty, U., R. Kownatki, M. Lechner-Doll, and M. L. Enss. (1994). Binding capacity of camel saliva mucins for tannic acid. J. of Camel Practice and Research, December, 121.
[26] Cianci, D., L. Goio, A.M. Hashi, S. Pastorelli, M. Kamoun, G.B. Liponi and M. Orlandi. (2004). Feed intake and digestibility in camels fed wheat straw and Meadow hay. J. Camel Science. 1: 52-56.
[27] Shawket, S. M., H.S.Ziwal, M. H. Ahmed, A. M. Nour and A.M. Abdel-Wahed. (2005). Camel's performance response to dietary protein level under semi-arid conditions. Egyptian Journal of Nutrition and Feeds, 8: 211- 223.
[28] Kamoun, M., P. Girard, and R. Bergaoui. (1989). Feeding and growth of the dromedary. Effect of concentrate feeding on dry matter intake and growth of camels in Tunisia Revue, d'Elevage et de Med. Vet. Des Pays Tropicaux. 42, 89-94.
[29] Etman, A.H.M.(1997). Effect of nutrition on the productive and reproductive efficiency of one humped camels. Ph. D. Fac. Agric. Al-Azhar Univ. Egypt.
[30] Kamoun, M. (1993). Reproduction and production of Maghrabi dromedaries kept on pastures of the

Mediterranean type. Etudes et Synthesrs de I' IEMVT. 1993, No. 41, 117-130; Actes de I' Atelier ''Peut on ameliorer les performances de reproduction des camels '' Paris, France, 10-12 September 1990.

[31] Kamoun, M. (1995). Dromedary meat: production, qualitative aspects and capacity for conversion. Options Mediterraneennes Serle-B, Etudes ET Recherches. 13:105-130.

[32] Khanna, N.N. (1988). Annual Report, Project Director, National Research Center on Camel Bikaner.

[33] Kyriazakis, I. and J.D. Oldham. (1993). Diet selection in sheep: the ability of growing lambs to select a diet that meets their crude protein (nitrogen × 6.25) requirements. British Journal of Nutrition, 69: 617-629.

[34] Stevens, D.R., J.C. Burns, D.S. Fisher and J.H. Eisemann. (2004). The influence of high- nitrogen forages on the voluntary feed intake of sheep. Journal of Animal Science, 82:

[35] Konig, K.W.R. (1993). Influence of saltbush (*Atriplex spp*.) as diet component on performance of sheep and goats under semi-arid range condition. Ph. D. dissertation, Reihe Agrarwissens chaft, Institute for Animal Production in the Tropics and Subtropics, Aachen, Germany (ISBN: 3-86 III-706-1).

[36] Alicata, M.L., G. Ahmato, A. Bona, D. Giambalvo and G. Leto. (2002). In vivo digestibility and nutritive value of Atriplex halimus alone and mixed with wheat straw. Journal of Agricultural Science. I39, 139-142.

[37] El-Faramawi, A.A. (1984). Effect of drinking bring water on energy and water metabolism in sheep. M. Sc. Thesis, Ain Shams Univ.

[38] Kandil, H.M., A.O. Sooud, M.FA. Farid, H.M. El-Shaer, and M.A. El-Ashry. (1985). Effect of drinking saline water on feed utilization and nitrogen and mineral balance in camels. First Inter. Congress on Animal Production in Arid zones 9 ZICAPAZ, Sep., 7 – 14, 1985, ACSAD, Damascus, Syria.

[39] Ahmed, M.H. (1984). Studies on nitrogen metabolism in ruminants: effects of drinking saline water on nitrogen metabolism and rumen kidney function in sheep. Ph. D. Thesis, Alex. Univ., Egypt.

[40] Abou El-Nasr, H.M., M. Shawket, Safinaz, M.F. Farid and H.S. Khamis. (1988). Studies on saline drinking water for camels and sheep: 1- Water intake and excretion. Alex. J. Com. Sci. Dev. Res., 23: 131–139.

[41] Greg, L. Charles, S. and Roxanne, J. (2008). Livestock and Water. NDSU Extension; 954.

[42] Ehrienbruh , R.,M . Eknaes, and T. Pollen ,N. L. Andersen, and K. E. Bøe, (2010). Water intake in dairy goats – the effect of different types of roughages. Italian Journal of Animal volume 9 issue 4 pp 400 – 403.

Molecular Identification of Phytophagous Scarabaeid from different regions of India

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Abstract**—***Identification of scarabaeid beetles is a challenging task due to variable morphological differences among species and delineation among the immature forms, the grubs and adults. A method for easy and accurate species-level identification at any life stage is required. In this study, a 658-base pair region of the mitochondrial cytochrome oxidase I (COI) gene was used to explore its utility in the identification of important beetles. Twenty seven specimens were collected from 25 locations in different states, were characterized using specific primers for their identification. Sequence analysis and divergence among the species was assessed. The composition of the mitochondrial sequence of the COI gene in the present study was expectedly AT biased. Genbank accession numbers were obtained for the species. Molecular sequence information from NCBI revealed relatedness in all the collected scarabaeids, accurately as revealed by their morphological characters. The studies indicate the relevance of DNA sequencing to match different forms of beetles and address ambiguities in morphological identification and information on species diversity would help plan strategies for pest management.*

***Keywords*— Beetle, Characterisation, COI gene, Scarabaeid, Sequence.**

I. INTRODUCTION

Scarab beetles are the most diverse and widely distributed insects which belong to the largest order *Coleoptera.* The family *Scarabaeidae* is composed of about 91% of all scarabaeoids and represented by 30,000 species worldwide (Pathania *et.al*., 2015). About 2500 species are reported from India (Chandra and Gupta, 2011) and a majority of these are phytophagous (sub families *Melolonthinae, Rutelinae,Dynastiae* and *Cetoniinae*) (Ali, 2000., Dadmal *et.al.*, 2013). The adult beetles and their grubs cause extensive damage to fruit crops, vegetables, ornamental plants, plantation crops pastures, turf and meadow grasses, lawns, golf courses and forest trees (Dashad *et.al*., 2008 .,Lawerence *et.al*., 2013). Adults of the sub-family *Melolonthinae* and *Rutelinae* are pre-dominantly leaf feeders where as those of Cetoniinae feed on flowers and fruits, and are popularly referred to as flower beetles, prefer nectar, sap or juice of ripening fruits and vegetables. Members of Dynastinae usually attack stems or roots of plants (Bhat *et.al.*, 2005). Grubs of Melolonthinae, Rutelinae and Dynastinae commonly referred to as white grubs are often soil dwelling and cause extensive damage to the roots of cereals, legumes, small fruit plants, shrubs and trees (Thakre and Zade, 2012). In India, the white grubs are pests of national importance (Chandra and Ahirwar, 2007., Chandra and Singh, 2010).

Lack of taxonomic understanding has been a major impediment to the study and management of scarabaeid beetles. Identification of scarabaeid species is a challenging task due to variable morphological differences among species and delineation among the immature forms, the grubs and adults. Species identification of larval specimens requires a sophisticated technique and also vast knowledge of the cephalopharyngeal skeleton morphology (Greenberg, 2002).

Morphological identification keys are often effective only for a particular life stage or gender (Hebert et.al.,2003., Chandra and Gupta, 2012., Gupta et.al., 2014.,). The use of taxonomic keys often requires proficiency to avoid inaccuracy for those similarities which cannot be easily deciphered. Under these circumstances, DNA analysis appears promising to solve the species identification problem owing to the durability and stability of the DNA

(Wallman and Donnellan, 2001). It also can solve the problems of morphological identification with damaged specimens (Judith and Nicola, 2008). This technique is based on the mitochondrial DNA (mtDNA) encoded cytochrome oxidase I gene (COI) (Wells and Sperling ,2001., Sharma *et.al*., 2004, Theurkar *et.al.*, 2013). Partial sequences of this COI gene have been shown to have sufficient discrimination power (Stijn and Matthias, 2009., Zahoor et.al., 2013), which makes it suitable for a diagnostic taxonomy. The COI gene has been used for inferring phenogram analysis at various taxonomic levels of many animal groups (Avise,2000). Efficient Mitochondrial DNA (mtDNA) -COI based methods in the delineation and identification of scarabaeid species have been reported (Blaxter et.al., 2004., Dittrich et.al., 2009, Dirk et.al., 2007, Dirk et.al., 2011, Tauz et.al., 2003, Fang, 2009., Paul et.al, 2009).

II. MATERIALS AND METHODS

Field survey and Collection of Scarabaeid Beetles:

The diversity of scarab beetles depends on the availability of food for larvae and adult, weather conditions and soil type. Collection of scarab beetles was made randomly by hand picking and light trapping. Grubs were collected from a soil depth of 0.25-0.05 m depth in cultivated fields. The beetles were collected during May-June which is the major activity period to assess the diversity. The populations were collected from different states and geographical locations of the country from various trees and crop plants (arecanut, coconut, groundnut, mulberry, millets, neem, soybean, sugarcane and vegetables).

Collection of Adult Beetles Using Light Traps:

Light traps were used for four months (May - September) to collect the beetle populations. The light traps were placed in the center of the fields at a height of about 3 meters above the ground and operated between 7:00 PM to 5:00 AM to attract the scarabaeid beetles which are positively heliotactic in nature. The light trap comprised of PVC plastic funnel of 25 cm in height, and 30 cm. diameter. The bottom diameter of the funnel was 5 cm. The rain shed cone for protecting the bulb was fixed at 17 cm above the funnel with the help of three white metal sheets. The diameter of the rain shed cone was 20 cm. The light source consisted of a 125-watt incandescent light bulb with copper wire choke. The light trap had three baffles (30 cm x 10 cm), placed at a uniform distance of 10 cm around the circumference of funnel. The baffles were fixed to emit light uniformly in all directions without any interference, when the beetles are attracted to light they collide with baffles and fall into the trap. A nylon bag was attached to the bottom of this funnel for collection of beetles. The collected beetles were preserved in a vial containing 70% alcohol and taken to the laboratory for morphological identification and a few samples stored at -80°C at the Division of Genomic Resources, ICAR-NBAIR, India for characterization and identification.

Identification of the Beetles:

The scarab adults and grubs collected from different locations were identified up to the genus level at the Department of Entomology, University of Agricultural Sciences, Bangalore and the Division of Entomology, Indian Agricultural Research Institute, New Delhi, based on the keys and characters listed by (Dirk, *et.al.*, 2011). Adult beetles were identified based on the morphological characters such as body size, coloration, surface sculpture and male genitalia, while the grubs were differentiated based on the color, size of the cephalic capsule, number and form of dorsal sensorial maculae of the last antennomere, distribution, stimulatory structures in the maxilla and mandible , raster pattern arrangement of bristles and hairs on the underside of the abdomen, shape of anal slit (crescent, Y shaped, strongly Y shaped), shape and size of the respiratory plates, proportions of each pair of legs and tarsungulus size (Dashad *et.al*, 2008, Dirk *et.al*, 2011).

Extraction of Genomic DNA:

Total genomic DNA was isolated using the method described by (Gavarāne *et.al*, 2011) . Genomic DNA was isolated using modified Qiagen DNeasy blood tissue kit method. The insects were washed thoroughly in double distilled water. Total genomic DNA was isolated from the leg portion of the insect. The cleaned insect leg portion was homogenized in 1.5 ml appendorf tube in 500 µl of TE (Tris-EDTA-pH 8), with hand pestle and the homogenate was centrifuged at 10,000 rpm for 10 minutes in cooling centrifuge (-40C). The supernatant was discarded and the pellet was dissolved in 500 µl of lysis buffer (400 µl of TE and 100 µl of 5% SDS), followed by the addition of 6 µl of Proteinase K, and the solution was incubated at 650C for one and half hours. A mixture of 120 µl phenol chloroform isomyl alcohol (25:24:1) was added and the tubes vortexed for 30 seconds and then centrifuged for 10 minutes at 10,000 rpm in cooling centrifuge. The upper aqueous layer was carefully transferred in to fresh tube, without disturbing the protein layer at the interphase. Isoprapanol (500 µl) was added to this aqueous layer and stored at -40C overnight and then centrifuged at 7000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with 70% alcohol and later the acohol was drained out, the pellet was dried and dissolved in 30 µl of TE-I was stored at -200C after checking on 0.8% agarose gel and visualized after staining with ethidum bromide.

DNA Quantification- PCR amplification and sequencing of COI gene fragment:

The isolated DNA samples were quantified in order to find out the amount of DNA using Nanodrop Spectrophotometer. The absorption was measured at 260nm as the nitrogenous bases in DNA show strong absorption at this wavelength.

The extracted DNA samples were subjected to PCR amplification of 658bp cytochromeoxidase I (COI) gene fragment. The amplification was carried out using the universal CO I primers: COI forward (LCO1490) 5'GGTCAACAAATCATAAAGATATTGG 3'and CO I reverse (HCO2198) 5'TAACTTCAGGCTGACCAAAAAATCA 3' obtained from M/S Bioserve biotechnologies (India) Pvt Ltd. Amplification was performed in 0.2 ml PCR tubes with total volume of 25 µl containing 2.5 µl of template DNA, 4 µl of GeNeiTM 10X Taq buffer containing 15mM of MgCl , 2.5 µl of GeNieTM 10mM dNTP mix, 1 µl of forward primer (10pmol/µl), 1 µl of reverse primer (10pmol/µl), 1 µl of GeNieTM Taq DNA polymerase and 13 µl of sterile water. Temperature conditions for amplification were as follows: initial denaturation at 95^0C for 2 minutes, denaturation at 95^0 C for 30 seconds, annealing at 50^0C for 1 minute, extension at 72^0C for 2 minutes followed by 34 cycles and final extension at 72^0C for 7 minutes were carried out in thermal- cycler (BioRad, USA). PCR-amplified products were purified using Bioneer's PCR purification Kit (www.Bioneer.com). Amplification of DNA was then checked by running the samples on 1% agarose gel using 250bp DNA ladder and visualized in gel dock. The amplified products were then sent to commercial sequencing at M/S. Eurofins Pvt Ltd, Bangalore where the chain termination method was used for sequencing.

Sequence analysis and data interpretation:

The most commonly used method of DNA sequencing is the dideoxy method or chain termination method. The amplified products of COI gene were got sequenced at M/s. Eurofin Pvt Ltd, Bangalore. The COI gene sequence data was retrieved in the form of Chromatograms. Several individuals from each species were sequenced and chromatograms were subjected to VSQual (Binneck *et.al.*, 2004) to evaluate the reliability of the data, and good quality fragments were used to construct a consensus sequence for each sample. Chromatograms were edited to discard ambiguous bases, and edited sequences were aligned by using the Basic Local Alignment Search Tool (BLAST), with the sequences of same or related genera retrieved from the nucleotide database (PUBMED) of National Centre for Biotechnology Information (NCBI). The sequence data's was submitted to NCBI and accession numbers were obtained. Consensus sequences of COI partial gene were multiple aligned using Clustal W (ver. 1.83) (Thompson *et.al.*, 1994., Tauz *et.al*, 2003).

III. RESULTS AND DISCUSSION

Molecular identification:

The genomic DNA was isolated from the populations of scarabaeids collected from different states viz, Andhra pradesh, Himachal Pradesh, Karnataka, Kerala, Meghalaya, Tamilnadu and Uttar Pradesh. Persusal of the data on the collection of scarabaeid beetles from the different geographical locations and crops and their identification had revealed the diversity of beetles in the country. An array of phytophagous beetles (Table 1) belonging to the subfamilies (Cetoniinae, Dynastinae, Melolonthinae and Rutelinae) were collected

In the present studies, the isolated genomic DNA of scarab beetles from various locations was characterised through COI gene fragment (648-656 bp size) was successfully sequenced for all the specimens, and the alignment of all specimens considered in this study lacked any insertion or deletion. A total of 27 species were sequenced over COI regions and the Blast done with NCBI database to decipher the identity of the scarabaeids from various locations is given in Table 1. Molecular sequence information from NCBI revealed relatedness in all the collected scarabaeids, accurately as revealed by their morphological characters. Our observations, corroborate with the reports of (Dittrich et.al., 2006, Zahur et.al., 2013, Wardani and Sugiyarto, 2009).

The utility of DNA data in taxonomy and species diagnosis in the scarabaeid beetles was reported by (Dirk et.al., 2007 and 2011), based on the sequence variation in DNA based groups which highly structured. The population of scarabaeids from various locations were characterised using Cytochrome C oxidase subunit I (COI) gene, which has been recognised as an effective marker not only for species identification but also for phylogenetic relationship (Hebert et al., 2003, Dhoj et.al., 2009, Maryati and Sugiyato, 2009, Imura et al., 2014).

Nucleotide Analysis:

Nucleotide analysis of the sequences was carried out in order to find out the MCL (Maximum Composite Likelihood) estimate of the pattern of nucleotide substitution, AT%, GC% and the AT content at first, second and third codon position. The MCL pattern showed the probability of substitution (r) from one base (row) to another base (column) (Khannal *et.al.*, 2012). The sum of r values was made equal to 100. Rates of different transitional are shown in substitutions which were 18.79, 21.13, 9.83 and 8.97 and the transversionsal substitutions are given in (Table 2). The nucleotide frequencies are 27.99% (A), 40.02% (T/U), 18.63% (C), and 13.36% (G). The transition/ transversion rate ratios are k 1 = 3.253 (purines) and k 2 = 2.558 (pyrimidines). The overall transition/ transversion bias is R = 1.288, where R = [A*G* k 1 + T*C* k 2]/[(A+G)*(T+C).

The analysis also revealed that the percentage of AT was comparatively more i.e., 33.25% ranging between 29.8-34.9% than that of GC which is 16.8% with a minimum of 15.1% and maximum of 19.4% . indicating that the sequences were AT biased. This difference was attributed to the AT percentage at different codon position. The AT content at first codon position ranged between46-48%withaverageof44%,andtheATpercentage at second and third codon position is nearly invariant 27% and 28% respectively.

The composition of the mitochondrial sequence of the COI gene in the present study was expectedly AT biased and this was generally observed in several previous studies (Williams *et.al.*,, 1990). In general, the frequency of transitional substitutions is known to be higher than transversion substitutions in the genome (Tauz *et.al*, 2003), According to 10X rule the percentage of nucleotide divergence between the intraspecies should be less than 3% and that of interspecies should be more than 3%. Hence the sequences analyzed in the present study exhibited high inter species variability on the basis of nucleotide sequences. Therefore, the intra specific divergence was higher enough to discriminate between the individuals.

Results indicated that the COI-based pest identification was extremely effective for the beetles based on the COI marker profile. DNA sequence data have been employed successfully to elucidate the relationships of many groups of insect species at generic level. Molecular sequence information from NCBI revealed relatedness in all the collected scarabaeids, accurately as revealed by their morphological characters. Our observations, corroborate with the reports of (Richards *et.al*, 1997., Zhu *et.al*., 2000, Blaxter *et.al*., 2004.,Monti *et.al*, 2005). Qiu , *et.al*, (2009), suggested that where sequence information is available in Genbank for morphologically defined species, which can be matched with some DNA based clusters, close relationship can be identified readily in sequence variation in field collected field samples and these clusters are likely to correspond to previously described unknown species. Mgocheki *et.al.*,(2012), reported that the sequence information based on mitochondrial markers can be utilized for species delineation of adults and grubs of scarabaeids inferring larval taxonomy. Our studies indicate the relevance of DNA sequencing to match different forms of scarabs and address the issues of having to depend exclusively on morphological features and avoid misdiagnosis.

The species diversity is influenced by the cropping pattern, the climatological factors and the altitude. The abundance was reported to be negatively and significantly correlated with altitude. Low temperatures at higher altitudes inhibit the growth and development of beetles (Dhoj *et.al.*, 2009., Khanal *et.al.*, 2012) .

Our findings contribute to a better understanding of the identification of pests by COI genes and aid in formulating better management strategies.

IV. CONCLUSIONS

The diversity of phytophagous scarabaeid beetles from various geographical locations of India occurring in crops were morphologically identified and characterised using molecular tools, Molecular sequence information from NCBI revealed relatedness in all the collected scarabaeids, accurately as revealed by their morphological characters. Phylogenetic tree revealed the genetic relatedness among the beetles and understand the evolutionary relationship. The relevance of DNA sequencing to match different forms of beetles and address limitations in morphological identification is indicated. Knowledge on species diversity, through surveys would be helpful in planning strategies for conservation of natural enemies, habitat management, design and develop pest management strategies.

Table 1. Scarabaeid beetles collected from various locations and their accession numbers

Sl.No	Species	Subfamily	Location	Latitude/Longitude	Acc.No.
1	***Holotrichia consanguinea***	**Melolonthinae**	Anakapalle	17 .38.^{0} N, 83.2^{0} E	KU35553
			Samarlakota	17 .5^{0} N, 82.2^{0} E	KU35552
			Tirupathi	13.65^{0} N, 79.42^{0} E	
2	***Holotrichia serrata***		Bangalore	12.97^{0} N., 77.57^{0} E	KT254245
3	***Schizonycha* sp.**		Shimoga	13.92° N, 75.56° E	1762749
4	***Maladera insanabilis***		Phasighat	25.34.11°N, 91°59.2E	KU35551
					KU35551
5	***Apogonia* sp.**		Aligarh (UP)	27.89° N, 78.08° E	1762764
6	***Leucopholis lepidophora***		Sringeri	12.57 -13.52^{0} N.,	KU665428
			Shivamoga	75.72 -75.22^{0} E	KU665428
			Thirtahalli		KU665428
			Sirsi	14.6196° N, 74.84° E	KU665428
			Sultan Betheri (Kerala)	11.67^{0} N, 76.28^{0} E	KU665428
7	***Leucopholis burmeisteri***		Chikmagalur	13.40^{0} N., 78.05^{0} E	KU665432
			Belgaum	15.51^{0} N., 74.29^{0} E	KU665432
			Kannur (Kerala)	11.8^{0} N, 75.32^{0} E	KU665432
8	***Leucopholis coneophora***		Thrissur	10.52^{0} N, 76. 2^{0} E	KU665428
9	***Heterorrhina sp.***	**Cetoninae**	Ooty(TN)	11.41^{0} N., 70.58^{0} E	KM657485
10	***Protaetia* sp.**		Tirupathi (AP)		KM657490

			Phasighat	28.069° N, 95.32° E	KT1762766
			Shillong	25.34.11°N, 91°59.2E	KM657489
			Ooty (TN)	11.41 ^{0} N., 70.58 ^{0} E	KM657486
			Gudalur	11.59 ^{0} N., 76.50 ^{0} E	KT1762776
					1762777
11	***Protaetia cuprea ignicollis***		Bangalore	12.97 ^{0} N., 77.57 ^{0} E	KT203778
12	***Adoretus cupreus***	**Rutelinae**	Anand (Gujarat)	22.56° N, 72.92° E	KT254249
13	***Adoretus falvus***				KT254250
14	***Anomala* sp.**		Shimla (HP)	31.18° N, 77.17° E	1762765
			Bangalore (Dasarahalli)	12.97 ^{0} N., 77.57 ^{0} E	KM657492
				25.34.11°N, 91°59.2E	KM657491
15	***Anomala ruficapilla***		Bangalore (Dasarahalli),	12.97 ^{0} N., 77.57 ^{0} E	KT254246
16	***Anomala dimidata***		Theni		KU517668
	Anomala dimidata		Theni (Tamilnadu)	15.51 ^{0} N., 77. 79 ^{0} E	KU517664
17	***Phyllopertha horticola***		Anekal (Tamilnadu)	12.70° N, 77.69° E	KT203779
18	***Exomala pallidipennis***		Bangalore		KU317746
			Valampari (T.N)	15.51 ^{0} N.,74.29 ^{0} E	KT203780
19	***Onthophagus nuchicornis***	**Scarabaeinae**	Chintamani	13°24 ^{0} N 78°04'12. E	KU517667
			Bangalore		KU517666
20	***Onthophagus auritus***		Mudhigeri	13.1365° N, 75.64° E	KU665401
			Mudhigeri		KU665398
21	***Onthophagus coenobita***		Bangalore (Nandi hills)	13.3702° N, 77.68° E	KU665397
22	***Copris tripartitus***		Bangalore (Nandi Hills)	13.3702° N, 77.68° E	KU665396
23	***Basilepta sp***		Mudhigeri	13.1365° N, 75.64° E	KU665400

24	***Calicnemis obesa***	**Dynastinae**	Theni	15.51 ^{0} N., 77. 79 ^{0} E	KU517665
25	***Oryctes rhinoceros***		Theni (TN)	15.51 ^{0} N., 77. 79 ^{0} E	KU517993
26	***Alissonotum sp***		Kapatganj (UP)	26.920° N, 83.77° E	1762754
27	***Aethina concolor***	**Nitidulinae**	Mudhigeri	13.1365° N, 75.64° E	KU665399

Table 2. Maximum Composite Likelihood estimate of the pattern of nucleotide substitution of COI

	A	**T**	**C**	**G**
A	------	8.26	3.84	8.97
T	5.78	----	9.83	2.76
C	5.78	21.13	-----	2.76
G	18.79	8.26	3.84	----

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REFERENCES

[1] Ali, A.T.M . (2001). Biosystematics of phytophagous scarabaeidae-An Indian Overview. In: Indian Phytophagous Scarabs and their Manage- ment. Present Status and Future Strategies (Sharma, G.,Y.S Mathur., Y.S. and Gupta, R.B.L. (eds). Agrobios, 5-37.

[2] Avise, J. C. (2000). Phylogeography: The history and formation of species. Harvard University Press, Cambridge. vii+453 p.

[3] Bhat, O.K., Bhat, A.A., Kaul, V.K. (2005). Faunistic studies of white grubs in Kashmir. Insect Environment, 11: 14- 15.

[4] Binneck,E., João Flávio Silva,V., Norman Neumaier, B., José Renato Farias and Alexandre Nep Omuceno, L. (2004). VSQual: a visual system to assist DNA sequencing quality control.Genetics and Molecular Research, 3 (4): 474-482.

[5] Blaxter. M.L. (2004). The promise of DNA Taxonomy. Proceedings of the Royal Society of America, Series B. 359: 669-679.

[6] Chandra, K. and Ahirwar, S.C. (2007). An inventory of scarab beetles (Coleoptera: Scarabaeidae) of Achanakmar-Amarkantak biosphere re- serve, Chhattisgarh, India state Fauna Series. 15: 273-300.

[7] Chandra, K. and Singh, S.P. (2010) Scarabaeid beetles (Coleoptera) of Achanakmar Wildlife Sanctuary, Chhattisgarh. Nature Journal of Life Sciences, 7: 71-74.

[8] Chandra, K. and Gupta, D. (2011). Study of Scarabaeid beetles (Coleoptera) of Veerangana Durgavati Wildlife Sanctuary, Damoh, Madhya Pradesh, India. Deccan. Current Science, 5: 272-278.

[9] Chandra, K. and Gupta, D. (2012). Diversity and composition of dung beetles (Scarabaeidae: Scarabaeinae and Aphodiinae) assemblages in Sing- hori Wildlife Sanctuary, Raisen, Madhya Pradesh (India). International Journal of Science and Nature, 3: 886- 891.

[10] Dadmal, S.M., Khadakkar, S.S., Ghuge, P.A. (2013). Occurrence of five Holotrichia species (Coleoptera: Scarabaeidae: Melolonthinae) in Maharashtra and their male genitalia characterization. The BioScan, 8: 349-352.

[11] Dashad, S.S., Chauhan, R. and Choudhary, O.P. (2008). Emergence of differ- ent species of white grub beetles in relation to abiotic factors based on light trap catches. Research Crops, 9: 688.

[12] Dirk Ahrens., Michael,T., Monaghan and Alfried, P,V. (2007). DNA-based taxonomy for associating adults and larvae in multi-species assemblages of chafers (Coleoptera: Scarabaeidae). Moleclar Phylogenetics and Evolution, 44: 436–449.

[13] Dirk Ahrens., Scott, M. and Vogler, A.P. (2011). The phylogeny of monkey beetles based on mitochondrial and ribosomal RNA genes (Coleoptera: Scarabaeidae: Hopliini). Molecular Phylogeny and Evolution, 60: 408-415.

[14] Dittrich, G., Schröde, D.E., Conlong, M.J., Way, J. and Du. Harrison,G. (2009). Identification key to scarabaeid beetle larvae attacking sugarcane in South Africa using DNA barcoding and integrative taxonomy. Proceedings of South African Sugar Technolgists Association, 82: 500-524.

[15] Dhoj, Y.G.C., Keller, S., Nagel, P. and Kafle, L. (2009). Abundance and diversity of scarabaeid beetles (Coleoptera: Scarabaeidae) in different farming areas of Nepal. Formosan Entomon, 29: 103-112

[16] Fang, X. M. (2009). Phylogenetic analysis of Chrysomelidae in China inferred from mitochondrial COI sequences (Coleoptera: Chrysomeloidea). Journal of Suzhou University, 24 (1): 113-117.

[17] Gavarāne, I. (2011). Inese Kokina, Kristīna Aksjuta, Arvīds Barševskis and Uldis Valainis, Acta Biologica Universitatis Daugavpiliensis. 2011, 11 (2): 141-145.

[18] Greenberg, B. (2002). Key to the eggs, larvae, pupae and adults of some forensically important flies, pp. 54–153. In: B. Greenberg & J. C. Kunich (eds). Entomology and the law flies as forensic indicators. Cambridge University Press, Cambridge. xii+298 p.

[19] Gupta,D., Kailash Chandra and Salma Khan (2014). An updated checklist of scarabaeoid beetles (Coleoptera: Scarabaeoidea) of Pench Tiger Reserve, Madhya Pradesh, India. Journal of Entomology and Zoology Studies, 2(5): 225-240

[20] Hebert, P.D.N, Cywinska, A., Ball, S.L., and DeWaard, J.R. (2003). Biological identifications through DNA barcodes. Proceedings of Royal Society America, B. 270: 313-321.

[21] Imura,O., Morimoto,N. Shi, K. and Sasaki, H. (2014). Landscape diversity of pasture dung beetle communities in the central region of mainland Japan and implications for conservation management. Biodiversity and Conservation, 23: 597-616.

[22] Judith, A.S., and Nicola, C. B. (2008). Molecular genetic identification of forensically important flies in the UK. Forensic Science International: Genetics Supplement Series 1 (1): 620-622.

[23] Khanal, D., Dhoj, Y.G.C., Sporleder, M. and Thapa, R.B. (2012). Distribution of whitegrubs in here ecological domains of Nepal. The Journal of Agriculture and Environment, 13: 40-46.

[24] Lawerence, J.F., Hastings ,A.M., Dallwitz, M.J., Paine, T.A., Zurcher, E.J. (2013). Beetle Larvae of the World: Descriptions, Illustrations, and Information Retreival for Families and Subfamilies. CSIRO Publishing, Australia.

[25] Maryati, K.T. and Sugiyarto (2009). Characterization of white grub (Melolonthidae; Coleoptera) in salak plantation based on morphology and protein banding pattern. Bioscience 1 (2):72-77

[26] Mgocheki,N., Conlong, D.E., Ganeshan, S. and Addison, P. (2012). Molecular identification of South African sugarcane white grubs (Coleoptera: Scarabaeidae). Proceedings of the South African Sugar Technologists' Association, 85: 108 – 113.

[27] Michael. M.T., Balke, Ryan Gregory and Alfried, P.V. (2005). DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462): 1925–1933.

[28] Monti, M.M., Nappo, A.G. and Giorgini, M. (2005). Molecular characterization of closely related species in the parasitic genus Encarsia (Hymenoptera: Aphelinidae) based on the mitochondrial cytochrome oxidase subunit gene. Bulletin Entomological Research, 95: 401-408.

[29] Pathania ,M., Chandel, R.S., Verma, K.S. and Mehta, P.K. (2015). Studies on the Preliminary Ecology of Invasive Phytophagus Scarabaeidae of North Western Himalaya. Arthropods, 4: 46-68.

[30] Paul, F., Rugman, J., Robert, W., Tom, V.N., Richard, S. (2009). Molecular differentiation of the Psyttalia concolor (Szépligeti) species complex (Hymenoptera: Braconidae) associated with olive fly, Bactrocera oleae (Rossi) (Diptera: Tephritidae), in Africa. Biological Control ,49: 17-26.

[31] Qiu, B., Chen, Y.L., Liu, W., Peng, X., Li, M.Z. (2009). Identification of three major Bemisia tabaci biotypes in China based on morphological and DNA polymorphisms. Progress in Natural Science, 19: 713-718.

[32] Richards, N.K., Glare, T.R. and Hall, D.C.L. (1997). Genetic variation in grass grub, Costelytra zealandica, from several regions. Proceedings of 50th NewZealand Plant Protection Conference, 1997: 338-343.

[33] Singh, M.P., Bisht, R.S., and Mishra, P.N. (2003). Distribution of white Grub fauna in Garhwal Hills of Western Himalayas. Indian Journal of Entomology, 65: 217-221.

[34] Sharma, R.N., Mitra, B. and Chakraborty,P. (2004). Fauna of Protected Areas - 12: A preliminary account of beetle (Coleoptera) fauna of Kalatop-Khajjiar Wildlife Sanctuary, Himachal Pradesh. Zoo’s Print Journal, 19: 1626.

[35]

[36] Stijin, D. and Matthias, G. (2009). COI sequence variability between Chrysomyinae of forensic interest. Forensic Science International: Genetics, 3 (2): 89-95.

[37] Tamura, K. and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees.Molecular Biology and Evolution, 1993.10 (3):512-26.

[38] Tamura, K., Nei, M. and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of National Academy of Sciences (USA) ,101: 11030-11035.

[39] Tamura,T.,Peterson,D., Peterson, N., Stecher,G.,Nei,M. and Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution, 28(10):2731-2739.

[40] Tautz, D., Arctander, P., Minelli, A.H., Thomas, R. and Alfried, P.V. (2003). A Plea for DNA Taxonomy. Trends in Ecology and Evolution, 18: 70-74.

[41] Thakare, V.G. and Zade, V.S. (2012). Diversity of beetles (Insecta: Coleoptera) from the vicinity of Semadoh - Makhala road, Sipnarange, Melghat Tiger Reserve, (MS) India. Bioscience, 3: 112-115.

[42] Theurkar, S.V., Ghadage, M.K., Madan, S.S., Bhor, G.L., and Patil, S.B. (2013). Occurrence of white grubs in ground nut Growing area of Khed Taluka, part of Northern Western Ghats. MS, India. Research Journal of Recent Sciences, 2: 1-3.

[43] Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Reearch, 11: 4673-4680.

[44] Wardani, S.and Sugiyarto.(2009).Characterization of white grub (Melolonthidae; Coleoptera) in salak plantation based on morphology and protein banding pattern . Nusantara Bioscience, 1: 38-42.

[45] Wallman, J.F., Donnellan, S.C. (2001). The utility of mitochondrial DNA sequences for the identification of forensically important blowflies (Diptera: Calliphoridae) in southeastern Australia. Forensic Science International, 20(1-2):60-67.

[46] Wells, J. D., Sperling, F.(2001). DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). Forensic Science International, 120 (1-2): 110-115.

[47] Williams, J.G.K., Kubelik, A.R., Livak,K.J., Rafalski, J.K. and Tingey, S.V. (1990). Nucleic Acids Research, 18: 6531–6535

[48] Zhu, Y.C.J., Burd, D., Elliott, N.C. and Greenstone, M.H. (2000). Specific ribo- somal DNA markers for early PCR detection of Aphelinus hordei (Hy- menoptera: Aphelinidae) and Aphidius colemani (Hymenoptera: Aphi- diidae) from Diuraphis noxia (Homoptera: Aphididae). Annals of Entomological Society of America, 93: 486-491.

[49] Zahoor, M.K, Suhail, A., Zahoor, S., Iqbal, A. and Awan, F.S. (2013). Molecular characterization of Scarab beetles (Scarabaeidae: Coleoptera) using RAPD. Pakistan Journal of Life Social Sciences, 11 (3): 238-243.

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Assessment of the current status of pesticide use in triple-rice crops in Hoa Long commune, Lai Vung district, Dong Thap province, Vietnam

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***Abstract*— *This study was implemented to evaluate the current status of pesticide use in triple rice cropping in Hoa Long commune, Lai Vung district, Dong Thap province, Vietnam. The findings showed that the common pesticides used by the farmersin the study area belonging to toxicity Group II and III (World Health Organization (WHO) classification) accounted for 67.6%. Some farmers also usedpesticides containing banned active ingredients such as 2.4D, carbosulfan and carbendazole. The frequency of pesticide use was 5.5 times/crop which was relatively high. Basing on the active ingradientcompounds present in the study area, the potential environmental impact of pesticides is very seriously. The farmers used masks when spraying pesticides, however, they did not have a full understanding of the harmful effects of pesticide exposure. Packaging and bottles of pesticides after use have not been collected and disposed appropriately;it was commonly thrown away in the canals, ditches, and bare land after use. Local authorityhas not yet taken comprehensive measures to completely handle pesticide packaging and bottles due to funding constraints. In order to reduce the environmental and health risks associated with the use of pesticides and its wastes in the study area, local authority needs to increase the propaganda of knowledge about the management and use of pesticides while developing appropriate program for effective management of pesticidewastes.*

***Keywords*— pesticides, environmental pollution, toxicity, triple-rice crop, Lai Vung, Dong Thap.**

I. INTRODUCTION

Dong Thap has a natural area of 3,374 km^2, of which aerable land accounts for 276,206 ha. The population of the province 1.7 million people, with more than 82.73% living in rural areas, and 73.59% of agricultural labor. The value of agricultural production accounts for over 50% of the total economic values. Agricultural production is the main source of income for the majority of the rural people. Therefore, the issue of agriculture, farmers and rural areas is currently crucial to the socio-economic development of Dong Thap. In agricultural production, pesticide is one of the important supplies and is used in large quantities annually.

Toan et al. (2013) showed that the residues of active ingredient quinalphos were all present in the surveyed water bodies with the decreasing detection frequency from rice fields, rivers and in-field canals accounting for 40%, 50% and 67%, respectively. The concentration of active ingredients quinalphos in in-field canals and rivers in Summer-Autumn crop was higher than in Winter-Spring crop. In particular, at a number of survey sites, the concentration of quinalphos in water exceeded the acute toxicity level effective dose (EC_{50}) for invertebrates (0.66 µg/L). In the annual surface water monitoring program of the province, it is necessary to monitor the residues of pesticides that are commonly used in surface water. The main cause of pesticide residues is due to the fact that people often use pesticides of type II and III toxicity according to the World Health Organization (WHO) classification. The pesticide is often not used appropriately in terms of frequency, duration and dosage. Insecurity in use and storage is an issue of concern among the interviewed households (Toan et al., 2013). In addition, waste from the use of pesticides is often not properly managed and disposed of in the field as well as in the storage site. These situations could pose risks to public health and the surrounding environment. Cong et al (2015) reported that plant protection pesticides containing the active ingredient chlorpyrifos ethyl on cholinesterase can seriously affect the growth and development of snakehead

(*Channastriata*). Trung and Huong (2009) showed that the active insecticide quinalphos could seriously affect the enzyme activity of cholinesterase and glutathione-S-Trasnerase of the carp.

Several studies on pesticides and their effects on the environment and biodiversity have been reported, however, such study on the use of pesticides on triple-rice crop in Long Hoa commune, Lai Vung district, Dong Thap province is still limited. In order to have information to help scientists, especially scientists on environmental toxicology research evaluate the potential impact of pesticides on environment and biodiversity, this study was conducted to investigate current status of using and managing pesticide bottles in the triple-rice crop growing areas in Hoa Long commune, Lai Vung district, Dong Thap province, Vietnam.

II. METHODOLOGY

The study was carried out in a triple-rice crop area in Hoa Long commune, Lai Vung district, Dong Thap province, where farmers have a long tradition of rice cultivation. Information on the type and amount of pesticides used by farmers was collected by interviewing using semi-structured questionnaires. The study conducted with the interview of100 rice-growing farmers in Hoa Long commune, Lai Vung district, Dong Thap province of types, dosages,duration, and frequency of pesticide use. The study also collected information regarding methods of pesticide use as well as safety measures when spraying pesticides in the field. The management of package and bottles of pesticides after use was recorded through direct interview and field survey. In addition, the study also conducted interviews to collect opinions of commune officials on environmental management in agriculture in Hoa Long commune, Lai Vung district, Dong Thap province. The information from the interview was synthesized using Miscrosoft Excel combined with using simple calculation formulas such as SUM, AVERAGE, COUNIF to summarize collected data.

III. RESULTS AND DISCUSSION

3.1 General information of the respondents

Results of interviews with the households indicated that the average age of the household head was 45 years old. In particular, the highest age was 76 years old, the lowest age was 28 years old. Household heads aged 50 and under account for 66% of the total interviewees. The age of 50 and under is the age of healthy and experienced for many years in agricultural production. Respondents were 82% male, and 18% female, which is an important factor in determining the reliability of interview information because male respondents were directly involved in the use of pesticides. The survey results presented that the education level of the farmers in the study area is still low. Primary and secondary school farmers accounted for 86% (the majority of the farmers aged 40 to over 50 years old had low education level), while the high school level only accounted for 14%. The low levels of education of the interviewer farmers could be due to difficult rural conditions, unfavorable travel conditions, and time-consuming and costly education. The majority of the farmers in the study area are engaged in agriculture work in addition to some other seasonal jobs such as pesticide spraying, porting, knitting, making roofs to earn extra income to support their families.

The results of the study showed that households with land area from 7,000m^2 to 10,000m^2 accounted for 40%, land area of 10,000m^2 accounted for 34% and the rest was from 4,000m^2 to 7,000m^2. The land area pattern indicated the disparity in the land area which could lead to the use of pesticides differently in terms of doses and types. The low land area farmers often skipped the training programs relating to rice production techniques including the use of rice variety, fertilizer, and pesticide. This could lead to lack important information in environmental protection in agricultural production. In the study area, the farmers often use rice varity of Dai Thom 8 in Winter-Spring crop while IR50404 variety is used in Summer-Autumn and Winter-Autumn crops. The rice farming experience of the interviewed farmers was very well.According to the interviewing results, the number of households with rice production experience above 10 accounted for 74%, while 26% of the interviewed households have rice cultivating experience from 5 to 10 years. There was no household with the rice production experience of less than 5 years. Recently, pests and diseases have developed strongly leading to the increase of the use of pesticides in the rice fields. Consequently, risks of environmental pollution affecting human health and ecosystems become high.

3.2Farmers' knowledge of pesticides

According to the survey, up to 56% of the farmers did not know about banned pesticides in the market but they were sure that they do not use them. Only 44% of households said that they knew well about prohibited pesticides and could name the banned substances such as 2,4D and benomyl. These active substances are very toxic, which could kill natural enemies immediately. Therefore, farmers who know about these pesticides have limited use due to its toxicity. The research results showed that 40% of the households said that they know well about the harmful effects of pesticides, 54% of the households responded that they only know about pesticide exposure harms through newspapers, television, radio and relatives. Only 6% of the households are unaware of the harmful effects of pesticides and are not interested in knowing the impact. These households with the farmers aged over 50 and have primary school education.

The interview results showed that up to 94% of the farmers understood and knew about the harmful effects of pesticides on environments and human health. But when asked about the way of treating packaging and bottles of pesticides after use, up to 40% of the farmers chose tothrowdirectly in the fields, rivers or surrounding areas. There were 38% of the interviewed farmers sold and 22% burned packaging and bottles of pesticides after use. As could be seen that the interviewed farmers did have a good awareness of environmental protection as well as the harmful and long-term effects of pesticides. The selling and burning could result in human exposure to the residual pesticides. In addition, the pesticide residues would be absorbed into water and soil environment. Especially, plastic bottles and packaging are difficult to decompose when being discharged into the environment, which couldseriously pollute the environments.

There was a training course on how to properly manage packaging and bottles of pesticide after use, however there were only 54% of the farmers participated in the training course. In addition, local authority also organized integrated pest management (IPM) to improve the quality of pest management, reduce the use of pesticides and to protect the environment. Most farmers are rightly aware that throwing away pesticide bottles into the environment is wrong (of which 88% of the farmers said that they understood it was wrong-doing action but they have no other appropriate treatment method available). The farmers aged over 50 and have low education level (primary and secondary) said they have no interest in this issue. The research results also showed that the collection sites and containersfor packages and bottles of pesticide after use are not sufficient.

3.3Dosage and frequency of pesticide use

According to the survey, pesticides were used diversely with 31 trade names belonging to 35 active ingredients. This includes pesticides, diseases, growth stimulants, herbicides and raticides. Of the 35 active ingredients, up to 94.3% of the active ingredients were listed in the list of pesticides used in Vietnam according to Circular 03/2018/TT-BNNPTNT of the Ministry of Agriculture and Rural Development. The study discovered 2 prohibited active ingredients of pesticides (accounting for 5.7%) that have been used in the study area. This showed that the farmers still use illegal pesticides in the market. This could happen because 56% of the farmers did not know what are banned pesticides. In addition, the sellers are illegally trading.

Among the active ingredients investigated in the study area, there is one active ingredient belonging to the toxic group I. The pesticides in the toxic group II were the most used by farmers, accounting for 42.8%, the toxic group III accounting for 22.8%, and group IV accounting for 24%. Compared to the results of Toan (2013), the situation of using pesticides in rice production in the Mekong Delta shares similarities that rate of pesticide use is still high for the group II and III. Table 1 summarizes some of the active ingredients, toxicological classifications and potential environmental impacts of the pesticide used in the study area. The results showed that the pesticides found in the study area could cause serious environmental problems which could lead to irreversible effects on health and the environment without early remedies.

Table 1. Active gradients, toxic classification and potential impact of the pesticides present in the study area

Active ingredients	**Toxic classification**	**Potential impact**
Carbosulfan	- Group I; LD_{50}: 11 mg/kg; Time isolation 14 days.	- Toxic to humans and warm-blooded animals, but safe to plants; accumulate in the environment and food chains.
Abamectinh	- Group II; LD_{50}(oral) 300mg/kg; LD_{50}(skin)>1800mg/kg; Time isolation 7 days.	- Toxic to fish and bees; Irritating to skin and eyes.
Tricyclazone	- Group II; LD_{50}(oral) 250-314mg/kg; Time isolation 14 days.	- Less toxic to fish.

Active ingredients	**Toxic classification**	**Potential impact**
Propiconazole	- Group II; LD_{50}(oral) 1517mg/kg; LD_{50}(skin) 4000mg/kg; Time isolation 7 days.	- Less toxic to the environment, human, cattle, fish and bees.
2.4D (2,4-Dichlorophenoxyacetic acid)	- Group II; LD_{50}(oral) 699mg/kg	- Relatively toxic to fish; considered carcinogen; is an ingredient in orange agent.
Fipronil	- Group II; LD_{50}(oral) 95-97 mg/kg; Time isolation 7 days.	- Bioaccumulation in the natural food chain, especially in fish and aquatic animals; Very toxic to bees, fish and other beneficial organisms; risk of causing cancer.
Carbendazim	- Group II; Time isolation 14 days.	- Causing infertility, cancer; Less toxic to bees and fish.
Metaldehyde	- Group II; Time isolation 14 days.	- Relatively toxic to fish and bees.
Profenofos	- Group II	- Very toxic to fish; toxic tobees.
Propanil	- Group II; Oral (rat): LD_{50} = 1.080 mg/kg; skin (rabbit /rat): LD_{50} > 2.000 mg/kg; inhalation (Rat): LC_{50} > 6.1 mg/L.	- Eye and skin irritation in rabbits.
Imidacloprid	- Group II	- Less toxic to fish; toxic to bees.
Quinalphos	- Group II; Time isolation 15 days.	- Very toxic to fish and bees.
Chlorfenapyr	- Group II; (oral) LD_{50}: 223mg/kg (male rat); LD_{50}(oral): 459mg/kg (female rat); LD_{50} (skin): ≥ 2000mg/kg (rabbit); Time isolation 14 days.	- Less toxic to fish; toxic to bees.
Isoprothiolane	- Group III; Time isolation 7 days.	- Toxic to fish; less toxic to bees.
Butachlor	- Group III	- Highly toxic fish; less toxic to bees.
Difennoconazole	- Group III; LD_{50} (oral) 1.453 mg/kg, LD_{50} (skin) 2.010 mg/kg; Time isolation 7 days.	- Toxic fish; less toxic to bees.
Metalaxyl	- Group III; LD_{50}(oral) 669mg/kg; LD_{50} (skin) 3100 mg/kg; Time isolation 7 days.	- Causes moderate eye irritation.
Propineb	- Group III; Time isolation 5 days.	- Toxic fish; less toxic to bees.
Hexaconazole	- Group IV; Time isolation 7 days.	- Less toxic fish and bees.
Copper oxychloride	- Group IV; LD_{50} (oral)1144.7mg/kg (mice), LC_{50}2.2 mg/g (carp) (48 h). - Time isolation 7 days.	- Less toxic to warm-blooded animals.

Active ingredients	**Toxic classification**	**Potential impact**
Kasugamyan	- Group IV; LD_{50} 4 mg/kg; Time isolation 14-21 days.	- Not toxic to fish and honey bees.
Chlorantraniliprole	- Group IV; LD_{50} (oral) > 550 mg/kg (rat); LD_{50} (skin) > 5000 mg/kg (rat); LC_{50} (inhalation) > 3,394 mg/l, (rat).	- Toxic to fish
Azadirachtin	- Group IV	- Less toxic to mammals. - Reduction of female ovaries, fallopian tubes, by seminal vesicles, glucose levels, the activity of several enzymes, affecting the male reproductive system.
Azoxystrobin	- Group IV; LD_{50} (oral)> 5.000 mg/kg, (skin)> 2.000 mg/kg	- Low toxicity for mammals, birds, bees, insects and earthworms. Very toxic to aquatic organisms.

The majority of the farmerssaid that the types of agrochemical use were highly varied. Pest and disease chemical accounted for 82%, plant growth regulators accounted for 34%, herbicides accounted for 38%. In addition, the farmers also used chemicals to kill rat and yellow snail. Regarding time of use of pesticides, it was responded that when there are occurrence of insects and diseases, the farmers would immediately spray pesticidesto control the potential outbreak that could lead to crop damage. The above evidence proves that the farmers did not know how to use pesticides properly. The finding in the present study showed that 74% of the farmers use pesticides empirically and 26% of the farmers use the pesticides under the guidance of the seller or from the instructions on the packaging.

According to the farmers in the study area, approximate 78% of the farmers used the thedose according to the instructions on the package and 22% of the farmers selected the dose based on the level of seriousness of the pests and diseases. Sometimes, the farmers empirically used the dose two-fold higher than the recommended dose to quickly and securely kill pests or diseases. The number of pesticide spraying times in each rice crop was 5.5 times/crop. Compared with the results obtained by the Mekong River Commission in 2007, the number of sprays per crop in the study area is similar to the frequency of spraying in the Vietnamese Mekong Delta (5.3 times/crop), 5.5 times higher than with the Red River Delta (1.0 times/crop). Previous study showed that the number of sprays per crop on rice was 7 - 8 times in HauGiang province (Nhan et al., 2018) and 7.1 times in Can Tho city (Binh, 2008). The use of pesticides in this study is still lower than in some other areas in the Vietnamese Mekong Delta.

3.4Farmers' understanding of health issues due to pesticide exposure

Up to 26% of the farmersunderstood the harmful impact of pesticides on health through dermal contact, inhalation, and ingestion. However, the farmers said that they did not observe any cases of pesticide poisoning. There are 56% of the farmers supposed that the pesticide has an unpleasant smell and causes discomfort when spraying, sometimes causing dizziness, guava, and headache. The remaining 18% of the farmers said that there was nothing happened when spraying pesticides (the majority of the farmers are over 50 years old and the education level is low). Up to 92% of the farmers used protective mask when spraying pesticides. There were no other safety devices. Some farmers supposed that the use of protective clothes would cause difficult for their spraying, for example, the confortable feeling. The majority of the farmers stored pesticides in the house where children could reach. Only 16% of the interviewed farmers stored pesticides in a separate place. Some farmers used after purchase so that the was no storate of pesticides. The finding showed that farmers did not really understand the potential impact of the use, storage of pesticides on health and environment.

3.5The management of pesticide bottles in the study area

Only about 12% of the farmers said that a container for pesticide packaging and bottles after use was placed near their fields. And 88% of the farmers answered that the containers of pesticides were placed far away from the fields. Although knowing the of disposal of pesticide packaging and bottles after use is wrong, but because the container is far away with a small volume and often filled with garbage. The results of the interview with local officials indicated that there is a container for farmers in the area to collect the packages and bottles of pesticides after use. However, budget is the main constraint for placing more and larger volume of the pesticide collecting containers.

Fig.1: Simple tank for containing pesticides bottles and packages

IV. CONCLUSION

The current status of pesticide use in Hoa Long commune, Lai Vung district is a matter of great concern. The common pesticides used by the famrers belonging to toxic group II and III accounted for 67.6%. The banned pesticides including 2.4D, carbendazim, carbosufan were being used in the study area. After the use, up to 45% of the farmers threw away bottles and packages into the environments, selling to the vendors or burning at the field sites. All the current practices are extremely inappropriate posing a potential risk to health and ecosystems. The frequency and dosage of pesticide use were still high. The protective measures for the farmers in the study area is not highly efficient since the farmers only used simple mask. The management of bottles and packages at home still has many potential health risks. The management of pesticide packaging and bottles after use is still limited due to budget limitation. In the coming time, the local environmental managers need to strengthen the management of pesticide bottles and packaging because they are hazardous wastes that may seriously affect health and the environment. Budget allocation for pesticide wastes and training for farmers in properly using pesticides are urgently needed.

REFERENCES

[1] Nguyen Quang TrungandDoThi Thanh Huong (2009). The effects of quinalphos on cholinesterase and glutathione-s-transferaseactivities in common carp (Cyprinus carpio). Scientific Journal of Can Tho University, No 22a (2012); 131-142.

[2] Nguyen Ngoc Huyen Tran (2017). Current status of pesticide use and economic efficiency of *Eryngium foetidumin* Tam Hiep commune, Chau Thanh district, Tien Giang province. University graduation thesis, Can Tho University.

[3] Nguyen Van Cong, Nguyen Anh Tuan and Chau Thanh Tuoi (2015). Effects of pesticides containing the active ingredient chlorpyrifos ethyl (CE) on cholinesterase (ChE) in snakehead fish during the breeding period. Scientific Journal of Can Tho University, No 23(2015), 33-39.

[4] Pham Van Toan, Bui Thi Ngaand Nguyen Phan Nhan (2014). The residue concentrations of pesticide Quinalphos in surface water in rice fields and rivers in HauGiang Province. Scientific Journal of Can Tho University,No. 33 (2014), 109-116.

[5] Pham Van Toan (2013).The situation of pesticide use and several of reduced measures for improper pesticide use in rice production in the Mekong Delta. Scientific Journal of Can Tho University, No. 28 (2013), 47-53.

Solid Waste Composition and Management in Phu Huu Eco-Tourism Area, Hau Giang Province, Vietnam

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Abstract— *The study was conducted to evaluate the current status of solid waste management in PhuHuu Eco-Tourism Area in HauGiang Province, Vietnam in 2019. The main research method was to interview managers and visitors to the place. In addition, the study also aimed to classify and estimate daily generated solid waste. The results presented that the rate of generation of solid waste was 0.3 kg/person/day, with total solid wastes of 70-100 kg/day for weekdays and 200-300 kg/day for weekends and holidays. The organic components accounted for 69% including rice, leftovers, spoiled vegetables, fruit peels and leaves while inorganic components occupied 31% including plastic cups, straws, plastic wrap and foam boxes. Results of interviews with visitors indicated that the majority of tourists did not agree with the arrangement of rubbish bins and solid waste management in the tourist area. Solid waste management is not efficient due to the arrangement and the size of the waste bins, as well as the methods of solid waste classification. There is a need for better management of solid in the waste tourism area to attract more tourists to visit.*

Keywords*— *Solid waste, inorganic components, organic components, PhuHuu eco-tourism area, HauGiang province.

I. INTRODUCTION

Tourism is an economic sector whose existence and development are associated with the ability to exploit resources and the characteristics of the surrounding environment. Therefore, tourism and environment have a very close relationship with each other and if inappropriate exploitation and development of tourism activities may cause depletion of resources, deterioration of environmental quality. According to the Vietnam Tourism Law (2005), ecotourism is a form of nature-based tourism, linked to the local cultural identity with community participation for sustainable development. Besides, the development of tourism activities generates waste. A combination of several studies shows that solid waste from tourism activities has a basic characteristic similar to the domestic waste of residential areas (Suarmatha, 1990; Jindal and Shikura, 1998). Some previous studies show that the average amount of bio-waste from tourists varies from 0.67 to 0.8 kg/person/day (Tran HieuNhue and Nguyen Quoc Cong, 2005). This can be considered as an important source of pollution from tourism activities, particularly in places where waste management and treatment are not yet appropriate.

PhuHuu eco-tourism area is located inPhuNghia hamlet, PhuHuu commune, Chau Thanh district, HauGiang province is a tourist destination with many specific characteristics. Thanks to the advantages of natural conditions and indigenous culture, PhuHuu today has become a familiar eco-tourism site not only for local tourists but also for visitors from other provinces. However, environmental protection issues such as solid waste management is the issues that need to be addressed to tourism management. The study was conducted to assess the rate of generation of solid waste and assess the current status of solid waste management including classification, collection, transportation and disposal of solid waste. The findings could provide important information on the waste generation rate and recommendation for better environmental protection in the tourism area.

II. MATERIALS AND METHODS

2.1 Introduction of PhuHuu eco-tourism

PhuHuueco-tourismis located on Provincial Road 925, belonging to PhuNghia hamlet, PhuHuu commune, Chau Thanh district, HauGiang province. Being located on relatively flat plain, there are many ponds and ditches with an area of 11ha. The tourist area includes food service areas, homestay resort services, sightseeing landscapes, playing service areas (including children's games, strong-feeling games, water games, terrestrial games, folk games ...), traditional craft villages, picnic camps, spiritual areas, aquaculture and garden canal systems fruit trees for tourism, buying and selling agricultural products and goods for tourists. PhuHuu eco-tourism officially put into operation on December 18, 2018.

Fig.1: Location of PhuHuu ecotourism

2.2 Data collection

The study was conducted by interviewing 60 tourists and tourism management board to collect information including environmental management, application of legal documents, personnel arrangement and assessment, and evaluation of the interviewed tourists on the environmental management at the eco-tourism.

Based on the number of the locations of the waste binsin the tourist area, three waste bins was randomly selected at the end of the day, at 5 pmto detetermine the total weight and the waste composition. The composition of solid waste was obtained by the followingsteps: (1) Weigh the weight of the waste in the bin and then use the shovel to mix the wastes in the bin thoroughlt ; (2) Divide the wastes into four equal parts and take two opposite parts (A + C) or (B + D), then these two selected parts werewell mixed by shovel; (3) repeat the step (2); (4) the two opposite parts of the wastes in step 3 were collected for detemination of composition of solid waste. The wastes were then classified according to TCVN 6705: 2009 on ordinary solid waste-sorting. The collected data wasinput and processed by Microsoft Excel software presented mainly as percentages through simple charts and tables.

III. RESULTS AND DISCUSSION

3.1 Sources of solid wastes

In the tourist area, solid waste mainly comes from business activities such as counters for drinking water and fast food; restaurant area; fruit garden; area for organizing outdoor activities. The survey showed that visitors visited most areas in the tourism area; However, the drinking water area and the restaurant area were the two locations with relatively higher number of visitors. Therefore, these two locations can be assessed as the places where most solid waste is generated. In addition, the amount of solid waste that can originate from outside since the visitors prepared their own food, but the quantity could be very small.

3.2 Solid waste composition

Results of interviews from officials and employees of the tourist area indicated that the average number of tourists visiting the area is approximate 300-400 guests/day, and the number of visitors can be up to 900-1000 guests/day on holidays and weekends. According to the report of the General Department of Environment (2008), the average rate of solid waste generation in the Mekong Delta region is 0.61 kg/person/day, if calculated according to this generation rate, the amount of waste at the study site is around 244 kg/day for normal days and 610 kg/day for holidays and weekends. Huong et al. (2018) and Long and Mai (2013) recorded at the tourist sites in HoaBinh and Binh Phuoc that the daily amount of solid waste was 201 kg/day and 261 kg/day; This difference could be explained due to the difference in the numbers of visitors.

The solid waste is measured by weighing the waste in three waste bins located at the stockpile (dimensions of each is Ø 600 x H 470 mm). The amount of solid wastes in the three waste bins (waste bin 1, 2, and 3) were 24 kg, 28.6 kg and 25.8 kg, respectively. The solid waste composition was presented in Table 1. According to a report on solid waste management research in Vietnam, JICA (2011) classified solid waste into two categories including inorganicsolid wastes such as stone, gravel, cement, glass, nylon and organic solid wastes such as food waste, vegetable and fruit wastes, agricultural wastes.

Table 1. Composition of solid wastes in the waste bins

No.		**Organic solid wastes**			**Inorganic solid wastes**		
		Compostion	Weight	%	Compostion	Weight	%
1	Waste bin 1	Rice and leftovers	4.7	19.58	Plastic cups and wrap	2.8	11.67
	Waste bin 2		5.8	20.28		4.2	14.69
	Waste bin 3		6.1	23.64		3.3	12.79
2	Waste bin 1	Vegetables	5.2	21.67	Plastic straws	1.1	4.58
	Waste bin 2		6.2	21.68		1.6	5.59
	Waste bin 3		5.2	20.16		1.5	5.81
3	Waste bin 1	Fruit wastes	4.8	20	Nylon	2.2	9.17
	Waste bin 2		3.5	12.24		1.9	6.64
	Waste bin 3		3.9	15.12		2	7.75
4	Waste bin 1	Leaves	2.3	9.58	Foam boxes	0.9	3.75
	Waste bin 2		3.9	13.64		1.5	5.24
	Waste bin 3		2.6	10.08		1.2	4.65

In waste bin 1, the major organic waste accounted for 70.83% of the total, with spoiled vegetables accounting for the highest of 5.2 kg (21.67%) and rice, uneaten food accounting for 19.58%. For inorganic components, plastic cups accounted for the highest (about 11.67%) compared to other similar components. The results of survey of waste bin 2 was similar to those recorded in waste bin 1, organic solid waste was approximate 19.4kg (accounting for 67.83%) and inorganic solid waste took 32.17% (9.2 kg). In waste bin 3, organic waste occupied a high proportion of 68.99% and very high inorganic waste took 12.79%. This survey result was similar to that of Dieu et al. (2013) on household waste in Ho Chi Minh City with organic solid waste accounting for 80.1 - 90%. In addition, Viet and Chiem (2013) also reported that the waste composition in the Mekong Delta is mainly composed of organic matters, accounting for 82.6%. In addition, when compared with other province (Ca Mau), the amount of solid waste generated an average of 1,028 tons/day (2014) with an organic content of 70-75% is also leftovers, vegetables and fruit peels (Ca Mau People's Committee, 2018).

Table 2.The mean proportion of solid wastes compostition in the study area

TT	Organic solid wastes (18,06kg accounted for 69,14%)			Inorganic solid wastes (8,06 kg accounted for30,86%)		
	Compostion	Weight	%	Compostion	Weight	%
1	Rice and leftovers	5,51	21,09	Plastic cups and wraps	3,43	13,13
2	Vegetables	5,55	21,25	Plastic straws	1,4	5,36
3	Fruit wastes	4,07	15,58	Nylon	2,03	7,77
4	Leaves	2,93	11,22	Foam boxes	1,2	4,59

The results of the survey on average waste composition showed that organic solid waste accounted for 69%, of which uneaten food (21.09%); vegetables, fruits (21.25%), leaves (11.22%) and fruit peels (15.58%). These types of solid wasteswere sourced from restaurants and partly from fast food stalls, which are easily biodegradable in nature, the decomposition process creating unpleasant odors especially in hot and humid weather. 31% of inorganic solid wastes includes plastic wrap, plastic cups, plastic straws, and foam boxes (Table 2).

Results of weighing and sorting wastes at the gathering place of tourist area, the amount of solid wastes generated for weekdays was approximate 70-100 kg/day and 200 - 300 kg/day for holidays and weekends. According to the calculated results, the rate of generation of solid wastes of the tourist area was approximate 0.3 kg/person/day. Compared to a study by the General Department of Environment (2008), the rate of generation of solid waste in the tourism area is lower, this may be due to the fact that visitors only stayed for short period of time (an average of 1 to 2 hours).

3.3 Awareness of solid waste management of visitors at PhuHuu tourist site

3.3.1 Gender and age

The interview results showed that the proportion of female accounted for 55% (33/60 people), male accounted for 45% (27/60 people). The age of the respondents in PhuHuu tourist area between the ages of 18 to 30 years old accounts for 73.33% (44/60), the percentage of people aged 31 to 50 accounted for 23.33% (14/60), the proportion of people> 50 years old who took part in the interview was 2 people, accounting for 3.33%. The above survey results showed that the people at ages of 18-30 years old tend to travel more than the other age groups.

3.3.2Education and occupation

The majority of interviewers are qualified from high school and university. Visitors with high school level accounted for the highest proportion with 41%, followed by the university level with 32%. The number of people with college and intermediate degrees is relatively low, the proportion of colleges accounts for 20% and intermediate accounts for 7%. The interview results showed that the educational level of the interviewees in the tourism area is relatively high.

In the tourist survey, visitors varied in occupations including office workers, business, housewives, workers, farmers and even students. Their main occupation is office workers accounting for 46.67%, this is a job with relatively high pressure so most office workers tend to relax by traveling. For those who have a freelance business, the proportion of traveling is also relatively high at 23.33%, with freelance work often not limited to working time, and high financial capacity. These are also people with relatively high qualifications, often traveling to many places, so they can also have an overview of life, including material and spiritual life, in which the spiritual life plays a part. The games in the tourist area are very important. Students also go on tours but this percentage is relatively low at only 13.33% which could be due to limited in financial capacity. Workers and farmers are the least traveled with workers only 6.67% and farmers 3.33%; For workers, the rest time is relatively short except for big holidays. As for farmers, most of them are not limited by time but very few travelingand this could be because financial problem.

3.3.3Awareness of environmental protection of the tourists

The awareness of environmental protection of the tourists in the study and the level of practice on environmental protection are also high. When asked how to use food (drinking water and fast food) at the counter or

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take away and after using the packaging, how the waste was handled and the results revealed that 100% of tourists put waste in the waste bins in accordance with the regulations. There were 100% of the tourists understood that it is harmful when indiscriminately discharging garbage into the environment. However, there were many different answers on the pollution of environmental compartments. For examples, 33.33% of respondents said that waste would pollute soil, 18.33% said it would deteriorate water environment, 45% supposed it would destroy fresh air while 3.33% said it would pollute soil, water and air. The study also recored that 100% of the respondents totally agree that solid waste affects human health and causes diseases. There were 86.67% of interviewed visitors agree to pay the additional charge ranging from 1,000 to 2,000 VND to the entry tickets for improving waste management systems. Regarding environmental management in the tourist area, 85% of tourists recommended to put extra waste bins in the tourist area.

3.4 Current status of solid waste management in PhuHuu tourist area

34.1Collection of solid waste

According to the results of interviews with tourists about the collection and cleaning in the tourist area, up to 52/60 people (86.67%) said that they did not see the cleaning staff. In addition, over 80% of the respondents said that the placement of the waste bins and the size of the bins were not reasonable. However, according to the survey of solid waste management staff, it was said that there were many trash bins around the aisles in the tourist area in the past but the high amount of organic matters had created environmental problem, especially the bad odors from the waste bins. This led to the reduction of the waste bins. Since then the waste bins only installed at the drinks and fast food counters. Solid waste collection in the resort is arranged with five employees, with frequency 1 time/day. Everyday in the morning from 6 am to 9 am the whole tourist area is cleanedand all the solid wastes were collected and placed at the designed place. In addition, for food and drink counters with waste bins arranged at the point of sale, the owners of these saleswould be responsible for cleaning and managing the solid wastes.

3.4.2Current status of solid waste classification

Classification is the important step in solid waste management. The purpose of solid waste classification is to separate the components according to their physical, chemical or biological characteristics to reuse, recycle or reprocessing. The survey results showed that the classification of solid waste at the tourist site has not been implemented, particularly in the tourist area, only one type of bin is installed for all types of wastes.

3.4.3 Current status of solid waste storage

After being collected, solid wastes were transported to the stockpile in the tourist site. The storage is arranged by the Tourism Management Board at the area away from the sightseeing and dining areas, with an area of about 2 m^2. At the storage site, solid waste is stored in the10 containers with dimensions of Ø 600 x H 470 mm. The survey revealed that the storage of solid waste in the tourist area is not hygienic, in particular the containers do not have lids, creating an unpleasant odor. Odors are formed because solid waste is stored for long, creating a special odor that increases sharply on high temperature days, due to anaerobic decomposition of organic matters.

3.4.4 Current status of solid waste transport and treatment

Through interviews with officials and employees of the Tourism Management Board, the tourist area has signed a contract with the Water Supply and Sewerage Joint Stock Company - Hau Giang Urban Works, Chau Thanh Branch to collect and treat solid wastes. The company is responsible for collection of the wastes at the gathering point of the tourist area so the collection rate reaches 100%, with the frequency of collection is once a day in the time frame from 17:30 to 18:00.

3.5 Evaluation of solid waste management

The issue of environmental sanitation at the tourist site has been concerned by the Tourism Management Board and is fully aware of the importance of environmental protection. In the study area, solid waste collection has been carried out; However, it is only carried out at a good level with the frequency of collection is not high and not reasonable. Besides, the arrangement of less waste bins also affects the collection since a few tourists could throw wastes indiscriminately. The classification of solid waste in the tourist area has not been implemented, only one type of waste bin is allocated to all types of wastes. Regarding the storage of solid waste at the stockpile area, although there has been a land area as well as the containers to store wastes, this storage area has not been hygienic because the containers do not have lids which could result in generating bad odors.

3.6 Proposing solutions for solid waste management in Phu Huu tourist area

In order to better management of solid wastes at the study site, the managers should improve the organization of collection and sanitation by the allocation of more staff for increasing the frequency of cleaning. In addition, the managers should minimize by imposing extra charge for the food and items brought in from outside. Promoting the propaganda, education to raise awareness and responsibility of environmental protection of the visitor community is necessary. Diversify the forms of propaganda and dissemination of tourism regulations to officials, employees and tourists regarding solid waste management is urgently needed.

Furthermore, the solid waste generated in the tourism area should be sorted at source, possibly based on the 3R principle (reduce, reuse and recycle). For organic solid waste that has leftovers, spoiled vegetables can be put in a covered container and then given to surrounding households as pet food, which can minimize the amount of organic solid waste generated in tourist areas, limiting odors. For plastic cups and plastic bottles, the users should pay for extra cost included in the tickets so the amount of the wastes could be efficiently reduced.

IV. CONCLUSION

The results of solid waste analysis in the tourist area showed that the amount of waste generated was 70-100 kg/day on weekdays and 200-300 kg/day for weekends/holidays. The rate of generation of solid waste in the tourist area averaged at 0.3 kg/person/day. Through practical surveys and analysis, the solid waste composition in the study area includedtwo types of inorganic wastes accounting for 31% comprising plastic wrap, plastic cups, plastic straws, foam boxes and organic waste occupied 69% including leftovers, vegetables, fruits, leaves, fruit peels. Current situation of solid waste management in tourism area was limited showing through the waste is not classified, waste bins and its size were not appropriate; the waste bins were not covered. The managers of the tourism area need need to fix all the existing problems including allocation of more staff working on solid waste collection, waste separation, and allocation of more waste bins with the appropriate covers. Extra fees should be charged on the food and stuff taken from outside, and plastic stuffs. Raising the awareness of environmental protection for visitors and staffs in the tourist area is essential.

REFERENCES

[1] Bui Huu Long and Phi Phuong Mai (2013). Assess the impact of ecotourism development on environmental components at My Le Ecological Tourism Area. Ho Chi Minh City University of Agriculture and Forestry.

[2] Jindal, R., Harada and Shikura, S. (1998). Solid waste management in several Asian countries. Review of environmental systems 42/43. Bangkok, Thailand: Information Center on Environmental Systems (ENVSIC), Asian Institute of Technology.

[3] Le Hoang Viet, Nguyen Huu Chiem (2013), Curriculum on solid waste management and treatment. Can Tho University Publishing House.

[4] Suarnatha, I.M. (1990). Hotel solid waste management. Case study of alternative model of Jimbarau, Bali, Indonesia.

[5] General Department of Environmental Protection (2008). Project of Modeling and pilot implementation of classification, collection and treatment of daily-life waste for new urban areas.

[6] Tran Hieu Nhue and Nguyen Quoc Cong (2005). Research and study on organic waste management of Bai Chay tourist area, Tp. Ha Long. Culture and Information Publishing House, Hanoi.

[7] Tran Thi Huong (2018). Assessing the impact of ecotourism activities on the natural and social environment in Ban Lac, Chieng Chau commune, Mai Chau district, Hoa Binh province. Journal of Forestry Science and Technology.

[8] Tran Thi My Dieu, Le Minh Truong and Nguyen Trung Viet (2013). Composition, volume of domestic solid waste from households and the ability to recover and recycle: Case study in District 1, Ho Chi Minh City Chi Minh.

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Touchdown treatment of root trainer shortened the formation time of root mass of rubber tree seedlings

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***Abstract*— *Root trainers are increasingly being used to avoid root coiling and distortion and lack of wind tolerance after field transplanting, when compared to the conventional polybag planting system. Root tips grew out of root trainer forming an intact root mass and then could be transplanted to the field with high survival rate. While the influence of spatial position on root tips growing out of budded stump root trainer was still unknown at nursery stage. Therefore, the bottoms of root trainer were placed in aboveground(20cm, current practice), touchdown(0cm) and underground(15cm) for budded stump nursery, respectively. Clone CATAS 7-20-59 budded stump was raised in root trainer and percentage of plants with root tips outgrowing, growth performance, leaf N content, leaf SPAD, leaf temperature and leaf surface humility were recorded, respectively. The results showed that root trainer touchdown of budded stump gave more percentage in root tips growing out of root trainer, more leaf N content(P <0.01), more leaf SPAD value (P <0.01)and higher leaf surface humility(P <0.01) when compared to underground and aboveground treatments. Thus, touchdown was the optimal choice for rubber budded stump root trainer nursery.*

Keywords*— *Hevea brasiliensis, budded stump, root trainer, touchdown, SPAD value.

I. INTRODUCTION

High-quality seedlings of *Hevea* are an important prerequisite for raising good plantations and enhancing the productivity. Root trainers are increasingly being used to avoid coiling and spiraling of roots and improve proliferation of lateral roots and ultimately survival rate and productivity of plants, and three month old plants raised in root trainers showed better growth than those raised in polybags(Soman and Saraswathyamma, 1999). The performance of root trainer plants (raised by planting budded stump) and direct-seeded green-budded polybags were comparable during the initial four years after transplanting in the field, but considering the practical convenience and cost involved, root trainer one-whorl plants appeared to be the ideal planting material for commercial planting of *Hevea*(Sherin et al., 2013). Root trainer plants of *Hevea* had only 55 percent less biomass than polybag plants due to the limited space(Sumesh et al., 2015). Root trainer is easy for transportation and transplant saving time and cost for rubber planter. The growth performance of rubber mini-budding raised in polytube was significantly related to the ground clearance(Zhou et al., 2015). Our previous nursery experience showed that root tips grew out of root trainer forming an intact root mass and then rubber budding raised in root trainer could be transplanted to the field with high survival rate(Fig.1), which was a standard rule for rubber root trainer for transplanting. However, the influence of ground clearance on root tips growing out of budded stump root trainer was still unknown.

Soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) is easy to operate, reliable, fairly stable and low cost, and widely used in many crops. SPAD value is positive correlation with chlorophyll content, which can be used for evaluation index instead of chlorophyll content when conducting elimination selection for germplasm sources of *Hevea brasiliensis*(Li et al., 2017). SPAD value also is suggested to divide growth phenology of *Hevea brasiliensis*(Zhang et al., 2019). For rubber root trainer nursery, shortening the time of root mass formation and improving efficiency is vital. Therefore, the present study was carried out to assess the influence of spatial position on root tips growing out of root trainer and growth of budded stump root trainer.

Fig.1: Root mass of budded stump in root trainer

II. MATERIAL AND METHODS

The experiment was conducted from October 2018 to June 2020 in the nursery base of natural rubber of Rubber Research Institute of Chinese Academy of Tropical Agricultural Sciences, Danzhou City, Hainan Province, China. Clone GT1 seeds were sown in sand bed for germination and about 20-25 days later the GT1 seedlings as rootstock were transplanted in ground nursery for budded stump nursery and meanwhile root trainer for seedling nursery. The containers are tapering polypropylene cups with a drainage hole at the bottom(top diameter 8cm*height 37cm*bottom diameter 2cm). The budded scion was clone CATAS 7-20-59. After budding successfully, they were raised in root trainer with pure coconut bran. The nursery of budded stump was according to the conventional practices. At nursery stage, the bottom of root trainer were placed in aboveground and touchdown for seedling nursery. While the bottom of root trainer for budded stump nursery were placed in aboveground(20cm, current practice), touchdown(0cm) and underground(15cm), respectively(Fig2.). Each treatment had three replications, and each replication contained 100 plants. At 2-3 mature leaf whorls stage of rubber seedling, rate of root tips growing out of root trainer at two spatial positions(aboveground and touchdown) were measured, respectively. At one stable leaf whorl stage of budded stump, non-sprouting rate, diameter, plant height and rate of root tips growing out of root trainer at three spatial positions(aboveground, touchdown and underground) were recorded, respectively. At one mature leaf whorl stage, a Portable Chlorophyll Meter(CY-YL04, China) was used for detecting chlorophyll content(SPAD value), nitrogen content, leaf temperature and leaf surface humidity of plants during 11:00-11:50 and each treatment contained 30 plants. Statistical analyses were performed with data processing system (DPS) statistical software package version 16.5 using one-way ANOVA followed by the Duncan's Multiple Range Test (SSR) to evaluate significant difference among different spatial position of budded stump root trainer. All data were shown in the mean ± SD of three biological replicates(each replication contained 100 plants for growth performance and 30 plants for Portable Chlorophyll Meter).

Fig.2: Spatial position of root trainer and root tips of budded stump

III. RESULT AND DISCUSSION

Spatial position on percentage of root tips growing out of root trainer As observed in Fig.3A, mini-seedling budding raised in root trainer, showed 80.0% root tips growing out of root trainer at touchdown and 23.3% root tips growing out of root trainer at aboveground. While seedling raised in root trainer, recorded 72.6% root tips growing out of root trainer at touchdown and 4.8% root tips growing out of root trainer at aboveground. To further investigate the effects of spatial position of root trainer on root tips, budded stump was transplanted in root trainer at underground, touchdown and aboveground (Fig.2), respectively. Seventy-six days after transplanting, root tips of root trainer were checked at stable leaf stage(Fig.3B). Bud non-sprout percentage of budded stump root trainer were 15.7% at aboveground, 4.7% at touchdown, and 7.0% at underground, respectively. The percentage of root-tips non-growing out of root trainer were 53.7% at aboveground, 44.0% at touchdown, and 42.7% at underground, respectively. The percentage of root-tips growing out of root trainer were 30.7% at aboveground, 51.3% at touchdown, and 50.3% at underground, respectively. In the present observation performed on different types of rubber seedling, the treatment of root trainer touchdown gave more percentage for root tips growing out of root trainer compared to underground and aboveground treatments.

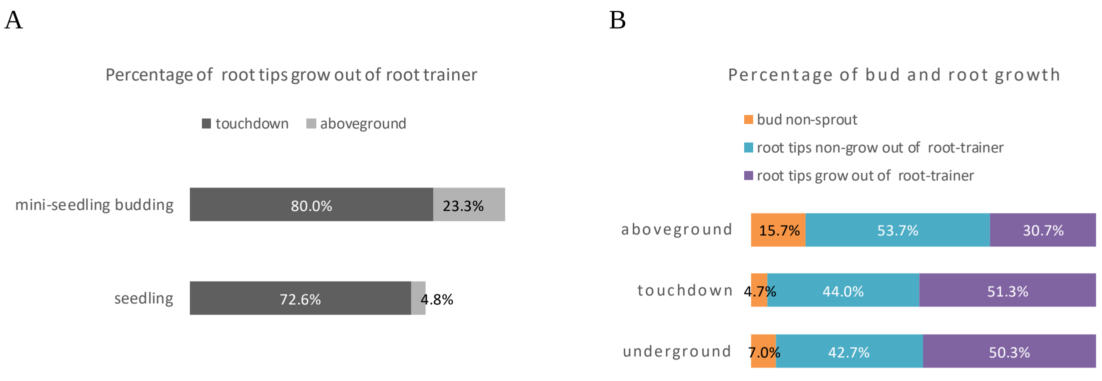

Fig. 3: Percentage of plants with root tips out growing of root trainer at two spatial positions(A) and three spatial positions(B)

Spatial position of root trainer on growth of rubber budded stump At one mature leaf whorl stage of budded stump, rootstock diameter, scion diameter and scion height were recorded to check spatial position of root trainer (aboveground, touchdown and underground) on growth performance. There was no significant difference in rootstock diameter and scion diameter among three spatial positions(Fig.4A, B). While scion height of budded stump root trainer at underground was 5.67% (P <0.05) and 6.51% (P <0.05) less than that at touchdown and at above ground (Fig.4C), respectively. There was no significant difference in scion height between touchdown and aboveground. Normally, the first leaf whorl growth of budded stump root trainer was provided by rootstock nutrient, and thus the rootstocks at three spatial positions gave no significant difference, which had no significant difference in scion diameter at the same scion clone. However, scion height of budded stump root trainer at underground was significantly lower than that at other two spatial positions(same plant density), which might induced by plant density for struggling for sunlight(Fig.2) at the same shade.

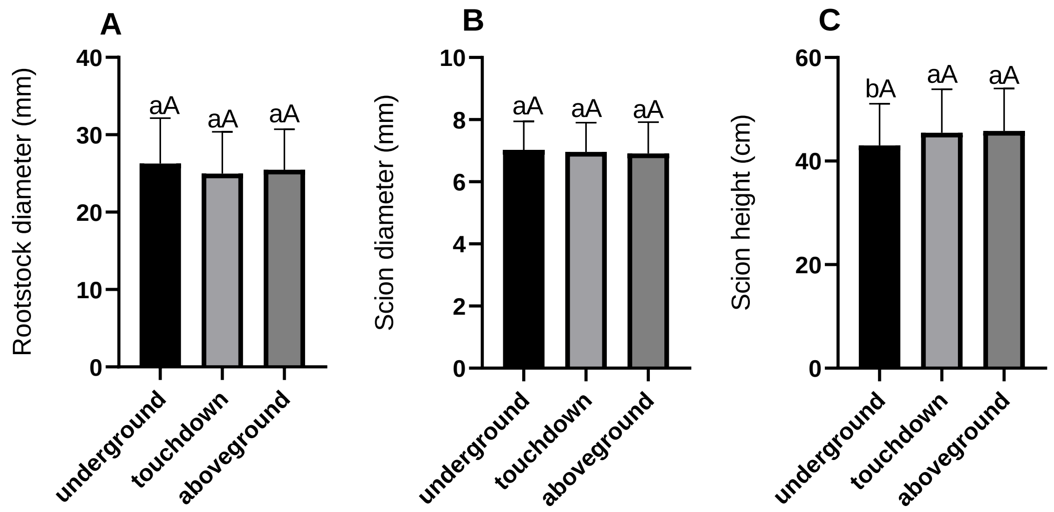

Fig. 4: Spatial position of root trainer on growth of budded stump

Spatial position of root trainer on N content and SPAD value of budded stump As shown in Fig.5A, leaf N content of budded stump root trainer at aboveground was 2.45% (P <0.01) and 5.20% (P <0.01) less than those at underground and at touchdown, respectively. Leaf N content of budded stump root trainer at underground was 2.60% (P <0.01) less than that at touchdown. Similarly, SPAD value was consistent with leaf N content in the present study. As shown in Fig.5 B, SPAD value of budded stump root trainer at aboveground was 3.02%(P <0.01) and 6.29%(P <0.01) less than those at underground and at touchdown, respectively. SPAD value of budded stump root trainer at underground was 3.18%(P <0.01) less than that at touchdown. Comparing with the above results of rootstock and scion growth, there was no significant difference among three spatial positions, but the treatment of root trainer aboveground gave less percentage for root tips growing out of root trainer, which might account for absorbing nutrient from the soil at underground and at touchdown, and thus might cause leaf gave more N content and SPAD value. Chlorophyll meter can estimate leaf chlorophyll content as a surrogate measure of the nitrogen (N) status of plants to assess the N requirements of crops and increase nitrogen-use efficiency(Maiti et al., 2004). Moreover, there was a consistent strong and positive correlation SPAD value and net photosynthetic rate(Pn) and thus could be used as surrogate trait for improved Pn under drought conditions(Puangbut et al., 2017; Songsri et al., 2009). Therefore, in the present study, touchdown treatment of root trainer made budded stump grew well than other two spatial positions.

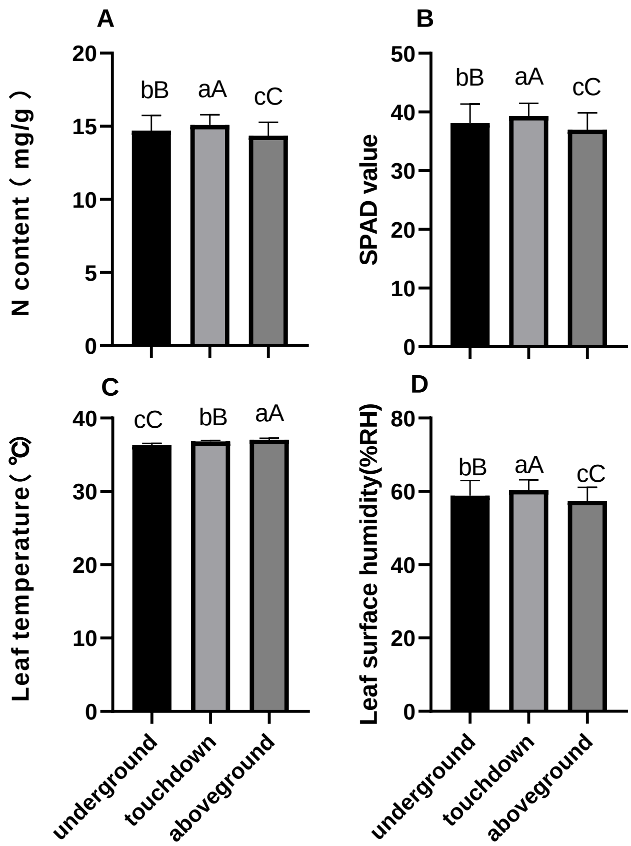

Fig. 5: Spatial position of root trainer on leaf N content, SPAD value, leaf temperature and leaf humidity of budded stump

Spatial position of root trainer on leaf temperature and leaf surface humidity of budded stump As shown in Fig.5C, leaf temperature of budded stump root trainer at aboveground was 1.98%(P <0.01) and 0.59%(P <0.01) higher than those at underground and at touchdown, respectively. Leaf temperature of budded stump root trainer at touchdown was 1.41%(P <0.01) higher than that at underground. On the contrary, as shown in Fig.5D, leaf surface humidity of budded stump root trainer at aboveground was 2.45%(P <0.01) and 5.12%(P <0.01) less than those at underground and at touchdown, respectively. Leaf surface humidity of budded stump root trainer at underground was 2.54%(P <0.01) less than that at touchdown. These results showed that spatial position of root trainer had significant effect on budded stump.

Correlation analysis among N content, SPAD value, leaf temperature and leaf surface humidity As mentioned in Table 1, SPAD value was significantly correlated with N content and leaf surface humility (P<0.001), irrespective of treatments. N content was also significantly correlated with leaf surface humility (P<0.001) ,whatever treatments. Leaf temperature was correlated with SPAD value, N content and leaf surface humility (P<0.05) at touchdown treatment. While leaf temperature was not correlated with SPAD value, N content and leaf surface humility (P>0.05) at underground and aboveground treatments, respectively. Plant water status must be taken into account to precisely monitor crop SPAD value and N needs with Chlorophyll Meter(Martínez and Guiamet, 2004; Nigam and Aruna, 2008). Chlorophyll content and SPAD value were reported to be positively correlated with drought resistance in potato (Zhang, 2007) and in *Hevea brasiliensis*(Yin et al., 2017). In the present study, leaf surface humility was significantly correlated with SPAD value and N content (P<0.001), which was consistent with previous study. Moreover, leaf temperature correlated with SPAD value, N content and leaf surface humility for touchdown treatment was obvious, respectively.

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Table 1 Correlation analysis among SPAD value, N content, leaf temperature and leaf surface humidity of budded stump

Treatments	**Factors**	**SPAD value**	**N content**	**Leaf surface humidity**	**Leaf temperature**
Underground	**SPAD value**		0	0	0.6619
	N content	0.9996		0	0.6823
	Leaf surface humidity	0.9996	1		0.6823
	Leaf temperature	0.051	0.0477	0.0477	
Touchdown	**SPAD value**		0	0	0.0215
	N content	0.9992		0	0.0205
	Leaf surface humidity	0.9992	1		0.0205
	Leaf temperature	0.2298	0.2315	0.2315	
Aboveground	**SPAD value**		0	0	0.1189
	N content	0.9995		0	0.1171
	Leaf surface humidity	0.9995	1		0.1171
	Leaf temperature	0.131	0.1316	0.1316	

Notes: The lower left corner is the correlation coefficient r and the upper right corner is the p-value.

IV. CONCLUSION

Root trainer touchdown of budded stump gave more percentage in root tips growing out of root trainer, more leaf N content, more leaf SPAD value and higher leaf surface humility when compared to underground and aboveground treatments. Thus, touchdown was the optimal choice for rubber budded stump root trainer nursery.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Li, X., F. Zhang, Y. Hu, Y. Qiu, C. Mao, T. Yang, S. Ni, and Y. Wu, 2017: Spatial Distribution Characteristics of Chlorophyll on Different Leaf Positions in Hevea brasiliensis. Journal of Northwest Forestry University 32, 52-58.

[2] Maiti, D., D.K. Das, T. Karak, and M. Banerjee, 2004: Management of nitrogen through the use of leaf color chart (LCC) and soil plant analysis development (SPAD) or chlorophyll meter in rice under irrigated ecosystem. The Scientific World 4, 838-846.

[3] Martínez, D.E., and Guiamet J.J., 2004: Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie 24, 41–46.

[4] Nigam, S.N., and R. Aruna, 2008: Stability of soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) and their association across varying soil moisture stress conditions in groundnut (*Arachis hypogaea L*.). Euphytica 160, 111–117.

[5] Puangbut, D., S. Jogloy, and N. Vorasoot, 2017: Association of photosynthetic traits with water use efficiency and SPAD chlorophyll meter reading of Jerusalem artichoke under drought conditions. Agricultural Water Manage 188, 29-35.

[6] Sherin, G., I. Sabu P., S. T. A., and S. V. K., 2013: Field performance of polybag and root trainer rubber plants at different stages of growth. Rubber Science 26.

[7] Soman, T.A., and C.K. Saraswathyamma, 1999: Root trainer nursery for Hevea. Indian Journal of Natural Rubber Research 12, 17-22.

[8] Songsri, P., S. Jogloy, C.C. Holbrook, T. Kesmala, N. Vorasoot, C. Akkasaeng, and A. Patanothai, 2009: Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. Agricultural Water Manage 96, 790-798.

[9] Sumesh, K.V., S.K. Soman, K. Annamalalainathan, and J. Jacob, 2015: Physiological comparison of root trainer and polybag plants of *Hevea brasiliensis.* Journal of Plantation Crops 43, 204-211.

[10] Yin, Z., X. Li, F. Zhang, C. Mao, and Y. Wu, 2017: The identification of *Hevea brasiliensis* clones to drought resistance by conductivity combined with leaf SPAD value. Tropical Agricultural Science and Technology 40, 6-9+13.

[11] Zhang, F., X. Li, C. Mao, Y. Wu, and S. Ni, 2019: Dynamic Changes of SPAD Value and Color of *Hevea brasiliensis* Leaves during Different Growth and Development Periods. Journal of Northwest Forestry University 34, 83-90.

[12] Zhang, W., 2007: Relations between chloropyll content, CAT activity of potato and drought resistance of variety. Research of Agricultural Modenization, 622-624.
[13] Zhou, J., J. Wang, W. Lin, and M. Cai, 2015: Effect of different suspension culture height on rubber mini-polytube budding growth. Chinese Journal of Tropical Agriculture 35, 1-3+8.

Effects of Dehaulming in Potato (*Solanum tuberosum*) Cultivation: A Review

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***Abstract*— *Potato is one of the most important commercial crops worldwide covering 20 million hectares cropping area. Series of cultivation practices are performed in potato cultivation where dehaulming is considered one of the prime-factors that affect the quality and size of tubers. It is also considered as a key factor in production of healthy potato seed in Seed plot technique methods. Dehaulming practice includes the act of detaching and defoliating the vegetative part lying above the ground of potato plant from the underground tuber. Effect of dehaulming is significantly found in the yield of seed tuber, the post-harvest quality of potato tuber and the disease, pest protection aspect of plants. The weight of tuber was found to be 384.20 g when haulm was cut at 65 days after planting (DAP), then significant increase in weight was found i.e. 533.00 g when the haulm was cut at 80 DAP. And the maximum seed yield was observed when dehaulming was done at 70 DAP i.e. 19.75 t/ha and similar to the non-seed yield. The post-harvest quality of tuber is also significantly improved when dehaulming was performed prior to harvesting. Mainly, the dehaulming practice when performed at 65 DAP was found comparatively safer from infestation and viral disease transmission through the sap sucking pest.*

***Keywords*— Potato, Dehaulming, Tuber, Yield, Post harvest.**

I. INTRODUCTION

Potato is one of most important commercial crops world-wide. With a total cropping area of about 20 million hectares globally, the potato is the fourth most important staple crop after rice, wheat, and maize (Stef de Haan et al,2016). The primary center of origin and diversity of potato crop is found widely prevalent in western region of South America (Hawkes,1990). The first domestication of potato was found to be in southern Peru and northwestern Bolivia between 4000 to 8000 BC. The gradual evolution of the diversity of potato in the farmer's hands is foretell to allow its higher adaptation to climate change and the increasing food security on the immoderate agroecology (John and Keen 1986; Zimmer, 2014).

Solanum tuberosum plant is herbaceous in nature which has height upto 0.4-1.4 m tall and it has prostate to semi erect structure of plant (Spooner and Knapp, 2013). Stem may be hairless to densely hairy with purple green or mottled green in color. It has a single bear terminal leaflet with three to four large ovoid leaves (Spooner and Knapp 2013; Struik,2007). The flowering pattern usually starts from branches near to the base of the plant and then proceeds upward. The flower remains open for 2 to 4 days, where the receptive stigma and pollen are produced for around 2 days (Plaisted,1980). The storage organ of *Solanum tuberosum* is tuber that is developed from the swollen underground stem, consisting of several eyes on tuber which are called buds that have potential to sprout and develop into new stem (Hoopes and Plaisted 1987). The tuber formation is much favored in short days. Among the entire cultivation practice dehaulming is considered one of the major practices that determines the qualitative and quantitative character of potato.

Dehaulming is the practice in which aerial parts of a plant are removed 10-15 days before harvesting ("Dehaulming in potato", 2018). Dehaulming can be done after the yellowing of aerial parts because yellowing of the vine indicates the maturity of the potato (Lutaldio et al, 2009). Timing of dehaulming varies according to varieties and in general, the varieties that are not disease resistant are dehaulmed earlier than the disease resistant varieties (Virtanen et al.,2014).

Generally, there are two methods of dehaulming and they are:

Mechanical methods:

- **Haulm cutting**: In this method, aerial parts are cut with sharp objects. Manual cutting takes around 40 man-hours per hectare and hence advanced haulm cutters are developed to increase field efficiency and cutting efficiency (Titiwa et al, 2019).
- **Haulm pulling**: Haulms are pulled instead of cutting in this method. Manual pulling is a time and labor consuming process. This method is most effective as it does not allow regrowth of foliage (Misener & Everett 1981; Halderson et al., 1988). There were more fragments of vines in harvested tubers and also tubers' exposure to light was higher in case of haulm pulling method (Halderson et al, 1988).

Chemical method: Through this method, various chemicals are sprayed in foliage, which leads to death of plants. Compared to other methods, it is quite slower ("Seed Potato Dehaulming Methods", 2019). Among various chemicals like sulfuric acid, dinoseb, diquat, endothal, etc., sulfuric acid showed the quickest effect with 42% of desiccation at day one after application (Haderlie et al, 1989). Compared to Glufosinate-ammonium, diquat had quicker effect on stem and leaf desiccation in 3 and 7 DAT, and after 14 DAT, both had same effect on leaf desiccation but stem desiccation was higher in case of diquat (Ivany, 2001). Chemical that is applied for haulm cutting also kills weed and thus this method has double advantage ("Vine kill: Method and timing", 2019).

Regrowth of foliage was observed upto 24% annually when only mechanical methods were used and little regrowth was observed even in chemical methods (Virtanen et al., 2014). So, it is better to use the combination of both mechanical and chemical methods for the most effective haulm destruction and shorter tuber maturation process (Zotarelli et al, 2019).

II. EFFECTS OF DEHAULMING

2.1. Effect of dehaulming on yield

2.1.1.Effect on tuber yield:

The yield and the starch content of tuber is found to have lower in content when the haulm cutting was done before the natural senescence of the plant at an early stage (Struik and Wiersema, 1999). The haulm cutting practice when performed at an early stage where there is low starch content in tuber will directly affect the vitality of tuber to generate new sprouts and roots for consecutive growing seasons. (Sabba et al, 2007). Basically, on a fresh weight basis the tuber growth is almost found to have occurred till 50 days so the tuber per hill parameter is less related to the dehaulming practice. The general tuber per hill number ranges from 12.08 to 15.06 (Beukama and Zaag, 1990). However, the tuber weight per hill increases gradually. According to the research done in Bangladesh, weight of tuber was found to be 384.20 g when haulm was cut at 65 DAP, then significant increase in weight was found i.e. 533.00 g when the haulm was cut at 80 DAP. However, the result of haulm cutting at 75 and 80 DAP gave statistically similar results i.e. 505.60g on an average. So according to the data the yield obtained was 28.02t/ha in haulm cutting at 75 DAP which is almost similar it was 26.02 tons for 70 DAP and the least was for 65 DAP (21.30 t/ha) (Mahumad et al, 2009). So on average a 10-14 days gap between the plant harvest and the dehaulming practice is suitable for the periderm maturity (Waterer, 2007). Also, the 10-14 days period is adequate for skin setting of the tuber which will definitely determine the plant protection tuber quality and seed quality of the yields (Halderson et al, 1988).

However in the northern Europe production condition, prime factor to control the tuber size, haulm cutting is considered as an important practice since tuber growth in long- day condition of north Europe is found to be higher and quicker than normal (Temmerman et al, 2002). Hence haulm cutting is mostly done on strictly immature plants while they may still be flowering which results in unsynchronized timing between the haulm cutting and foliage senescence or maturation of potato tubers (Viraten et al, 2014). In the environment where there is a short growing season, haulm killing is considered as a means for early harvesting, building up the tuber skin before harvesting and obtaining a suitable size of tuber (Struik and Wiersema).

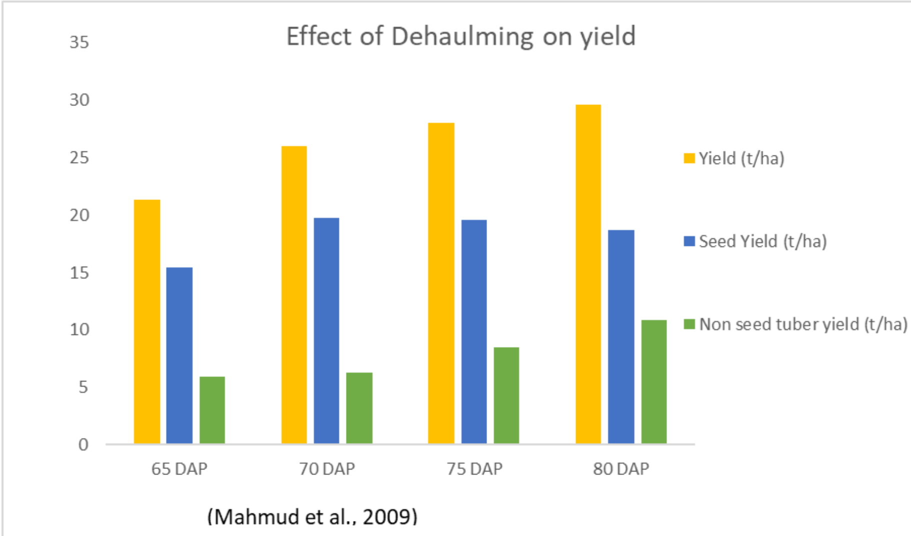

Fig 1: graphical representation of dehaulming effect on yield

2.1.2. Effect on seed production:

Quality of seed potato is primarily important in the aspect of yielding a crop and hence the seed production, harvesting (haulming and harvesting) and storage should be carefully carried out (Corrêa et al., 2007). When there is a favorable climatic condition, potatoes usually mature between the interval of 85 to 95 days after planting (Rashid, 1974; Ahmad 1977; Hussain, 1985). Maximum seed yield was observed when haulm cutting was done at 70 DAP i.e. 19.75 t/ha and similar to the non-seed yield. The yield data was statistically similar between haulm cutting at 75 and 80 DAP i.e. 19.56 t/ha and 18.69t/ha simultaneously. However, data as per the research conducted showed the poor seed yield when haulm was cut at 65 DAP i.e. 15.40t/ha. From the data, the maximum yield of non-seed tuber obtained from haulm cutting at 80 DAP was 10.89 t/ha which is equivalent to 37%. Also the ratio of seed and non-seed was found to be 1:0.318 for haul cutting at 70 DAP and the poor ratio was found for haulm cutting at 80 DAP that is 1:0.583) (Mahumad et al, 2009).

Dehaulming has significant effect on the emergence of seed potato.Those seed potato whose halumed were pulled out three weeks after flowering (75 days after planting) recorded emergence i.e 10.7 days however those seed potatoes whose halum were allowed to mature naturally until the harvest (95 DAP) or were pulled out during the flowering time (50DAP) recorded slow emergence i.e (11.6-12.6days) (Virtanen, E., et al, 2013).The root and stem bulk production is also influenced by the dehaulming where the physiologically older seed potato produced larger root and stem bulk at 95 days after planting and the seed potato whose haulm has been destroyed at the time of flowering, produced lower root and stem bulk (Virtanen, E., et al, 2013).

2.2. Effect of dehaulming on post-harvest and qualitative factors

Dehaulming hardens the potato tubers and increases the shelf life ("A Guide to Potato Production and Post Harvest Management in Kenya", 2013). After dehaulming, tubers are left in the field for 2-3 weeks for the skin to harden and such hardened skin reduces injuries during post harvest handling (Potato Vocabularies Explained, 9 Mar 2020). It generally takes 10-15 days for skin to set (Halderson et al, 1998 ; Virtanen et al, 2014). Respiration rate was minimum in tubers harvested after 90 days and dehaulmed before 10 days of harvesting (Mehta et al, 2003). Weight loss is one of the significant factors that determines the storability of potato tubers. Weight loss was seen minimum in potatoes harvested or lifted 9 days after dehaulming and maximum in potatoes harvested at 0 days after dehaulming (Nipa et al, 2013). However, starch content was found higher in the naturally senesced potato than in mechanically or chemically dehaulmed potato (Virtanen et al, 2014).

Dry matter content is considered a very important characteristic of potatoes when it is grown for industrial purposes. For processing potatoes into chips and French fries, dry matter content should be at least 20% (Gaur et al, 1999). Higher dry matter is associated with the crispiness

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and lower oil uptake by the fried products (Pope et al,1971; Mehta et al, 2003). Potato with lower dry matter is good for canning purpose (Kumar et al, 2013). There is a significant effect of dehaulming in dry matter content of tubers (Neenan,1965). Mean dry matter increased when dehaulming was delayed from 70 DAP to 80 DAP (Marwaha et al, 2012). Dry matter weight percentage of flesh of potato was found to be 20.55, 21.28, 22.31, 24.60 and 27.32% in the potato harvested after 12 days after dehaulming and 12.85, 13.52, 15.52, 16.55 and 18.81% harvested after 0 days after dehaulming at 0, 30, 60, 90 and 120 days after storage respectively (Nipa et al, 2013).

Potato should have reducing sugar less than 100mg/100gm for development of proper color in chips (Marwaha et al, 2005). When the amount of reducing sugar is high, it promotes Millard's reaction and results in darker chips and also in formation of a compound called acrylamide (Kumar et al,2013) which is a neurotoxin and a carcinogen (Vainio,2003 ; Bethke et al, 2010). When dehaulming was delayed from 70 to 90 DAP, there was significant reduction in the reducing sugar level, with increase in quality of tuber (Marwaha et al, 2012).

Phenolic compounds are generally found in the skin of potatoes and major compounds are phenolic acids and flavonoids (Akyol, Hazal, et al, 2016). Such compounds cause enzymatic discoloration after cutting or peeling (Marwaha et al, 2010). Total phenol content is seen lower when dehaulming is done after 70 DAP and highest when done after 90 DAP (Marwaha et al, 2012).

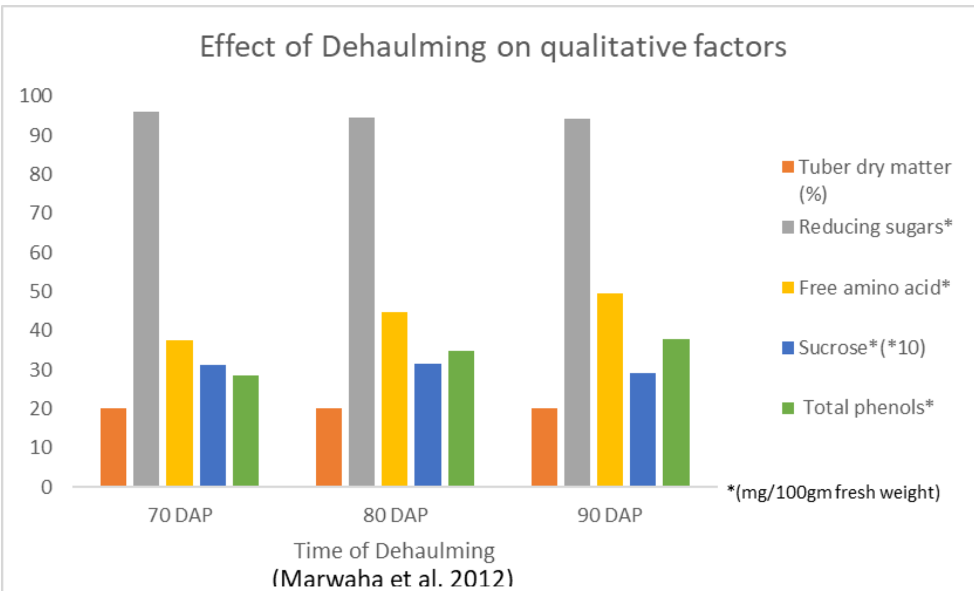

Fig.2: Graphical representation of dehaulming effect on qualitative factors

2.3. Effect on plant protection:

Different genera of different soil borne seed borne pathogen and many bacteria and fungus like *Rhizoctonia solani* (black scurf), *Phytophthora infestans* (late blight), *Phoma foveata* (gangrene) and *Verticillium dahlie* (Verticillium wilt) as well as bacterial diseases are controlled through the practice of haulm killing (Kempenaar & Struik 2007).

Several researches have been conducted to maximize benefits of haulm killing and minimizing soil transmitted and seed borne disease in seed potato production (Virtanen et al, 2014). However some of the haulm killing methods have been found to increase the plant disease incidence (Kempenaar et al, 2008; Dijst , 1998). Black scurf is most prevalent in case of mechanical- chemical dehaulming practices (Virtanen et al, 2014). Cutting the stem along with chemical haulm killing aided with the extended period of time between dehaulming and plant harvesting increases the incidence of black scurf disease (Dijst, 1988). Also, Dijst explained that sclerosis formed on the surface of tubers due to the formation of water- insoluble components will ultimately make tubers more prone towards the disease. Immature tubers are more prone towards microbial infection and their resistance gradually increases with maturity (Hide and Lap wood, 1992). Pathogens can attack foliage, root systems and tubers, therefore disease and its control can be important throughout the crop cycle. Majorly, the dehaulming practice when performed at 65 DAP was found comparatively safer from infestation and viral disease transmission through the sap sucking pest (Mandal et al, 2020). According to the study conducted in New Alluvial Zone of West Bengal, it was concluded that for quality seed production and less disease pest incidence use of 50cm * 15 cm spacing along with haulm cutting at 65 DAP, while planting has to be done in the first week of November (Mandal et al, 2020).

Also, research has found that the incidence of aphids as a vector which carries potato leaf roll virus and the potato tuber moth infestation is controlled by dehaulming and plant part before harvesting. Generally, dehaulming is done in crop immediately when the aphids cross the critical limit of 20 aphids per 100 compound leaves (Awasthi LP, 2017). Also, some research has explained that the viral incidence was 1% which is regarded to be within permissible limit when the haulm cutting was done by Dec 25 than after the aphid buildup (Awasthi LP, Verma HN, 2017). Thus, the maximum mitigation of crop damage from the leaf roll and severe mosaic was obtained when the seed crops were planted at the end of October and haulm were cut by date when the critical limit of vector is just surpassed (Khurana et al, 1997).

III. CONCLUSION

Potato has always been one of the most important foods. It can be consumed as a vegetable or can be processed into various types of products. There are many different cultivation practices and in this article, we discussed dehaulming and its effect on yield, post harvest and qualitative factors and in plant protection. Haulms are destroyed when vines turn yellow indicating the maturity of the plant. Haulm destruction is done by either cutting, pulling or chemical application. Proper dehaulming helps to increase the yield both in terms of tuber size and seed tuber and also minimize injuries during post harvest handling by thickening of skin. Reducing sugar, phenol content, dry matter, free amino acids, etc. determine the quality of potato in industrial level and dehaulming helps to maintain optimum level for these elements. In addition to that, it also plays a significant role in plant protection. Various pathogen carrying pests can be avoided by dehaulming at the right stage that will help to produce disease free tubers.

REFERENCES

[1] Akyol, Hazal, et al. “Phenolic Compounds in the Potato and Its Byproducts: An Overview.” *International Journal of Molecular Sciences*, vol. 17, no. 6, 2016, p. 835., doi:10.3390/ijms17060835.

[2] Awasthi LP, Verma HN (2017) Current status of viral diseases of potato and their ecofriendly management -A critical review. Virol Res Rev 1: DOI: 10.15761/VRR.1000122

[3] Bethke, P.C., Busse, J.S. Vine-Kill Treatment and Harvest Date have Persistent Effects on Tuber Physiology. Am. J. Pot Res 87, 299–309 (2010). Retrieved from https://doi.org/10.1007/s12230-010-9137-4

[4] Beukema H.P. and V.D. Zaag de. (1990). Crop ecophysiology, In: Introduction to potato production. PUDOC, Wageningen, The Netherlands. pp. 42-60.International Year of the Potato 2008, Retrieved from www.fao.org/potato-2008/en/

[5] Corrêa RM, Pinto JEBP, Faquin V, Pinto CABP, Reis ÉS (2007) The production of seed potatoes by hydroponic methods in Brazil. *Fruit Veg Cereal Sci Biotech 3*, Special Issue 1: 133–139.

[6] “Dehaulming in potato”. (2018, December 08). Retrieved from https://www.agriedu4u.com/2018/11/14/dehaulming-in-potato/

[7] Dijst, G. (1988). Effect of periderm and water-soluble exudates of potato tubers on black scurf formation before and after haulm destruction. *Netherlands Journal of Plant Pathology*, 94, 247-266.

[8] Gaur PC, Singh SV, Pandey SK, Marwaha RS, Kumar D and Kumar D (1999) Kufri Chipsona-2: A new high dry matter potato variety for chipping. *Curr Sci* 76: 722-24

[9] “A Guide to Potato Production and Post Harvest Management in Kenya”. (2013), Retrieved from www.npck.org/Books/potato%20production%20manual.pdf.

[10] Haan, Stef & Rodriguez, Flor. (2016). Potato Origin and Production. 10.1016/B978-0-12-800002-1.00001-7.

[11] Haderlie, L. C., et al. “Chemical Desiccation of Potato Vines.” *American Potato Journal*, vol. 66, no. 2, 1989, pp. 53–62., doi:10.1007/bf02854424.

[12] Halderson, J. L., Haderlie, L. C., & Skrobacki, A. (1988). Mechanical vine killing of potatoes. *American Potato Journal*, 65, 415-423.

[13] Hide G.A, Lapwood D.H, 1992- Disease aspects of potato production, pp. 403-407, - In: HARRIS P.M (ed.) the potato crop: scientific basic for improvement, chapman & Hale, London

[14] Hussain M.M. (1985). Alu Utpadayanay Pariparshikatar Provab (Influence of environment on potato production). In: Alu Utpadayan, Sangrakkhan O Babohar (Potato production, storage and utilisation). Meer Tochimon Ghani, 85/1, Kakarail, Ramna,Dhaka-2, Bangladesh, pp. 44-50

[15] Ivany, Jerry A., and J. Brian Sanderson. "Response of Potato (Solanum Tuberosum) Cultivars to Glufosinate-Ammonium and Diquat Used as Desiccants1." *Weed Technology*, vol. 15, no. 3, 2001, pp. 505–510., doi:10.1614/0890-037x(2001)015[0505:ropstc]2.0.co;2.

[16] Kempenaar C & Struik, P.C (2007) The canon of potato science: haulm killing. Potato Res 50: 341–345. Kerby NW, Dale MFB, Lees AK, Taylor MA & Bradshaw JE (2005) Breeding and diagnostic developments for better storage of potatoes to meet future industry needs. In Haverkort A.J & Struik PC (eds) Potato in progress: science meets practice.The Netherlands, Wageningen Academic Publisher: 76–85.

[17] Kempenaar, C., & Struik, P. C. (2007) Haulm killing. Potato Research, 50, 341-345.

[18] KHURANA, S.M PAUL, editor. "Virus and Virus like Disease of Potato and Their Control." DISEASE OF HORTICULTURAL CROPS - Vegetables, Ornamentals and Mushrooms, Indus Publishing Co, New Delhi, 1999, pp. 82–121.

[19] Kumar, Dinesh & Kumar, Parveen & Pandey, Subodh Kumar & Singh, SV. (2013). Processing Potatoes in Sub-Tropical Climates.

[20] Lutaladio, NeBambi, and Oscar Ortiz. Sustainable Potato Production Guidelines for Developing Countries. (2009), Retrieved from www.fao.org/3/a-i1127e.pdf.

[21] Mahmud, Abdullah-AL & Akhter, Sajeda & Hossain, M.J. & BHUIYAN, M. & Hoque, Md. Azizul. (2009). Effect of dehaulming on yield of seed potatoes. *Bangladesh Journal of Agricultural Research*. 34. 10.3329/bjar.34i3.3970.

[22] Mandal, M. and Das, S.K. (2020). Effect of intra row spacing, dates of haulm cutting and fertilizer dose on disease free quality seed tuber production of potato (Solanum tuberosum L.) under New Alluvial Zone of West Bengal . *Journal of Applied and Natural Science*, 12(1): 1 - 8 Retrieved from https://doi.org/10.31018/ jans.v12i1.2204

[23] Marwaha R.S., Pandey SK, Singh SV and Khurana SMP (2005) Processing and nutritional qualities of Indian and exotic potato cultivars as influenced by harvest date, tuber curing, pre-storage holding period, storage and reconditioning under short Days. *Adv Hort Sci* 19(3): 130-40

[24] Marwaha, R. S., et al. "Potato Processing Scenario in India: Industrial Constraints, Future Projections, Challenges Ahead and Remedies — A Review." *Journal of Food Science and Technology*, vol. 47, no. 2, 2010, pp. 137–156., doi:10.1007/s13197-010-0026-0.

[25] Mehta, A., & Kaul, H. (2003). Physiological losses and processing quality of potatoes under ambient temperature storage as influenced by tuber maturity. *Advances in Horticultural Science*, 17(4), 196-203. Retrieved July 4, 2020, Retrieved from www.jstor.org/stable/42883364

[26] Misener, G. C., & Everett, C. F. (1981). Vine pulling as a means of top killing potatoes. *American Potato Journal*, 58, 103-109. http//dx.doi.org/10.1007/BF02854379

[27] Nipa, J & Roy, Tuhin & Amin, A.K.M. & Hasanuzzaman, Md. (2013). Effect of Lifting Time and Tuber Size on Ambient Storage Performance of Potato Derived from True Potato Seed. Int. J. Sustain. Agric.. 5. 1-9. 10.5829/idosi.ijsa.2013.05.01.314.

[28] Neenan, M. (1965). Effect of Premature Haulm Destruction on Yield, Tuber Size and Dry Matter Content of Potatoes. *Irish Journal of Agricultural Research,* 4(1), 67-80. Retrieved July 4, 2020, Retrieved from www.jstor.org/stable/25555358

[29] "Potato Varieties." The Potato: Varieties - International Year of the Potato 2008, (n.d), www.fao.org/potato-2008/en/potato/varieties.html.

[30] "Potato Vocabularies Explained". (9 Mar. 2020), Retrieved from www.npck.org/potato-vocabularies-explained-2/.

[31] RW, Hopes. "Root and Tuber Crop." ROOTS AND TUBER CROP, edited by Plaisted RL and J.E Bradshaw, Scottish Crop Institute, 1980, pp. 99–600.

[32] Sabba, R. P., Bussan, A. J., Michaelis, B. A., Hughes, R., Drilias, M. J., & Glynn, M. T. (2007). Effect of planting and vine-kill timing on sugars, specific gravity and skin set in processing potato cultivars. *American Journal of Potato Research*, 84, 205-215. Retrieved from https://dx.doi.org/10.1007/BF02986270

[33] Sandhu, K.S. & Chinna, G.S. & Marwaha, Raman & Pandey, Subodh Kumar & Kumar, Parveen & Singh, R.K.. (2012). Effect of staggered planting and dehaulming schedule on yield and processing quality of potato cultivars in Punjab. *Potato Journal*. 39. 39-47.

[34] "Seed Potato Dehaulming Methods". (2019), Retrieved from www.npck.org/seed-potato-dehaulming-methods/

[35] Spooner, David & Ghislain, Marc & Simon, Reinhard & Jansky, Shelley & Gavrilenko, Tatjana. (2014). Systematics, Diversity, Genetics, and Evolution of Wild and Cultivated Potatoes. *The Botanical Review*. 80. 10.1007/s12229-014-9146-y.

[36] Struik, P. C., & Wiersema, S. G. (1999). Seed Potato Technology. The Netherlands: Wageningen Pers.

[37] Temmerman, L., Wolf, J., Colls, J., Bindi, M., Fangmeier, A., Finnan, J., Ojanperä, K., & Pleijel, H. (2002). Effect of climatic conditions on tuber yield (Solanum tuberosum L.) in the European "CHIP" experiments. *European Journal of Agronomy*, 17, 234-355. Retrieved from https://dx.doi.org/10.1016/S1161-0301(02)00064-3

[38] Titiwa, K P, et al. Development of Potato (Solanum Tuberosum L.) Haulm Cutter. IOP Conference Series: Earth and Environmental Science, vol. 301, 2019, p. 012009., doi:10.1088/1755-1315/301/1/012009.

[39] Vashisth, K.S., AK Verma, IP Chaube and BB Nagaich. 1981. Comparative degenerative effects of viral and MLO-Diseases during Autumn and Spring seasons of Indian potato varieties in Punjab. Seeds and Farms 11: 25-28.

[40] "Vine Kill: Method and Timing." Potato Country, (3 Sept. 2019), Retrieved from

www.potatocountry.com/2019/09/03/vine-kill-method-and-timing/.

[41] Virtanen, E., et al. "Effects of Production History and Gibberellic Acid on Seed Potatoes." *Journal of Agricultural Science*, vol. 5, no. 12, 2013, doi:10.5539/jas.v5n12p145.

[42] . "Effects of Haulm Killing on Seed Potato Quality." *Journal of Agricultural Science*, vol. 6, no. 3, (2014), doi:10.5539/jas.v6n3p168.

[43] Waterer, D. (2007). Vine desiccation characteristics and influence of time and method of top kill on yields and quality of four cultivars of potato (Solanum tuberosum L.). *Canadian Journal of Plant Science*, 87, 129-135. Retrieved from https://dx.doi.org/10.4141/P06-074

[44] Lincoln Zotarelli, Steven Sargent, et al. Potato Vine Killing or Desiccation. University of Florida IFAS Extension, Feb. 2019, edis.ifas.ufl.edu/hs181.

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Fall armyworm: Menace to Nepalese farming and the integrated management approaches

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***Abstract*— *Having worldwide spread from its native American distribution to Africa and Asia since 2016, the fall armyworm (Spodoptera frugiperda) is a crop pest species that has entered Nepal in May 2019 and distributed all over in a rapid way. Nepal is an agrarian nation and the majority of the farmers grow maize on a large scale. This pest has found to affect the maize substantially and damages all the crops entirely declining the yield heavily. This is a menace to maize farmers and poses a major threat to food security and agricultural trade. Thus this review focuses on the assessment of biology of the pest and the possible management approaches which the smallholder maize farmers could afford. Integrated pest management approaches, the integration of physical, chemical, and biological method, is adopted by the majority of the corn producers to reduce the impact of the pest on the crops. Different national and international organizations and institutions have been working to develop the strategies for effective control of such a harmful insect in Nepal.*

***Keywords*— biology, control, fall armyworm, farmer's nightmare, maize, strategies.**

I. INTRODUCTION

Fall Armyworm (FAW), *Spodoptera frugiperda,* an insect belonging to class-Insecta, order-Lepidoptera and family-Noctuidae, is native to tropical and subtropical regions of America (CABI, 2017; FAO, 2017) in the western hemisphere (Capinera, 2001). It is a polyphagous natured insect (Hoy, 2013) which feeds on 186 plant species from 42 families (Early et al., 2018). Even though it is ubiquitous in distribution (Dhungel et al, 2019),it is predominantly present in the zones which have a climate of very little frost cover, a minimum annual temperature of 18-26ºC and 500-700 mm rainfall (Early et al., 2018). The record reported that it canfly 100 km per night (Capinera, 2001). Severe damage is seen on plants by FAW at its larval stage, especially on maize, rice, and sorghum (Dhungel et al., 2019). Nepal, being an agrarian nation, the majority of the people depends on farming for their livelihood. GDP from the agriculture sector contributes 27% (Khatiwada, 2019). The major cereal crops––Rice, maize, wheat, millet, etc. contribute majorly in the agriculture sector of Nepal. The temperature range is highly suitable for the establishment of this pest. In this degree, Nepal is considered as vulnerable to this pest. Maize strain and rice strain are the two common strains of FAW (Frerot et al., 2017).Maize is the third most important cereal crop next to wheat and rice globally, known as 'Queen of cereals (Jeyraman, 2017). Particularly, it is cultivated in hilly areas which are used in major food and feed industry. Loss of yield in maize results in higher demand for maize and dependency of it on other countries. Biodiversity is threatened by biotic pollution caused by fall armyworm (FAW).If FAW infestation spreads in this way, there will be major threats for the farmers of Nepal. In Argentina, food security was threatened by the highest yield loss (72%) and 15-73% yield loss is shown by FAW's infestations in maize (Hruska and Gould, 1997). Farming in Nepal is based on subsistence type, the most cultivated product is consumed by a family member and only the rest of them are for selling. The farmers of Nepal would be impacted by damage of FAW if infestation and spreading of this pest increase to a greater extent. Farmers are confronting challenges on "how to control this pest?" In Nepal, there is aninadequate knowledge of pest and its management option. There is a lack of sound contingency and long term plans, coordinated researches, development, and intervention. There is a paucity of financial and material resources. So, it is time to think about pests that cause damage to the produced high-value crops. As fall armyworm cause major damage to this crop, the livelihood of a farmer is majorly affected. Sustainable crop management should be done to control this pest.

The effective control of fall armyworm is difficult but surely not impossible. Different related institutes like IRRI, CIMMYT, FAO,etc. are emphasizing on its control measure. Various insect resistant varieties have been developed to date. Also, different trainings, awareness programs, workshops, and conferenceshave been conducted to increase the general knowledge of pests for farmers by different organizations. Plant quarantine measures and legislation is made by government of Nepal in different quarantine centers of Nepal in the boarder of India-Nepal to prevent invasion of this detrimental pest. Several approaches by which integrated practices of economic control are done to suppress pest population, which is known as integrated pest management (IPM).However, government should put in 'place emergency plan'. These include monitoring with pheromone traps to determine the spread of FAW, roadside show to increase public awareness, a temporary ban on transportation of crop between two nations, restriction on transportation of plant material without checking properly. Economic and effective management of this pest should be done otherwise; it would ruin farmers' lifestyle.IPM is the integrated use of different techniques to suppress pest population which cause less harm to environment and people. Objectives of IPM are to prevent or avoid pest population, implement routine scouting to identify and respond to damaging pesticide and to suppress the pest using combination of biological, physical, cultural, and if necessary chemical approaches (Prasanna et al., 2018). Surveillance, scouting, and monitoring system should be promoted. Sustainable management option should be practiced on the basis of integrated pest management system. Government should empower and invest on research of this pest. Proper extension service should be provided to the farmers. Avoid of this pest is done by choosing quality seed in many cases. This paper aims to assess the threats of fall armyworm to maize production and the possible management approaches to reduce the attack of pests.

II. RESEARCH METHODOLOGY

This review completely uses secondary sources of information. Pieces of Literature were collected from different Journal articles, Agricultural institutes, other sources like FAO, CIMMYT, and relevant reports were studied and the major findings were summarized. Also, suggestions from related professors and officers were considered in the paper.

III. DISCUSSION

1. Scenario analysis of maize productions in Nepal

Maize cultivation is a way of life for most farmers of Nepal, especially in the hilly region. Maize is the most widely grown crop in Nepal after paddy in terms of acreage and productions and also a staple food for many people of the country. Although the report showed the slight increment in the yield of maize over the past decade, there has been very little improvement in yield when compared to average yield of the nation few years ago. The only plausible explanation behind the statement is probably the expansion of maize cultivation into less suitable terrain, degrading soil fertility status, sluggish adoption of advanced management approaches, and most importantly; the outbreak of severe pests and diseases such as fall armyworm. The 2020 maize crops, ready to be harvested from August onwards, are growing under appropriate weather conditions. The area cultivated is approximated at a high level, enduring the constant increase observed during the past eight consecutive years, signifying the strong demand by the feed industry (FAO, Global Information and Early Warning System, 2020).

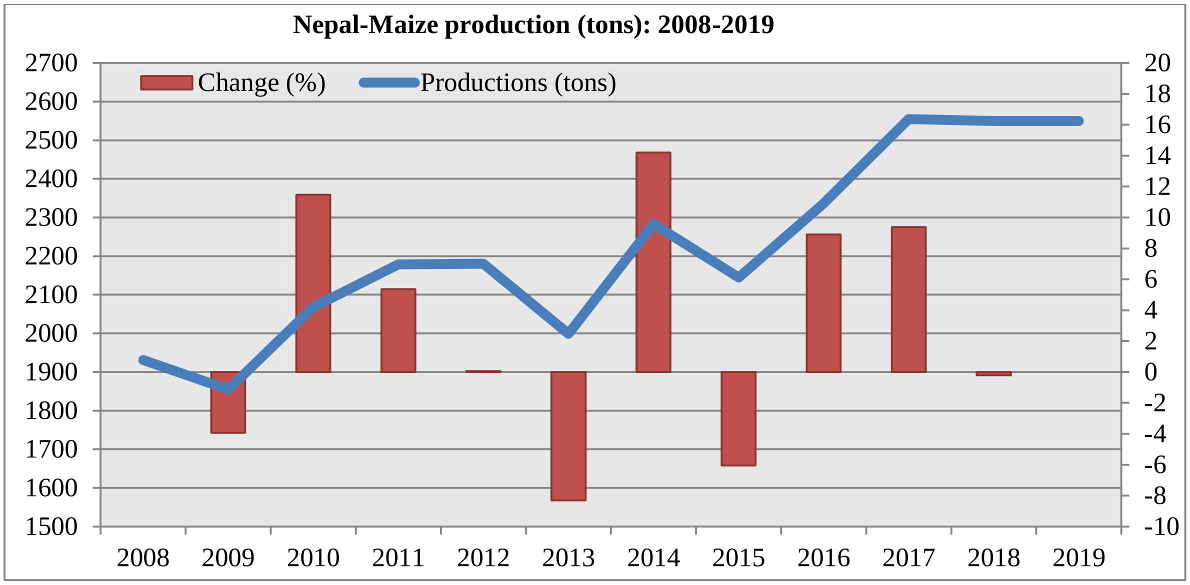

Fig.1 Scenario analysis of maize productions in Nepal: 2008-2019 Source: (Knoema, 2020)

In 2019, maize production for Nepal was 2,550 tons. Maize production of Nepal increased from 1931 tons in 2008 to 2,550 tons in 2019. Since a few years, the production has not increased as expected though there is advancement in agricultural technologies and the development of new innovations. The crop has been largely affected by the severe outbreak of dangerous insect-like fall armyworm. Maize cultivating areas are also increasing representing 25% of the total field area of cereal. 20% food and energy of people and 80% poultry and animal feed is supplied by maize. Maize productivity is declining year wise due to pest infestation among which FAW is a major pest. Young leaf whorls, ears, tassel and almost all parts are eaten by FAW, which is considered as a major feed for FAW, resulting in occasional total yield loss.

2. Origin and distribution of Fall Armyworm

This pest was limited to America until 2015 AD and recorded first time in Nigeria in 2016. According to Goergen et al. (2016), the FAW originated from the tropical regions of the Americas from the United States to Argentina and the Caribbean region. In 2018 AD FAW was recorded in Asia from Karnataka, India. In 9th August, 2019 FAW was first recorded in Nawalparasi, Nepal. Including Nepal, it has invaded 10 countries in the Asia (Poudel, 2020).It is a prime noctuid pest of maize and has remained confined there despite occasional interceptions by European quarantine services in recent years and has been recently introduced into the African continent and has already moved to at least 21 countries where the pest has been reported for the past 16 months (Abrahams et al., 2017). The genus Spodoptera comprises of 31 species with seven species previously recorded from the Afro-tropical region while six species are known to occur in West and Central Africa (Pogue, 2002). *Sopodoptera exempta* or African armyworm is the most common and well known amongst them in Africa.

Table 1 distribution of Fall Armyworm

Countries	First reported
Nigeria	2016
Benin	2016
Angola	2017
India	2018
Nepal	2019 (May)
Thailand	2018
Pakistan	2019
Srilanka	2019 (January)
China	2019 (January)
Australia	2020 (February)
Papua New Guinea	2020 (April)

Source: (FAO, 2020)

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In July 2018, it was observed in India and Yemen. By December 2018, FAW was recorded in Bangladesh, Sri Lanka and Thailand. As of June 2019, it was seen in Myanmar, China, Indonesia, Laos, Malaysia, Viet Nam, Egypt and the Republic of Korea. Japan recorded the outbreak of FAW in July 2019. FAW was officially reported in Australia and Mauritania in February 2020 and in Timor-Leste in March 2020. The FAO map above shows the areas affected by Fall Armyworm.

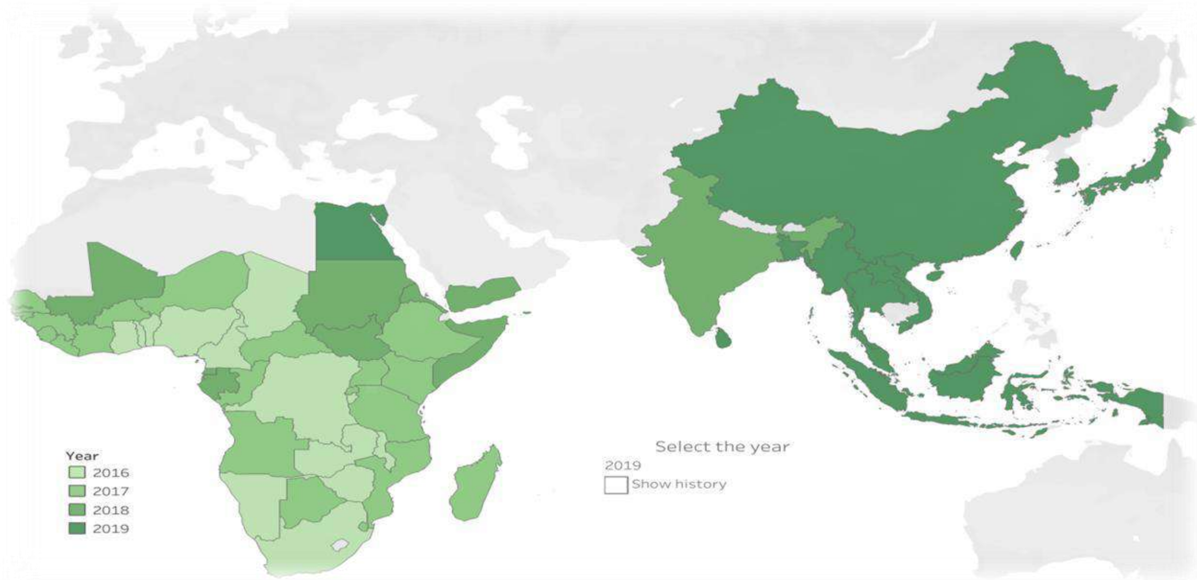

Fig2 Global status of FAW Source: FAO (2020)

Classification of Fall Armyworm

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Lepidoptera

Family: Noctuidae

Genus: *Spodoptera*

Species: *Frugiperda*

3. Identification of insect

Eggs of FAW can be recognizedon the basis of the clustered laying nature of the eggs ranging from few to hundreds in numbers (Sparks 1979; Sharanabasappa et al., 2018). Eggs are laid on single or multiple layers creamy colored with anal tuft of hairs or sometimes without hair cover (Firake, 2019, p. 9). Eggs are usually spherical in shape and laybeneath the leaves, near the base of the plant, close to the junction of the leaf and the stem (CABI, 2019, p. 21). Identification of larvae in the field needs expertise and skills as FAW is easily confused with similar species such as the African armyworm (Spodoptera exempta), and the cotton leaf worm (Spodoptera littoralis), as well as species of other noctuid genera. First instar larvae are greenish with a black head capsule, and later turned greenish-brown in the second instars. Larvae darken in color as they feed and appear greenish (Luginbill, 1969) .The third instars are brownish with three dorsal and lateral white lines. Fourth to the sixth instars are brownish black and had three white dorsal lines and alight lateral line (Sharanabasappa et al., 2018). The mature larva has a dark head with an upside down pale Y-shaped marking in head area and black four spots arranged in square in last abdominal segment (CABI, 2017). In male moth, the forewings generally shaded grey and brown, with the triangular white spot at the tip and near the center of the wing while the forewings of the females are less distinctly marked, ranging from the uniformly greyish brown to fine mottling of grey and brown (Prasanna.et.al., 2019).

4. Biology of the pest

The lepidopteron pest, fall armyworm has four stages in life cycle; viz: egg, larva, pupa, and adult. The fall armyworm feeds on leaves and stems of more than 80 plant species, causing significant damage to maize, rice, sorghum,

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sugarcane but also other vegetable crops and cotton (CABI,2020). Its life cycle is completed in about 30 days during the summer. However, it takes 60 days in the spring and autumn, and 80 to 90 days during the winter (Capinera, July 1999).

Egg:

Eggs are spherical in shape and the number of eggs per mass varies often between 100 and 200. Total egg production per female over her lifetime averages about 1500, with a maximum of over 2,000 (CABI, 2019, p. 21). Incubation period ranged from 2-3 days with a mean of 2.50 days with favorable temperature 20-30°C (Sharanabasappa et al., 2018).

Larva:

There are six larval instar of fall armyworm. The duration of the stage of larva tends to be almost 14 days during the summer and 30 days during cool weather. The mean development time was estimated to be 3.3, 1.7, 1.5, 1.5, 2.0, and 3.7 days for instars 1 to 6, respectively, when larvae were reared at 25ºC. The larvae in the back consists of 3 yellow stripes followed by a black and again yellow stripe on the side whereas on the second to last segment, four dark spots are seen that forms a square(FAO,2018).

Pupa:

The larva binds the particles of soil together to form a loose, oval and 20-30 mm long cocoon inside which a reddish-brown pupa measuring 14 to18 mm in length and 4.5 mm in width resides. The duration of the stage of pupa is nearly eight to nine days during the summer, but reaches 20 to 30 days during the winter (CABI, 2019).

Adult:

Adults are nocturnal, and are most active during warm, humid evenings. Females deposit most of their eggs during the first four to five days of life, but some eggs may be laid for up to three weeks. Females can mate multiple times during this period and lay multiple egg masses, with a potential fecundity of up to 1,000 eggs per female (Heinrichs.A.E, 2018, p. 12). The adult can liveupto an average 10 days, with a range of about 7-21 days (Prasanna et al., 2018).The favorable temperature for adult is less than 30°C(CABI,2017).

Table 2 Characteristics of different stages of Fall armyworm

Stage	**Shape**	**Color**	**Duration (days)**	**Suitable temp.(^{0}C)**	**Special characteristics**
Egg	Spherical (diameter: 0.75 mm)	Greenish gray in early stage and later turns into brownish black	2-3	20-30	Female covered a layer of scales (downy materials on the egg mass and give moldy appearance.
Larvae	3-4 cm long	greenish to brownish with longitudinal stripes	14-21	26-28	Yellow colored inverted Y-shape structure on the head, black dorsal pinaculate with long primary and four black spots arranged in a square on the last abdominal segment.
Pupa	1.3-1.7 cm long	Brownish in the early stage and later turned into back	9-13	13-16	A loose cocoon in an earthen cell
Adult	1.5-1.7 cm long	Dark grey to brown, straw	12-14	below 30	Distinctive white spot near the dorsal tip, or apex, of the wing, Forewing is mottled. Hindwings are straw colored with a dark brown margin

Source: (CABI, 2017)

5. Threats of fall armyworm: Farmer's nightmare

An intrusive pest, fall armyworm (Spodoptera frugiperda) was first recorded in Nepal from Nawalpur district which are presumed to have entered from India (Poudel, 2020). As the world is confronting the perils of climate changes in agricultural productions, the outbreak of detrimental insect, fall armyworm, has added the challenges in farming sector. Fall armyworm is a hazardous trans boundary insect with a high likelihood to spread expeditiously due to its natural distribution capacity. (FAO, 2019) portrays a real menace to food security and livelihoods of millions of smallholder rural peasants. Several researchers have performed a research on destruction level of fall armyworm on various crops in Indonesia (Maharani et al., 2019), Nepal (Bhusal and Bhattarai , 2019), Kenya (Groote et al., 2020), and many other countries.High fecundity of the insect at favorable environment condition is foreseen to result a severe damage to crops (Goergen et al., 2016). The larvae consumed both vegetative and reproductive structures of the crops. The young larvae mostly preferred the epidermal leaf tissues and make holes in the leaves; peculiar damage symptom of fall armyworm (Bhusal and Bhattarai , 2019). Loss of photosynthetic area, diminished reproduction, crop damage, lodging and structural damage in the whorl are shown on different researches by different researcher (Chimweta et al., 2019).

The fall armyworm (FAW) has harshly affected maize production across the country this year, 2020, as compared to previous year, 2019, hitting farmers who have already had to confront the burden of the coronavirus pandemic (The Himalayan Times, 2020). The catastrophic outbreak of the pest has threatened many small to large maize producers of the country. It has destroyed over thousands of hectares of maize fields from different districts across the country. In 2018, the Democratic republic of Congo reported that 45% of maize harvest losses occurred due to FAW attacks that resulted in a loss of 0.89 million tons of maize during harvest season (FAO, 2018).Similar cases were recorded in Nicaragua; yield loss of over 70% (Hruska and Gould , 1997), America; yield loss of 39% (Ivan et al., 2012), and Argentina; yield loss of 72% (Chamberlain et al., 2006). It causes substantial damage to maize by feeding on leaf whorls, ears and tassel which sometimes results in total yield loss (Sarmento et al., 2002). Although the direct foliar damage in maize from the attacks of fall armyworm is menacing too many farmers, the damage in many cases doesn't result in dramatic yield reduction (Hruska , 2019).The larvae, being voracious in nature, consume almost all the vegetation in their path. The consumption rate is high and the major damage is due to the feeding on the foliage. At the beginning, young larvae feeds on leaf tissue from one side and second or third instar larva make series of holes in the leaves and feeds on the edge of the leaves inwards. The older larva makes a huge damage leaving only ribs and stalk of corn. Because of its cannibalism nature, one or two larva is found per plant. Larvae also burrow the growing parts such as buds, whorl, etc. and hindered the growth of corn. It often infests ears as well. Such ears aren't consumed by humans. That's why the fall armyworm doesn't directly affect the food safety of maize; rather it can make the maize more susceptible to aflatoxin presence (Zanolli, 2018). The pest will consequently affect natural capital through economic yield losses and the capacity of farm lands to respond to shocks, and through increasing the cost of production which will ultimately affect household's social and physical capital (the household's assets). The pest also impact the import and export of the maize within or outside the country as it carries the risk of introducing pests to areas where the pests are not yet present. For the reasons, it has become a great nightmare particularly to maize crop farmers. The alarming pest has a voracious appetite for corn and other cereal crops and its impact would be noteworthy for the Nepalese farmers and country's economy (Beshir et al., 2019). The insects prefer maize - a key food crop in Nepal - as well as rice, sorghum, millet, potato, sugarcane, vegetable crops and cotton.

Fig 3: Corn leaf damage caused by the fall armyworm, Spodoptera frugiperda

6. Control/management

Sensing Fall Armyworm infestation before it causes heavy damage is the key to their management and control. Its management should be done cautiously as it is a dangerous pest. Only one method is not sufficient for the control of this pest. Different system should be integrated to control effectively.

Integrated management of fall armyworm

The best and most effective strategy to control FAW is taking preventive measures and immediate actions when the fall armyworm is detected. IPM focuses on the growth of a healthy crop with the least possible disruption to agroecosystems and encourages natural pest control mechanisms. Management of this pest should be done in such a way that sustain for eco-friendly environment, causes low risk to human environment, and also should be cost effective. Control of this pest is possible through many physical, biological, chemical, cultural means. Proper timing, selection of crop variety, crop management and proper use of bio-pesticides, and synthetic pesticides are important parameter of IPM. Right planting date is also important for its control. Late planted and late maturing varieties are susceptible to FAW infestation. Therefore, cultivation of maize variety which is genetically modified or early maturing type would be effective to control this pest. Bt-maize has been found resistant to FAW (FAO, 2018). Good soil condition is another important aspect. Crop variety which completely covers maize cob should be selected. Unbalance use of fertilizer especially Nitrogen can lead to oviposition by female fall armyworm. Thus, it is recommended to use balance fertilizer which is organic. Proper knowledge of larval cycle of FAW and time of day for application of pesticide is also important parameter. If 5% seedlings are cut or 20% whorls of small plant are infested, it is recommended to apply an effective management practice to prevent further damage (FAO, 2018).

6.1 Monitoring and scouting:

Frequent observation and estimation of pest population and losses should be done in the maize field. FAW field monitoring is one of the key components of IPM. Three methods; Scouting, Pheromone trap and Light trap are used for the monitoring of the pest (Abrahams, 2017). The efficient method of monitoring this pest in the field is by using black light trap and Pheromone trap. Upto four pheromone traps per acre should be suspended at canopy height during the whorl stage of maize (Mwangi, 2019, May, p. 9). Mating interruption is possible through pheromone trap in which chemicals or sex pheromones are produced by female to attract males are placed which can move long distance through air and makes monitoring easy (Shorey, 1994). Insect catches indicate the presence of moths in the area but may not be accurate indicators of density (Global Fall Armyworm Management , 2019, p. 11). Light traps can be used to control the adult fall armyworm which helps to trap both male and female insects. Nocturnal behavior of FAW moth makes it possible to monitor easily through Black light trap (Hunt et al. 2001; Qureshi et al. 2006).

Scouting is the use of science based protocols by trained individual which is the process of precisely assessing pest pressure and crop performance for the effective solution of pest and disease control. Scouting promote farmers knowledge on biology of pest in the field and their ecology, which makes easier for farmer in decision making process for high yield, lower wasted resources and sustainability (FAO, 2018a). It is typically performed in order to evaluate both the economic risk of pest infestation and the potential efficiency of pest control interventions, with the goal of informing practical crop management decisions at the individual field and farm level (Prasanna et.al., 2019, p. 12). Usually, it can be done by walking in "W" pattern in the field after leaving 4-5 outer rows. . Action is taken if 5% plant are damaged at seedling to early whorl stage, 10% whorl are damaged in mid whorl stage, 20% are damaged at late whorl stage (Kumbhar, 2019).

6.2. Early warning system

By warning farmers earlier on the futurerisk of FAW outbreaks, they have the likelihood to prevent crop damage. On the basis ofrisk level, farmers are encouraged to scout their fields routinely for eggs and larvae and take precautionary measures where possible. Food security and food safety are very important for day–to–day life but outbreaks of harmful diseases and pest has imperiled farmer's life. Along with diagnosis-controlling, early forecasting is crucial which is known as Early Warning System (Li et al., 2007). Consolidating the principles of biology, ecology and mathematics is the basis of early warning system (Wang et al., 2013). It is a process in which collection and sorting of data, and generation of early warning information is done (Wang et al., 2013). Android application -"Fall Armyworm Monitoring and Early Warning System(FAMEWS)" was used in Madagascar and Zambia and successfully applied by African country which was developed by FAO (FAO, 2018c). Similar application can be developed in Nepal to monitor FAW effectively.

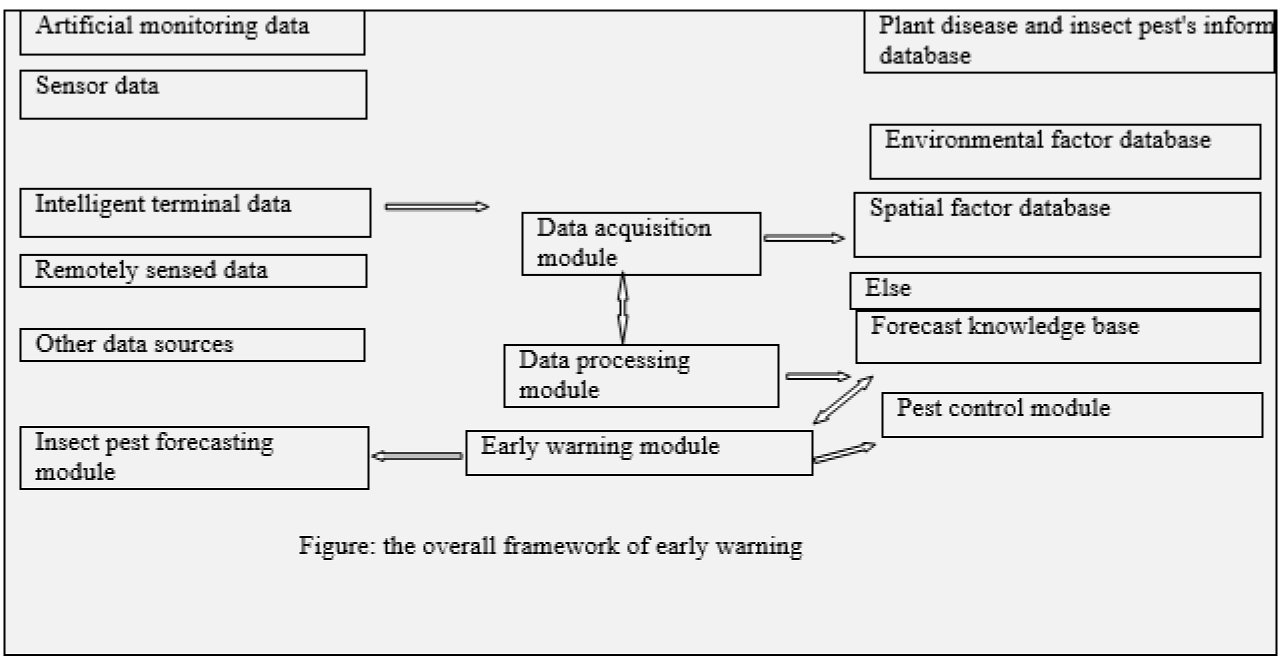

Fig 4 Framework of Early Warning System

Source: (Wang, 2013)

6.3. Physical method of control

It is the simplest method of pest control where FAW eggs and larvae are killed mechanically. During first week after planting, eggs which are laid on mass on maize leaves are immediately crushed. Young larvae are picked off the leaves, before invades inside whorl. Hand picking and destruction of egg masses during monitoring is done which helps to control the pest. In Ethiopia, 15% of the farmers practiced only handpicking for FAW management (Rwomushana, 2018) and crushing or immersing of larvae in masses. Application of dry sand in whorl is another method of controlling FAW by disturbing them. Soil application inside the whorl is done to control somehow.

6.4. Cultural method

Cultural control is an effective component of a pest control strategy for FAW. Deep ploughing has shown effective for controlling eggs and pupal stages of FAW. Planting of legumes as a trap crop and ploughing field rightly before planting the field can be an effective possible cultural method for managing the pest. Cultural practices like clean cultivation and proper use of fertilizers, grown of maize hybrids with tight husk cover will reduce ear damage by FAW (Firake, 2019). Timely sowing of seed could help to reduce pests. In whole one region planting date should be same which help Maize should be planted early. Balanced fertilizer should be applied. Neem based pesticide (Azadirachtin 1500 ppm) should be applied if there seems papery window on leave. Most farmers lack access to market and chemical pesticides. Use of Intercropping with leguminous crop i.e. French bean, soyabean, groundnut and other beans provide better protection to the crop compared to mono-cropping (Hailu et al., 2018). Erection of bird perches, sowing of trap crops, clean cultivation and balanced use of fertilizers, cultivation of maize hybrid with tight husk cover will reduce ear damage by FAW. Another important aspect is selection of crop variety. In case of Nepal, proper evaluation hasn't been done yet. Some GMO's including Bt-maize reported resistant in Africa, however FAW has overcome Bt-maize in case of America (FAO, 2018).

***Push-Pull Strategy*: It is one of the important strategies for controlling the infestations of FAW. In this method, maize is intercropped with pest-repellent *Desmodium spp*. (Push-crop), surrounded by pest-attractive Napier Grass, *Pennisetum purpureum* or *Bracharia spp*. as pull-crop (Dively, 2018). Napier grass attracts stem borers and FAW to lay egg on it but doesn't permit larval growth due to poor nutrition, so very few larvae remain alive. Desmodium emits volatile compound which is disliked by stem borer or FAW and repelling action happens thereafter. Combination of Desmodium green leaf and Bracharia cv. Mulato II known as 'Push-pull climate smart' was proved to be effective from different researches. Reduction of 82.7% in average number of larvae per plant and 86.7% in plant damage per plot have been observed in push-pull plot , compared to maize monocrop plots. Likewise, maize grain yields are recorded to be significantly higher, 2.7 times, in push-pull plots (Midega et al., 2018). Some of the promising practices identified are given in Table1 below:

Table 3 Cultural method of FAW management

S.N.	Method	Description	Reference
1.	Intercropping with compatible companion crops and fertilizer trees.	Planting additional crops in strips, rows, or stations between the main crop (e.g., pigeon pea, cassava, sweet potatoes, cowpea etc.) helps to reduce the infestation of FAW. Intercropping with repellent plants such as *Tephrosia* and *Desmodium* repel the adult female FAW moths and reduce the number of eggs lay on host plants.	Pichersky and Gershenzon (2002); Landis et al. (2000);
2.	Deep ploughing	Deep ploughing before sowing helps to expose the pupae to birds or predators.	(Mooventhan.P, 2019, p. 13)
3.	Planting at the recommended/ optimal time	Do not delay planting: take advantage of planting with the first effective rains, as FAW populations build up later in the crop season.	Van den Berg and Van Rensburg (1991)

4.	Conservation agriculture	Combined use of no tillage, residue retention, and rotation increases and diversifies biological activity of macro-(spider, beetles, and ants), meso-(fungi), and micro fauna (bacteria). These practices also lead to improvement of soil health, which contributes to more vigorous growth of the crop.	All (1988); Rivers et al. (2016)
5.	Increased ground cover	Cover crops like mucuna, lablab beans, jack bean, sun hemp, etc., contribute to plant species diversity that enhances biological activities and provides shelter for natural Enemies (spiders, beetles, ants).	Altieri et al. (2012)
6.	Push and pull strategy	In this strategy maize crop is intercropped with pest-repellent “Push crop” i.e. *Desmodium spp* and “Pull crop” i.e. Napier grass (*Pennisetum purpureum*) is panted in the border which attracts the pest thus, FAW can be killed by spraying insecticides in Napier grass only.Reductions in FAW infestation are reported to be 82.7% in the average number of larvae per plant and 86.7% in plant damage per plot in climate-adapted push-pull compared to maize monocrop plots.	(Dively, 2018)

6.5. Biological control

Biological control can be considered as a powerful tool and one of the most important alternative control measures providing environmentally safe and sustainable plant protection (Assefa.F .et .al, 2019). In situ protection of natural enemies by habitat management support to increase the plant diversity by intercropping with pulses and ornamental flowering plants which help in buildup of natural enemies (G. Ravi, 2019).Among several groups *Telenomus remus* and *Trichogramma spp* are effective to control pest in field. Biological control agents (BCAs) include the following:

a) predatory insects and mites, which eat their prey;
b) Parasitoids, which are insects with a free living adult stage and a larval stage that is parasitic on other insects.
c) Parasites and microbial pathogens, such as nematodes, fungi, bacteria, viruses and protozoa, which cause lethal infections. (FAO, 2018).

Predator:

Predators of the Fall Armyworm kill several individuals either as eggs, larvae, pupae or adults. The predators include earwigs, ladybird beetles, ground beetles, assassin, flower bugs, and predatory wasps. Birds, skunks, and rodents also feed on larvae and pupae of FAW among the vertebrate predators. Pair and Gross found 73% FAW pupal mortality mainly due to predators. Generally,they are non-selective or generalists, so they feed opportunistically on more than one host species, often even on their own kind. The most preferred site of FAW in maize is the whorl inside which a predatory earwig, *Doru luteipes* (Scudder) lays its eggs (Reis et al., 1988) and occurs throughout the maize crop cycle. Nymphs of *D. luteipes* consume 8–12 larvae daily, while in the adult stage they consume 10-21 larvae of *S. frugiperda* daily (Reis et al., 1988).

Table 4 Predators controlling FAW

S.N	Natural Enemy	Life Stage
1	*Calleida decora*	Larva
2	*Calosoma alternans*	Larva
3	*Calosoma sayi*	Larva
4	*Carabidae*	Larva/Pupa
5	*Doru luteipes*	
6	*Doru taeniatum*	
7	*Ectatomma ruidum*	
8	*Geocoris punctipes*	
9	*Steopolybia pallipes*	
10	*Podisus maculiventris*	

Source: (CABI, 2019)

Parasitoid:

Parasitoids are organisms whose adults lay eggs either inside or attached to a single host organism. To enable development, the resultant larvae feed on the tissues of the host until they are fully grown and pupate. Parasitoid larvae always kill their host as the outcome of their development. The majority of parasitoids known to be associated with the FAW are wasps, and less frequently flies (FAO, 2018). The parasitoid *Cotesia icipe* (Hymenoptera: Braconidae) found to parasitize 33–45% of FAW larvae (Global Fall Armyworm Management , 2019). Use of Parasitoids for the control of pest is effective for the environment and human health.

Table 5 Parasitoids controlling FAW

S.N.	Natural Enemy	Life stage	Host
1	*Archytus incertus*	larva	maize
2	*Archytus marmoratus*	larva/pupae	maize/sorghum
3	*Campoletis flavicincta*	larva	maize
4	*Chelonus curvimaculatus*	eggs/larva	maize
5	*Chelonus insularis*	eggs/larva	maize/sorghum
6	*Cotesia marginiventris*	larva	maize
7	*Cotesia ruficrus*	larva	maize
8	*Euplectrus platypenae*	larva	maize
9	*Glyptapanteles creatonoti*	larva	maize
10	*Lespesia archippivora*	larva	maize
11	*Microchelonus heliopae*	eggs/larva	maize
12	*Brachymeria ovata*	pupa	
13	*Telonomus remus*	eggs	maize/vegetable
14	*Trichogramma achaeae*	eggs	maize

15	*Trichogramma chilotraeae*	eggs	maize
16	*Trichogramma pretiosum*	eggs	maize
17	*Trichogramma rojasi*	eggs	maize

Source: (CABI, 2019)

Pathogen:

Pathogens are everywhere. Entomopathogens are those which affect insect. Pathogens are viruses, fungi, bacteria, nematode and protozoans. Mostly, virus, fungus, and bacteria play important role in controlling FAW. They are farmer-friendly pathogen which can be recycled by farmers easily in this process, dead-decayed larva which are found in field and contain full of viroid particle of fungal spore are taken from field and then they are grinded by kitchen blender. The liquid strain from these are taken and mixed with water. Then they are sprayed in infected plants. Nuclear polyhedrosis virus (NPVs) and Spodoptera frugiperda multi capsid nucleo polyhedrosis virus (Sf MNPV) are reported lethal to the FAW. The host-specificity of pathogens is quite high, usually restricted to a few closely-related insect species (FAO, 2018). The larvae of the Fall Armyworm infected by a pathogen change the color with increasing paleness and decreasing movement, especially, when touched. However, the best way to identify a diseased larva is when it is already dead. Particularly for FAW larvae infected with Baculovirus the dead larvae will generally be observed in the upper parts of the maize plant and will hang upside down. Dead larvae covered with a powdery white or greenish mass suggest fungal infection (Prasanna et.al., 2019, p. 78). The major entomopathogens helpful for management of FAW are listed below in table.

Table 6 Pathogens controlling FAW

S.N.	**Natural Enemy**	**Life Stage**
1	*Bacillus cereus*	Larvae
2	*Bacillus thuringiensis*	Larvae
3	*Bacillus thuringiensis alesti*	Larvae
4	*Bacillus thuringiensis darmstadiensis*	Larvae
5	*Bacillus thuringiensis thuringiensis*	Larvae
6	*Bacillus thuringiensis kurstaki*	Larvae
7	*Beauveria bassiana*	Eggs/Larvae
8	*Granulosis virus*	Larvae
9	*Metarhizium anisopliae*	Eggs/Larvae
10	*Nucleopolyhedrosis virus*	Larvae

Source: (CABI, 2019)

Botanical:

Natural pesticide that is derived from plants having defensive properties is known as Botanical pesticides. More than 6000 plant species from at least 235 plant families have been screened for pest control properties.According to the laboratory studies neem seed powder is found to be the effective in killing FAW larvae causing over 70% of mortality (Maredia, 1992). Azadirachtin (from neem) and pyrethrins (from pyrethrum) are the most widely used products (Assefa et al., 2019).The botanical pesticides are biodegradable, environmentally safe, less harmful to farmers and consumers, and often safe to natural enemies and hence amenable for use in biocontrol based on IPM strategies (Prasanna et.al., 2019, p. 76). Bio-pesticides are made from plant-derived pesticides and are harmless to natural environment. Bio-pesticides are substance or mixture of substance that are intended to suppress pest and prevent the damage or loss that they caused. Plant derived pesticides like neem based bio-pesticides can be used to control the larva of FAW as it is easily available to the local market. Different researches conducted by researcher shows that seeds or leaves of plants of Meliaceae family (Azadirachta indica) and Asteraceae family (Pyrethrum) and other plants such as *Tephrosia vogelii* or *Thevetia nerifolia* are showing capacity in the management of FAW. Further research needs to be done to promote botanical pesticide.

Table 7 Botanical method of FAW control

S.N.	Species	Family	Extract	Mode of Action
1	Neem, *Azadirachta indica*	Meliaceae	0.25% neem oil	Larvicidal with upto 80% mortality in the lab
2	*Aglaia cordata Hiern*	Meliaceae	Hexane and ethanol extracts of seeds	Larvicidal with upto 100% mortality in the lab
3	*Annona mucosa Jacquin*	Annonaceae	Ethanolic extract from seeds	Larval growth inhibition
4	*Vernonia holosenicea*, *Lychnophora ramosissia*, and *Chromolaena chaseae*	Asteraceae	Ethanol extracts from leaves	Ovicidal
5	*Cedrela salvadorensis* and *Cedrela dugessi*	Meliaceae	Dichloromethane extracts of wood	Insect growth regulating(IGR) and larvicidal with upto 95% mortality
6	Long pepper, *Piper hispidinervum*	piperaceae	Essential oil from seeds	affects spermatogenesis and hence egg laying
7	Chinaberry, *Melia azedarach*	Meliaceae	Ethanolic extracts of leaves	Antifeedant to larva; synergistic with pesticide
8	*Jatropha gossypofolia*	Euphorbiaceae	Ethanolic extracts of leaves	Antifeedant to larva; synergistic with pesticide
9	Castor, *Ricinus communis*	Euphorbiaceae	Castor oil and Ricinine (seed extracts)	Growth ihibition and larvicidal

Source: (Prasanna et. al., 2019)

6.6. Chemical control

Chemical pesticides expose the major hazard in sustainable agriculture. However, in severe condition, the steps should be taken quickly in that case chemical pesticides are used. Until more sustainable solutions is developed, it is recommended to alternate application of contact/systemic insecticides based on pyrethroids, carbamates or organophosphates as an immediate management measure (Evans, 2017). The exact timing for applying chemicals is very important for effective pest control; both the life cycle and the time of day matter i.e. spraying when larvae are deeply embedded inside the whorls and ears of maize is ineffective; and spraying during the day is ineffective because larvae only come to feed on plants at night, dawn or dusk. Chemical pesticide provide a protection level which cannot be guaranteed by other methods, however, they are expensive to afford by poor farmers. There use isn't economically feasible to smallholder farmers, cause environmental contamination; develop resistance to chemicals and often pest resurgence. The use of chemical pesticides can be used as a last option if all mentioned measure cannot maintain the pest below economic threshold level if there seems heavy damage on leaves and whorls of leaves. One should always wear aprons and other safety measures while applying insecticides as it is harmful to human health. Synthetic pesticides like methomyl, acephate, cyfluthrin, benfuracarb, methyl parathion, carbaryl carbosulfan, lindane, chloropyrifos, diazinon, and methyl parathion are found effective to control FAW (Dhungel et al., 2019). New insecticides such as chlorantraniliprole, flubendiamide and spinetoram reported better mortality than traditional insecticide like lamba and novaluron (Hardke et al., 2014). However, during selection of chemical pesticide we should use less hazardous pesticide reading from level. FAO has been assigned authority by the council in 2006 and again in 2013 to assist member countries in reducing risks posed by highly hazardous pest (FAO, 2018).

6.7. Host plant resistance

The most effective and ideal method of combating insects that attack plant is by developing insect-resistant varieties (Luginbill, 1969). The antibiotic mechanism of plant resistance offers a biologically, economically, and environmentally sound alternative to conventional pesticides for controlling the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith)in corn, *Zea mays L*. Recent studies in Brazil showed cross resistance among Cry1f, Cry1Ab, Cry1Ac and Cry1A indicating current Cry1 based plants face a challenge in managing fall armyworm but in contrast Bt maize containing the Vip 3Aa20 protein remains effective against FAW.Transgenic maize varieties with Bacillus thuringiensis (BT) have been successful in controlling FAW. There are resistant varieties on the market that suppress/control FAW and other lepidopteran pests.However, FAW resistant varieties of other crops have not yet been developed (Global Fall Armyworm Management, 2019).

7. Fall armyworm management efforts in Nepal

Most of people in Nepal depend on agriculture for their livelihood. Farmers of Nepal have been combatting the threat of devastating pest for long; however attack of fall armyworm across the field of maize raises the serious concern threatening food security to millions of people. In Nepal, the fall armyworm has the potential to cause maize yield losses of 20-25%, which translates to the loss of more than half a million tons of the annual maize production estimated at around $200 million (Pradhan, 2020). If the pest is left unrestrained, its impact will be huge for farmers and the economy.At present major problems affecting FAW management efforts in Nepal islack of knowledge about the plant disease and pest, their diagnosis and management practices the farmers, lack of sound contingency, lack of proper coordinated plans and policy, scientific research, surveillance, laboratory and adequate practices for management of pest which may be result of low financial status. However NGO and INGOs have been working together in the field for efficient management options in Nepal, some programmes conducted are present below:

- International Maize and Wheat Improvement Center (CIMMYT) have been focusing in creating awareness, disseminating appropriate technologies and management techniques, and strengthening the capacity of communities, institutions and government.
- The Ministry of Agriculture and Livestock Development has established a national taskforce to fight the pest. Most provinces have established similar taskforces that include researchers, agriculture extension agents, farmers and entrepreneur associations.
- Through the Nepal Seed and Fertilizer (NSAF) project, CIMMYT staff is working closely with the Ministry of Agriculture and Livestock Development, the Nepal Agricultural Research Council (NARC), the PQPMC, provincial governments, and other USAID-funded projects and development partners in Nepal. Together, they have developed integrated pest management packages, informative factsheets and surveillance guidelines.
- CIMMYT researchers have shared experiences on pest management, surveillance and scouting techniques from other countries in Asia and Africa. They have also demonstrated digital tools that will help map the spread of the pest and build accurate interpretation for better management.
- CIMMYT researchers collaborated with the Prime Minister Agricultural Modernization Project (PMAMP) to implement outreach campaigns in Banke district. This included a mobile information booth, local dissemination of audio messages, and distribution of posters and fact sheets about fall armyworm. The two-day campaign successfully raised awareness about the pest, reaching more than 1,000 farmers from four villages in maize growing areas.
- Further Plant Quarantine and Pesticide Management center has published factsheet for proper identification and management practices such as; Handpicking, monitoring and scouting, intercropping, pull-push strategy and biochemical.
- Chemical pesticide such as; Spinosad, Spinetoram, Emamectin Benzoate, Chlorantraniliprole has been recommended in suitable amount by Government of Nepal.
- "Fall Armyworm in Africa: A Guide for Integrated Pest Management," jointly produced by Feed the Future, the

United States Agency for International Development (USAID), the International Maize and Wheat Improvement Center (CIMMYT), and the CGIAR Research Program on Maize (MAIZE), provides tips on fall armyworm identification as well as technologies and practices for effective control. This can be helpful to Nepalese farmers to control the outbreaks of the pest.

IV. CONCLUSION

Nepalese farmers, especially maize producers have encountered the problem of fall armyworm infestations on a large scale in different parts of the country. Furthermore, the environmental condition of Nepal is found very favorable for the introduction, establishment, and spread of FAW. The colossal majority of farmers in the country are smallholders. These farmers have limitations to access to infrastructure which restrict their options for management of FAW. Due to very rapid spreading capacity its complete control is very difficult. Its entry in Nepal may bring up to 100% yield decline in maize as warned by FAO. Although the loss assessment of the pest in Nepal has not been estimated yet, CIMMYT has constantly been working to manage the pest in Nepal through evaluation of push-pull strategy in which Napier grass and Desmodium are cultivated with maize crop. Integrated pest management approach is practiced by most of the farmers which include; physical, chemical, botanical, and biological method. Biological approach of management is most effective as it uses different parasitoids, pathogens, and predators to control the best. Chemical method of control is not generally preferred as it is expensive and environmentally toxic. Plant quarantine has been established in India-Nepal border to check the entry of such harmful pest. Different related institutions and organizations have been supporting the government and the smallholder farmers to help in managing and controlling the fall armyworm in Nepal.

REFERENCES

[1] Abrahams, P., Bateman, M., Clottey, V., Cock, M., & Colmenarez, Y. (2017). Fall Armyworm: Impacts and Implications for Africa. UK aid and CABI

[2] All JN. 1988. Fall armyworm (Lepidoptera: Noctuidae) infestations in no-tillage cropping systems. Florida Entomologist 71: 268-272.

[3] Altieri et al, M. F.-M. (2012). Agro-ecologically efficient agricultural systems for smallholder farmers: contributions to food sovereignty. Agronomy for Sustainable Development.

[4] Assefa.F .et .al. (2019). Status and control measures of fall armyworm (Spodoptera frugiperda) infestations in maize fields in Ethiopia: A review. Cogent Food and Agriculture.

[5] Batista-Pereira, L., Stein, K., Paula, A., Moreira, J., Cruz, I., & Figueiredo, M. (2006). Isolation, identification, synthesis and field evalution of sex pheromone of the Brazilian population of Spodoptera frugiperda. J Chem Ecol., 32(5), 1085-1099.

[6] Beshir, A. R., Shrestha, H. K., & Pradhan, B. (2019, January 9). Destructive Pest Alert: Fall Armyworm (FAW) is paving its way to Nepal. Retrieved June 22, 2020, from Research program on Maize: https://maize.org/destructive-pest-alert-fall-armyworm-faw-is-paving-its-way-to-nepal/#:~:text=The%20invasive%20agricultural%20pest%20Fall,threat%20to%20global%20food%20security.&text=The%20deadly%20pest%20has%20a,the%20Nepalese%20farmers%20and%20economy.

[7] Bhusal, K., & Bhattarai , K. (2019). A review on fall armyworm (Spodoptera frugiperda) and its possible management options in Nepal . Journal of Entomology and Zoology Studies , 7(4), 1289-1292.

[8] CABI, F. (2019). Community-Based Fall Armyworm (Spodoptera frugiperda)Monitoring, Early warning and Managemen. Training of Trainers Manual, p. 21.

[9] CABI. (2017). Invasive Species Compodium. Retrieved from CABI: https://www.cabi.org/isc/datasheet/29810

[10] CABI. (2017). Spodoptera frugiperda. Retrieved from Invasive species Compendium: https://www.cabi.org/isc/datasheet/29810

[11] Capinera, J. (2001). Order Lepidoptera- Caterpillars, moths and butterflies. In handbook of Vegetable Pests (pp. 353-510). Academic Press.

[12] Capinera.L.J. (July 1999). fall armyworm. Featured creatures .

[13] Chamberlain , K., Khan , Z., Pickett , J., Toshova , T., & Wadhams , L. (2006). Diel periodicity in the production of green leaf volatiles by wild and cultivated host plants of stem borer moths, Chilo partellus and Busseola fusca. J. Chem. Ecol, 32, 565-577.

[14] Chimweta, M., Nyakudya, I., Jimu, L., & Mashingadze, A. (2019). Fall armyworm [Spodoptera frugiperda (J.E. Smith)]damage in maize: management options for flood-recession cropping smallholder farmers. International journal of pest management, 1-13.

[15] Cruz, I., Figueiredo, Maria, d. L., da, S., Rafael, B., & da, s. (2012). Using Sex Pheromone Traps in the Decision-Making Proces for Pesticide Application against Fall Armyworm (Spodoptera frugiperda [Smith] [Lepidoptera:

Noctuidae]) Larvae in Maize. Faculty Publications: Department of Entomology, 530.

[16] Dively, G. (2018). Management of Fall armyworm (Spodoptera frugiperda) with emphasis on Bt Transgenic Technology. Retrieved from https://usunrome.usmission.gov/wp-content/uploads/sites/54/2018-Africa-FAW-Talk-Rome-pdf.pdf

[17] Early, R., Gongalez-Moreno, P., Murphy, S., & Day, R. (2018). Forecasting thhe global extent of invasion of the cerear pest Spodoptera frugiperda, the fall armyworm. Neo Biota, 40, 25-50.

[18] Evans.M.O. (2017, may 16). Fall Armyworm: Most Effective and Recommended Pesticides and Controls. Myfarm.

[19] FAO. (2017). Food Chain Crisis : . Retrieved from http://www.fao.org/food-chain-crisis/how-we-work/plant-protection/fall-armyworm/en/

[20] FAO. (2018). Integrated management of the Fall Armyworm on maize: A guide for farmers field schools in Africa. Retrieved from Food and Agriculture Organization of United nation.

[21] FAO. (2018, October 25). Fall Armyworm - an Emerging Food Security Global Threat. Retrieved June 21, 2020, from https://www.ippc.int/en/news/fall-armyworm-an-emerging-food-security-global-threat/

[22] FAO. (2018a). FAO launches mobile application to support fight against Fall Armywor in Africa. Retrieved from http://www.fao.org/news/story/en/item/1106850/icode/

[23] FAO. (2018c). FAW Guidance Note 2: Fall Armyworm Scouting. Rome: Food and Agriculture Organization (FAO).

[24] FAO. (2019, December 19). Fall armyworm. Retrieved 18 June, 2020, from Food and Agriculture Organizations of the United Nations: http://www.fao.org/fall-armyworm/en/#:~:text=Fall%20armyworm%20(FAW)%2C%20Spodoptera,of%20millions%20of%20smallholder%20farmers.

[25] FAO. (2020). Food and Agriculture Organizations of the United Nations. Retrieved July 17, 2020, from http://www.fao.org/fall-armyworm/monitoring-tools/faw-map/en/

[26] FAO. (2020, May 13). GIEWS - Global Information and Early Warning System. Retrieved June 21, 2020, from Food and Agriculture Organizations of the United Nations: http://www.fao.org/giews/countrybrief/country.jsp?code=NPL

[27] Firake.M.D, B. (June 2019). Fall Armyworm: Diagnosis and Managementt (An extension pocket book). Umiam-793 103, Meghalaya, India: The Director, ICAR Research Complex for NEH Region.

[28] Firake.M.D, B. (2019). Fall Armyworm: Diagnosis and Management. Umiam-793 103,Meghalaya, India: ICAR Research Complex for NEH Region.

[29] Frerot, B., Leppik, E., Groot, A., Unbehend, M., & Holopainen, J. (2017). Chemical Signatures in Plant-Insect Interactions. N. sauvion, D. Thiery, and P.-A. Calatayud (pp. 139-177). Advances in Botanical research .

[30] G.Ravi, J. R. (2019). Bio rational integrated pest management modules and on-farm biorational inputs. National Institute of Plant Health Management,.

[31] GC, Y., Dhungel, S., Ghimire, K., Devkota, S., & GC, A. (2019). Fall aaaarmyworm: Global Status and Potential Threats For Nepal. The Journal of Agriculture and Environment, 20.

[32] Global Fall Armyworm Management (2019). Programmatic Pesticide Evaluation Report And Safer Use Action Plan (Persuap), 11.

[33] Goergen , G., Kumar , P. L., Sankung, S. B., Togola, A., & Tamò, M. (2016). First Report of Outbreaks of the Fall Armyworm Spodoptera frugiperda (J E Smith) (Lepidoptera, Noctuidae), a New Alien Invasive Pest in West and Central Africa. PLOS ONE, 11(10).

[34] Groote, H. D., Kimenju, S. C., Munyua, B., Palmas, S., Kassie, M., & Bruce, A. (2020). Spread and impact of fall armyworm (Spodoptera frugiperda J.E. Smith) in maize production areas of Kenya. Agriculture, Ecosystems & Environment, 292, 106804.

[35] Gross, H., & Pair, S. (1986, Sep). The Fall Armyworm: Status and Expectation of Biological Control with Parasitoids and redators. Florida Entomological Society, 69(3), 502-515.

[36] Hailu, G., Niassy, S., Zeyaur, K., Ochatum, N., & Subramanian, S. (2018). Maize-legume intercropping and push-pull for management for fall Armyworm, Stemborers, and striga in Uganda. Agron J, 110(6), 2513-2522.

[37] Hardke, J., Lorenz, I., & Leonard, B. (2014). Opportunities to manage fall armyworm (Lepidoptera: Noctuidae)On Bollgard IIVR cotton with reduced rates of insecticides. J. Cotton Sci., 18, 59-67.

[38] Heinrichs.A.E. (2018). Pest Risk Assessment of the Fall Armyworm,Spodoptera frugiperda in Egypt. Innovation Lab for Integrated Pest Management, 12.

[39] Hoy, M. (2013). Insect population ecology and molecular genetics. M.A. Hoy, Insect Molecular Genetics (Third Edition) (pp. 591-659). Academic Press.

[40] Hruska , A., & Gould , F. (1997). Fall armyworm (Lepidoptera: Noctuidae) and Diatraea lineolata (Lepidoptera: Pyralidae): impact of larval population level and temporal occurrence on maize yield in Nicaragua. Journal of Economic Entomology, 90(2), 611-622.

[41] Hruska, A. (2019, September). Fall armyworm (Spodoptera frugiperda) management by smallholders. CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources , pp. 1-11.
[42] Hunt, T., Highley, L., Witkowski, J., Young, L., & Hellmich, L. (2001). Dispersal of adult European corn borer (Lepidoptera:CRambidae) within and proximal to irrigated and non-irrigated corn. J Econ. Entomol., 94, 369-377.
[43] Ivan, C., Figueiredo, Corrêa, M. d., Silva, d., & Braga, R. (2012). Using Sex Pheromone Traps in the Decision-Making Process for Pesticide Application against Fall Armyworm (Spodoptera frugiperda [Smith] [Lepidoptera: Noctuidae]) Larvae in Maize Faculty Publications. (p. 530). Department of Entomology.
[44] Jeyraman, S. (2017). Field crops Production and Management. New Delhi: Oxford and IBH publishing Co. Pvt Ltd., I.
[45] Khatiwada, D. Y. (2019, Nov 11). Economic Survey 2018/2019. Retrieved from Ministry of Finance: https://mof.gov.np/uploads/document/file/compiled%20economic%20Survey%20english%207-25_20191111101758.pdf
[46] Knoema. (2020, Februry). Nepal - Maize production quantity. Retrieved June 21, 2020, from https://knoema.com/atlas/Nepal/topics/Agriculture/Crops-Production-Quantity-tonnes/Maize-production#:~:text=In%202019%2C%20maize%20production%20for,average%20annual%20rate%20of%202.64%25.
[47] Kumbhar, R. A. (2019). Current Status and Management of Fall Armyworm in India. Research Gate.
[48] Li, M.; Zhao, C.; Li, D.; Yang., X.; Sun, C.; Wang, Y. (2007). Towards developing an early warning system for cucumber disease for greenhouse in china. International conference on Computer and computing Technologies in Agriculture: CCTA 2007: Computer and Computing Technologies in Agriculture VII, (pp. 1375-1378).
[49] Luginbill, p. (1969). Developing resistant plant-the ideal method of controlling insects. USDA-ARS Prod.
[50] Maharani, Y., Dewi, K. V., Puspasari, L., Rizkie, L., Hidayat, Y., & Dono , D. (2019). Cases of Fall Army Worm Spodoptera frugiperda J. E. Smith (Lepidoptera: Noctuidae) Attack on Maize in Bandung, Garut and Sumedang District, West Java. Jurnal Cropsaver, 2(1), 38-46 .
[51] Malo, E., Bahena, F., Miranda, M., & Valle-Mora, J. (2004). Factor affecting the trapping of males of Spodoptera frugiperda (Lepidoptera. Noctuidae) with pheromones in Mexico. Florida Entomol., 87(3), 288-293.
[52] Maredia KM, S. O. (1992). Effects of neem,Azadirachta indica on six species of insect pests. Tropical Pest Management.
[53] Midega, C., Pittchar, J., Pickett, J., Hailu, G., & Khan, Z. (2018). A climate-adapted push-pull system effectively controls fall armyworm, Spodoptera frugiperda (J E Smith), in maize in East Africa. Crop protection 105, 10-15.
[54] Mihm, J. A. (1994). Insect Reistant Maize Recent Advances and Utilization. International symposium. International Maize and WHeat Improvement Centre (CIMMYT).
[55] Mooventhan.P, S. B. (2019). Integrated Management of Fall Armyworm in Maize. NATIONAL INSTITUTE OF BIOTIC STRESS MANAGEMENT.
[56] Mwangi.K.D. (2019,may). Fall armyworm Identification,monitoring and management option for maize in Kenya. Fall Armyworm Technical Brief for Maize in Kenya.
[57] Poudel, S. (2020, January 15). Fall Armyworm: A Menace For Nepali Farmers. Retrieved June 17, 2020, from The Rising Nepal: https://risingnepaldaily.com/detour/fall-armyworm-a-menace-for-nepali-farmers#:~:text=An%20invasive%20pest%2C%20Fall%20armyworm,to%20have%20come%20from%20India.&text=According%20to%20pest%20experts%2C%20Fall,kinds%20of%20crops%20including%20maize.
[58] Poudel, S. (2020, june 19). Fall Armyworm: . Retrieved from The Rising Nepal: https://risingnepaldaily.com/detour/fall-armyworm-a-menace-for-nepali-farmers
[59] Pradhan.B. (2020, april 7). Collective efforts to fight fall armyworm in Nepal. CIMMYT.
[60] Prasanna, B., Huesing, J. E., Eddy, R., & Pcschko, V. M. (2018). Fall Armyworm in Africa: A Guide for integrated Pest Management,First Edition. Mexico,CDMX: CIMMYT.
[61] Prasanna, B., Huesing, J., Eddy, R., & Virginia, R. (2019). Fall Armyworm in frica, A guide for integrated Pest Management. Mexico:USAID and CIMMYT.
[62] Reis, L., Oliveira, L., & Cruz, I. (1988). Doru luteipes Biology and potential in Spodoptera Control. Brazilian Agriculture Research, 23, 333-342.
[63] Rwomushana, I., Bateman, M., Beale, T., Beseh, P., Cameron, K., Chiluba, M., et al. (2018, October). Fall Armyworm: Impacts and Implication for Africa; Evidence Note Update. CABI: Oxfordshire.
[64] Sarmento , R. D., Aguiar , d. R., Vieira, S., de Oliveira , H., & Holtz , A. (2002). Biology review, occurrence and control of Spodoptera frugiperda (Lepidoptera: Noctuidae) in corn in Brazil. Biosci. J, 18, 41-48.
[65] Shorey, H., Summers, C., Sisk, C., & Gerber, R. (1994). Disruption of pheromone communication in Spodoptera exigua (Lepidoptera:Noctuidae) in tomatoes, alfalfa, and cotton. Environmental Entomology, 1529-1533.

[66] The Himalayan Times. (2020, May 25). Armyworm severely affects maize production. Retrieved June 19, 2020, from https://thehimalayantimes.com/business/armyworm-severely-affects-maize-production/

[67] Wang, D., Chen, T., & Dong, J. (2013). Research of the early warning analysis of crop disease and insect pests. International Conference on Computer and Computing Technologies in Agriculture: CCTA 2013: Computer and Computing Technologies in Agriculture VII, (pp. 177-187).

[68] Zanolli, G. (2018). Integrated management of the Fall Armyworm on maize. Rome, Italy: Food and Agriculture Organizatiosn of the United Nations (FAO).

Cashew (*Anacardium occidentale* L.) Production, Processing Facilities and Quality Assessment in Kafubulum Chiefdom, Sierra Leone

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Abstract— *The research was conducted to investigate the cashew production, processing facilities and quality assessment in Sierra Leone Agricultural Research Institute (SLARI)/Kenema Forestry and Tree Crops Research Centre (KFTCRC), Sierra Leone. A total of 80structured questionnaires on cashew production, processing facilities and quality assessment were administered to farmers including men and women in the Kafubulum Chiefdom, Port Loko District. Data obtained were analysed descriptively and quantitatively. Simple arithmetic like percentages were used to analyze the data. Tables were then used to present the facts and finding recording the research. The research revealed the Red and Yellow cashew colours were the varieties cultivated in the study area and red cashew gave better yield in the Kafubulum Chiefdom from the interview conducted. Upon the two colours, Red reported to be high yielding with 55% respondents. Also, cashew farmers have no processing facility except for roasting and drying the cashew kernel 100% each from the results obtained. This was shown from the analyses that 100% of these producers were willing to process their cashew to kernel.In general, the quality of the cashew kernels were high as farmers were able to maintain the exact required kernel 8% moisture content through colour observation and taste and Premium with 52.5% kernel grades were discovered.*

Keywords— ***Cashew, nut, production, processing, quality.***

I. INTRODUCTION

1.1 Background to the study

Cashew (*Anacardiumoccidentale* L.) is one of the important commercial plantation crops, which today is deemed to be the source of dollar earning crop for the country. It is in fact, a poor man's crop and the rich man's favorite snack food all over the world. The cashew is a kidney shaped drupaceous nut, greenish gray in colour. A single nut is 3 to 5 cm in length and 2 to 3.5 cm in width measures about 3 to 20 g weight.The cultivated cashew is a low spreading ever green tree of the tropics. It is presumed that cashew was originally introduced into India mainly for checking soil erosion. But gradually it gained commercial importance and is one of the ten top foreign exchange earners. In the earlier years of cashew production in India, the apple was considered valuable onto the 107th century when the cashew kernel gained commercial importance, and hence found favour among consumers. Prior to the First World War quantities of cashew kernel was exported from India to USA.

The French, Portuguese and Dutch seafarers described cashew from Brazil in the sixteenth century and the first illustrative description of cashew was given by a French naturalist in 1558 A.D. Cashew was introduced to the Malabar Coast of India in the sixteenth century by the Spaniards, and probably served as a locus of dispersal to other centers in India and South East Asia. The Spaniard who have been aware of the use of cashew in medicine, foods and beverages, probably visualized the potential importance of this crop to India. "Acaju" is the name given to cashew by the native Tapi Indians of Brazil and the French name “Acajou” is the nearest equivalence of the original name.

The Portuguese dropped the letter ‘A’ and became ‘Caju’ in Portuguese. The Kashmiri, Panjabi, Hindi, Marathi and Gujarati lexicons in India also referred to the cashew as ‘Caju’. It is probable that the use of kernel spread from Goa to Maharashtra, then to Gujarat, Rajasthan, Punjab and Kashmir and other Hindi speaking areas. In Kerala, it is called “Parangi Andi” meaning foreign or “Portuguese nut”. It is also known as “Kasu Andi”, “Kasu” meaning money and “Andi” the nut. In Tamil, it is known as “Mundiri” indicating the position and shape of the nut. In Oriya it is known as “Lanka Beeja” indicating that cashew reached Orissa by sea from Sri Lanka.

The Bengalis know cashew as “Hijlibadam” and Assamese refer to the nut as “CajuBadam”. Thus, most of the names used in India were derived from the Portuguese name “Caju” for cashew.

This serves as a piece of evidence that the cashew originated in Brazil (Nambiar, 1979). When the price of the nuts became attractive, it wasnot uncommon to see some cashew growers harvesting immature nuts the apple of which has not even started developing. In the absence of a market for cashew apple, farmers do harvest nuts early causing loss to themselves as well as to the processors.

Some intelligent farmers harvest nuts from the fully ripened cashew apple or ripe fallen cashew apples, so these matured nuts give good quality bold kernels on processing and it is profitable to both farmers and processors (Vaidehi, 2000).

At present, the major cashew growing countries of the world are India, Brazil, Indonesia, Vietnam, Nigeria, Mozambique and Tanzania.

The productivity of cashew nut at national level is 593 kg per ha which is quite less than the potentiality of more than 1000 kg per ha if the correct planting materials and latest technologies could be adopted (Directorate of Cashew Development, Cochin, 2008).

1.2 Statement of the Problem

Cashew nut production and processing is another source of employment and income generation especially to the rural people in Port Loko, Bombali and Kambia Districts. It makes poor rural farmers realize foreign exchange earnings. So, its role is important in the determination of economic development of the districts. Similarly, in Kambia and the other Districts, it plays a key role in employment generation and economic empowerment. The economic importance of the cashew nut production and processing is based on the great number of workers employed and the amount of foreign currency earned through cashew nut exports.

The majority of all cashew processors are women i.e. about 95%. This is also true in the case of cashew processors in Kambia, Port Loko and Bombali Districts. Except in roasting but, women workers are engaged in shelling, peeling, grading, and packaging sections.

The problem of the present research is to analyze the cashew nut production and processing facilities in the aforementioned district in Sierra Leone.

The most employed types of cashew nut processing stages are drying, roasting, shelling, peeling, rehumidification, grading, packaging and fabrication of processing equipment. Moreover, time engaged in cashew processing is from 8:30 AM to 5:30 PM. Hence, through this research earnest attempt will be made by the researcher to find out the factors that determine the status of cashew production and processing facilities in the Port Loko District.

1.3 General and Specific Objectives of the Research

1.3.1 General Objective of the Research

The main aim of this research wasto investigate cashew (*Anacardiumoccidentale* L) Production, Processing Facilities and Quality Assessment in Sierra Leone.

1.3.2 Specific Objectives of the Research

In order to achieve the study goal, the following specific objectives were taken into cognizance as thus below:

- determined the availability of cashew processing equipment and fabrication units.
- assessed the quality of cashew nut produced in the chiefdom.
- identified the constraints associated with production, processing and marketing of cashew nut.

1.4 Justification of the Research

Although several efforts have been made in different parts of the world to improve on the cashew nut production and processing but, serious attention has not been given to fabrication units. This research unearthed some of the problems faced in the processing of Cashew Nut and the Cashew Apple in the study area. The current research will serve as the point of entry for the introduction of appropriate processing technology to upgrading cashew nut products to premium. It will also serve as a baseline for future work on post-harvest technology of cashew nut production and processing facilities in Sierra Leone.

1.5 Limitations of the Research

This research was limited on the following factors:

- Availability of secondary data was a big issue as people have not really have written on this topic.
- Financial constraint in getting stationaries and even getting to website was not easy.
- Transportation: It was difficult to move from Kenema to Port Loko District for data acquisition.

II. MATERIALS AND METHODS

2.1 Study Area

This research was conducted in the Kafubulum Chiefdom, Port Loko District, North of Sierra Leone.

2.2 Data Collection

2.2.1Sample Size

80 Cashew Farmers including men and women were administered structured questionnaires on cashew production and processing facilities.

2.2.2Instrumentation

This includes:

2.2.3 Questionnaire

A well-structured questionnaire was tailored according to the directives of this research. The questionnaires were distributed among respondents in order to solicit their responses.

2.3 Data Analysis

Data were analysed descriptively and quantitatively. Simple arithmetic like percentages were used to analyze the data. Tables were then used to present the facts and finding and recording of the research.

III. CHARACTERISTICS

3.1 Demographic Characteristics

Table 3.1: Demographic characteristics of cashew farmers

Variable	Frequency (n=80)	Percentage
Sex		
Male	80	100
Female	0	0
Total	100	100
Age Bracket (Yrs)		
15-25	0	0
26-35	48	60
36-45	32	40
46+	0	0
Total	80	100
Experience in Cashew farming (Yrs)		
0-2	40	50
3-5	0	0
6-8	40	50
9+	0	0
Total	80	100

Table 3.1 above shows that male cashew farmers were highly engaged in cashew production with 100% respondents. It was observed that there was no gender balance in cashew production from the interview conducted. Accordingly, 60% (48) of cashew farmers interviewed were within the age brackets of 26-35 years while 40% (32) of them were within 36-45 years old. It can also be depicted that young people within the ages of 15-25 years do not involve in cashew processing likewise the age of 46 years and above. Half (50%) the total respondents have spent two or less years in cashew production and in the same vain 50% of them have six to eight years of experience in producing cashew in that part of the country.

3.2 Cashew Production

Table 3.2: Shows the estimated size of the cashew farm

Size (Acre)	No. of respondent	Percentage (%)
1-5	30	37.5
6-10	50	62.5
Total	80	100

According to table 3.2 above, 37.5% of the cashew farms were within 1-5 acres while 62.5% reported on the farm size ranging from 6-10 acres. It can be depicted form the data analysis that cashew farmers in the study area did not grow cashew in large scale, it could be access to land availability or lack of capital to invest in cashew production and processing.

Table 3.3: Shows the varieties of cashew grown

Variety	No. of respondent	Percentage (%)
Red	44	55
Oblong	0	0
Yellow	36	45
Pyriform	0	0
Variegated	0	0
Tall	0	0
Round	0	0
Dwarf	0	0
Other	0	0
Total	80	100

On the table 3.3, 55% of the Red Colour cashew variety was produced in the Port Loko District in Northernof Sierra Leone and 45% was Yellow variety. It shows that only two varieties of cashew were produced in the district. This was so because of the availability of cashew varieties in Sierra Leone. Like in Ghana there are numerous cashew varieties available such as SG 266, SG 276, SG 185, SG 278, SG 273, BE 079, BE 107, SG 261, TAN 393 and SG 265 and many more. However, these cashew varieties are available and recently established in the Kpuwabu and Pendembu Research Stations of Kenema Forestry and Tree Crops Research Centre (KFTCRC) of the Sierra Leone Agricultural Research Institute (SLARI).

Table 3.4: Shows the time of cashew harvesting

Time (moth)	No. of respondent	Percentage (%)
January-March	0	0
April-June	80	100
July-September	0	0
October-December	0	0
Throughout	0	0
Total	80	100

Table 3.4 above shows the harvesting time of cashew which was between April-June and indicated 100% response from the interview conducted. However, harvesting of cashew may vary based on the variety and climatic condition. It was reported that a community called Gbogoma Junction in the Kenema District, cashew tree bears two times every year. Also, a cashew tree at the Ministry of Agriculture and Forestry (MAF)'s Compound in IDA Kenema, bears two times a year and is a Red Colour Cashew.

Table 3.5: Shows methods used in cashew harvesting

Method	No. of respondent	Percentage (%)
Hand picking	32	40
Knife	0	0
Fall	48	60
Total	80	100

The analysis above revealed that 60% of the farmers did not harvest their cashew; instead they wait until when the cashew nut and its apple fall on the ground. However, 40% violated the rule of ripe cashew by the method of hand picking which is not recommended. The principle of wait until when the ripe cashew apple falls has not been implemented yet all over in the study area as demonstrated with the result above. Except for juice production, ripe cashew apple has to be harvested by hands.

3.3 Cashew Processing

Table 3.6: Shows the respondents who processed and those that do not process cashew

Option	No. of respondent	Percentage (%)
Yes	80	100
No	0	0
Total	80	100

From table 3.6 above, it came out clearly that all cashew producers with 100%normally process their cashew before sales. The results also showed that cashew is a crop that is processed before packaging and marketing.

Table 3.7: Shows stages of cashew processing done by farmers

Stage	No. of respondent	Percentage (%)
Roasting	54	67.5
Drying	26	32.5
Collection of kernels	0	0
Total	80	100

The analysis above revealed that 67.5% of cashew farmers roasted their cashew nut, but however, 32.5% donot roast the nut before drying. It also showed that not all cashew processing stages were done in the processing of cashew nut with 0% respondent for the remaining processing stages.

Table 3.8: Shows the methods used in cashew separation by farmers

Method	No. of respondent	Percentage (%)
Cutlass	0	0
Stick	0	0
Knife	24	30
By hand	56	70
other	0	0
Total	80	100

According to table 3.8, the respondents agreed that they normally separated their Cashew Apple from the Cashew Nut with rope with 70% value and the remaining 30% use their hands for separating the Nut from the Apple. This was so because cashew farmers were trained how to separate the ripe cashew apple from the nut. No other method (s) were discovered from the cashew farmers for the aforementioned cashew processing stage.

Table 3.9: Shows the problems faced in cashew separation

Problem	No. of respondent	Percentage (%)
Kernel damage	0	0
Apple damage	80	100
CNSL damage	0	0
Total	80	100

Apple damage was a major problem faced by cashew farmers during separation as reported intable 3.9 with 100% agreed by the respondents interviewed. However, cashew farmers needed more training on the separation of ripe cashew apple from the nut to avoid the 100% damage. However, Kernel damage and Cashew Nut Shell Liquid (CNSL) were not experienced as problems faced in cashew separation as 0% reported for both cases.

Table 3.10: Shows the respondents normally dry cashew nut

Option	No. of respondent	Percentage (%)
Yes	80	100
No	0	0
Total	80	100

The option yes was chosen by the respondents that in deed the cashew farmers were not only producing cashew but also processed the nuts as value addition of cashew production, processing and marketing systems.

Table 3.11: Shows where the respondents dry their cashew nut

Place	No. of respondent	Percentage (%)
Drying floor	80	100
Raised platform	0	0

Solar dryer	0	0
Wood dryer	0	0
Total	80	100

Drying floor was the only place where cashew producers dried their produce with 100% agreed while other drying methods were not practiced. For quality cashew product, it is not recommended to dry cashew nuts on drying floor rather under shade for better quality and avoid foreign particles.

Table 3.12: Shows the duration of cashew nuts drying

Duration (day)	No. of respondent	Percentage (%)
1	0	0
2	0	0
3	22	27.5
4	0	0
5	0	0
1 week	58	72.5
Total	80	100

From table 3.12 above, most cashew farmers dry their cashew nuts for a period of 1 week with 72.5% during the interview conducted in Port Loko District. Also, few cashew farmers dry their cashew nuts within a period of 3 days before sold to their contact partners or buying agents.

Table 3.13: Shows problems faced duration cashew nuts drying

Problem	No. of respondent	Percentage (%)
Swelling	20	25
Theft	60	75
Burnt	0	0
Total	80	100

Theft case was reported as a major problem faced by cashew farmers and shows 75% value on table 3.13. However, swelling was also reported as another major problem faced by cashew producers. Here, sorting has to be considered as great practice before, during and after the cashew drying.

Table 3.14: Shows if cashew nuts is soak before roasting

Option	No. of respondent	Percentage (%)
Yes	0	0
No	80	100
Total	80	100

Apart from other cashew processing stages practiced by cashew farmers, soaking was not recognized as a major processing stage in cashew processing which is the initial processing stage for kernel production. Farmers preferred not to soak the cashew nuts and this indicates 100% on the table. Soaking should be the first cashew processing stage followed by roasting.

Table 3.15: Shows cashew nut is roasted

Option	No. of respondent	Percentage (%)
Yes	50	62.5
No	30	37.5
Total	80	100

Roasting was considered an important processing stage with 62.5% respondents and has been practiced in the Port Loko District for quite some time now. However, some farmers did not consider it as an important stage in cashew processing with 37.5% respondents.

Table 3.16: Shows where cashew nuts is roasted

Place	No. of respondent	Percentage (%)
Drum	66	82.5
Pot	6	7.5
Roaster	2	2.5
Other	6	7.5
Total	80	100

From the survey conducted, it shows that the main place for roasting cashew nuts wasthe drum with 82.5% respondents. Some cashew farmers also roasted their nuts in their pots, while others didit with roaster.

Table 3.17: Shows problems faced with cashew nuts after roasting

Problem	No. of respondent	Percentage (%)
Cracking	50	62.5
Bad odor	30	37.5
Total	80	100

Cracking wasshown to be a problem faced by cashew farmers although bad odour also reported to be another problem during roasting with 62.5% and 37.5% respectively on table 3.17.

Table 3.18: Shows that the farmers deshelled their cashew nuts

Option	No. of respondent	Percentage (%)
Yes	50	62.5
No	30	37.5
Total	80	100

Even though cracking has been a serious problem as a processing stage faced by cashew farmers, majority of the processors managed to deshell their cashew nuts for value addition with 62.5% agreed.

Table 3.19: Shows methods of deshelling cashew nut

Method	No. of respondent	Percentage (%)
Mechanically	16	20
Manually	50	62.5
Other	14	17.5
Total	80	100

Manual deshelling was the method used in the Kafubulum Chiefdom in the Port Loko District according to the interview conducted. However, some farmers also practiced mechanical method of deshelling. Also, there were other cashew farmers who practiced other methods of deshelling with 17.5% reported on the analysis.

Table 3.20: Shows problems faced in cashew nut deshelling

Problem	No. of respondent	Percentage (%)
With stone	50	62.5
With hand	30	37.5
Other	0	0
Total	80	100

The other methods of deshelling practiced by cashew farmers although reported to pose problem by the cashew farmers and such problems occurred with the use of stones with bear hands. Both of them showed significant values as indicated in table 3.20 above.

Table 3.21: Shows if kernel grading was done

Option	No. of respondent	Percentage (%)
Yes	50	62.5
No	30	37.5
Total	80	100

Despite series of problems faced by the cashew producers, it was reported that the cashew producers went ahead in grading their cashew kernel with 62.5% agreed during the interview conducted.

Table 3.22: Shows methods of kernel grading

Method	No. of respondent	Percentage (%)
Sorting by size	50	62.5
Colour	10	12.5
Weight	4	5
Oil content	4	5
Palatability	4	5
Other	4	10
Total	80	100

The aforementioned cashew kernel grading was done by sorting the sizes as indicated in table 3.22with 62.5% value. Some farmers also graded their cashew kernels through colour observation, weight, oil content and palatability but all with minute percentages.

Table 3.23: Shows the problems in kernel grading

Problem	No. of respondent	Percentage (%)
Theft	52	65
Price	28	35
Total	80	100

After cashew kernel grading, the other problem faced by the producers was theft with 65% reported and also followed by the price of the cashew kernels which made the producers lose lots of income during the sales.

Table 3.24: Shows cashew kernel packaging

Option	No. of respondent	Percentage (%)
Yes	80	100
No	0	0
Total	80	100

All Cashew farmers agreed to packaging their cashew kernel products as indicated in the above table.

Table 3.25: Shows the packaging materials of cashew kernels.

Place	No. of respondent	Percentage (%)
Jute bag	0	0
Plastic bag	80	100
Carton	0	0
Box	0	0
Total	80	100

The only place for cashew kernel packaging was plastic bag with 100% responded from the survey conducted in Kafubulum Chiefdom, Port Loko District, Sierra Leone.

Table 3.26: Shows problems faced in kernel packaging

Problem	No. of respondent	Percentage (%)
Yes	80	100
No	0	0
Total	80	100

It was reported that even though cashew kernel was packaged in plastic bags but the producers also faced serious challenges during this process. This was so as a result of acquisition of plastic bags.

Table 3.27: Shows if moisture content of the cashew kernel is normally determined

Option	No. of respondent	Percentage (%)
Yes	50	62.5
No	30	37.5
Total	80	100

In cashew processing, the most paramount objective is the determination of the moisture content of the kernel, as 62.5% of the respondents interviewed agreed that they normally determine the moisture content of the cashew kernel. However, very few disagreed with 37.5% reported.

Table 3.28: Shows how moisture content of cashew kernel determined

Instrument	No. of respondent	Percentage (%)
Moisture Metre	30	37.5
By observation	50	62.5
Total	80	100

No instrument was available during the determination of the moisture content of the cashew kernel except by observation with 62.5% reported. But however, very few cashew farmers have access to the Moisture Metre to determine the moisture content of dry cashew kernel as shown above.

Table 3.29: Shows the specific percentage moisture content of cashew kernel

Moisture content (%)	No. of respondent	Percentage (%)
1	4	5
2	6	7.5
3	4	5
4	6	7.5
5	10	12.5
6+	50	62.5
Total	80	100

The specific percentage moisture content of cashew kernel for most cashew farmers was reported as 6% and above and the corresponding percentage was 62.5% which is within the recommended moisture content of dry cashew kernel. However, some farmers normally dried their cashew kernel below the recommended moisture content of dry cashew kernel.

3.4 Storage of cashew nut

Table 3.31: Shows storage of cashew kernels

Storage	No. of respondent	Percentage (%)
Jute bag	0	0
Drum	30	37.5
Plastic bag	50	62.5
Total	80	100

It has been uncovered that two cashew kernel storage systems were practiced in the study area of this research. But never the less, plastic bag was the most practicable cashew kernel storage system with 50% respondents agreed on table 3.31 above. However, cashew can be stored in jut bag and the final product (cashew kernel) can be stored in cartoon for export purposes.

Table 3.32: Shows the problems in the storage of cashew kernel

Problem	No. of respondent	Percentage (%)
Germination	56	70
Mould	24	30
Total	80	100

Problem was encountered during the storage of cashew kernel when plastic bags were used and one of these problems was germination which had a70% score. It has been indicated that the preferred storage system was not appropriate which also caused mould development as a result of Internal Moisture Migration (IMM) within the dried cashew kernels inside the plastic bags.

3.5 Cashew Quality

Table 3.33: Shows if cashew kernel is normally assessed and qualified

Option	No. of respondent	Percentage (%)
Yes	80	100
No	0	0
Total	80	100

The quality of cashew kernels was assessed by the cashew producers as 100% respondents have been reported on the table above. The cashew farmers assess and qualify their cashew products before being taken to the market for sale.

Table 3.34: Shows how cashew kernel is normally assessed and qualified

Quality	No. of respondent	Percentage (%)
Flavour	0	0
Colour	52	65
Taste	28	35
Aroma	0	0
Total	80	100

It can also be depicted from the table above that colour was used to assess and qualify the quality of cashew kernel by producers through the interview conducted. Some cashew farmers also use taste as another means of assessing and qualifying cashew kernel quality.

Table 3.35: Shows how cashew kernel is graded

Grade	No. of respondent	Percentage (%)
Premium	42	52.5
Grade 1	15	18.75
Grade 2	8	10.0
Grade 3	15	18.75
Total	80	100

Premium grade was the highest cashew quality reported on table 3.35 which shows 52.5% number of respondents.

Grades 1 and 2 qualities were also reported on the data collected and analyzed.

Table 3.36: Shows the problems faced after kernel grading

Problem	No. of respondent	Percentage (%)
Shortage	60	75
Weight	20	25
Total	80	100

It has also been depicted on table 3.36 that after premium, grade1 and grade 2 obtained normally shortage and this was another serious threat or loss faced by the cashew producers. Also, weight loss was another problem discovered during the analyses and discussions of these data.

3.6 Cashew Marketing

Table 3.37: Shows sales in cashew kernel

Option	No. of respondent	Percentage (%)
Yes	80	100
No	0	0
Total	80	100

From the table above,it shows that 100% of the farmers sold their cashew products to nearby contact partners and buying agents within the area.

Table 3.38: Shows where the kernel is sold

Sales	No. of respondent	Percentage (%)
Contact partners	40	50
Buying agents	40	50
Local processors	0	0
Total	80	100

According to the data above on table 3.38, it shows that, all cashew kernel products were sold to contact partners and buying agents within their vicinities.

IV. CONCLUSION

4.1 Summary

After data analysis and discussions, the following summary were reached and provided sound based on the results obtained from the research.

Processing Facility

From the analyses and discussions of the results, it was summarized that the cashew farmers in the Kafubulum Chiefdom of Port Loko, North of Sierra Leone have no processing facility to produce cashew kernel except for roasting and drying the cashew nuts.This was shown from the analyses that 100% of these producers were willing to process their cashew products. There is therefore the need to avail processing facilities to cashew farmers so that they can add value to their produce and by extension earn additional income.

Quality Assessment

Never the less, the cashew farmers did assess their cashew kernel based on colour and taste. Grades like Premium, Grade 1, Grade 2 and Grade 3 were obtained from the assessment made. For quality management, Premium is the accepted cashew kernel grade. In general, the quality of the cashew kernel was high as farmers were able to maintain the exact required kernel moisture content from the analyses made.

Cashew Yield

The Red and Yellow Colour cashew gave better yield in the Kafubulum Chiefdom from the interview conducted. Upon the two colours, Red Colour reported to be high yielding with 55% respondents.

It can be concluded by [12] from the literature that over 30 cashew varieties were planted in India whereas for Kafubulum Chiefdom, 2 cashew varieties were discovered. Generally, the mode of production, collection and storage practices affect the quality of cashew nuts. Smallholder farmers may harvest apple to meet urgent cash needs, without minding the maturity status of the nuts. From the literature review, all post-harvest operations relating to cashew kernel production were practiced, but from the survey conducted, most practices were skipped. This practice contributes to about 40% post-harvest losses of cashew nuts from the literature while for this research no post-harvest losses were discovered in the study area.

Cashew nut processing is generally done on a home scale and factory scale. In the home scale, the dried nuts are burnt in an open fire and hand shelled, from the research conducted, small scale kernel production was discovered.

Cashew tree yield of 1.5 to 4 kg of nuts/tree have been reported for Africa [1], and 7 to 11 kg of nut/tree for South Asia [1]. In Nigeria, mature cashew tree nut yields of <1kg to over 20 kg of nuts were obtained [5]. Nut yield in the range of 7.8 to 14.0 kg/tree were also observed in some Nigerian cashew germplasm collections [6]. [8] obtained nut yields of 0.25, 2.41, 8.65, 10.02 and 30.50 kg/tree for some Tanzanian varieties. In India, cashew varieties with tree yield capacity of 10 to 13 kg of nuts/tree have been distributed to farmers [13], but for this research focus was made on the production, processing and quality management but little on cashew yield.

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REFERENCES

[1] African Cashew Alliance. “Competitiveness of the African Cashew Sector, African Cashew Initiative (ACi).” February, 2011.
[2] Aliyu, O.M. and J.A. Awopetu, 2007. Assessment of genetic diversity in three populations of cashew using protein-isozyme electrophoresis analysis. Genetic Resource. Crop Evol,54: 1489-1497.
[3] Aliyu OM, 2000. Development of effective vegetative propagation technique (budding) in cashew. Annual Research Report of the Cocoa Research Institute of Nigeria, Ibadan, Nigeria. Pp. 35-35.
[4] Aliyu OM, 2001. Development of rapid method of propagating improved cultivars of cashew through budding, grafting and marcotting. Annual Research Report of the Cocoa Research Institute of Nigeria, Ibadan, Nigeria. Pp. 30-31
[5] Aliyu OM, 2004 Characteristics and compatibility studies in Cashew (Anacardium occidentale L.). Ph.D. Thesis, University of Ilorin, Nigeria. 266 pp.
[6] Aliyu OM, Awopetu JA (2007a). Multivariate Analysis of Cashew (Anacardium occidentale L.) Germplasm in Nigeria. Silvae Genetica 56: 3-4.
[7] Chemonics International Inc, 2002, Subsector Assessment of the Nigerian Cashew Industry. Available at http://pdf.usaid.gov/pdf docs/PNACY675.pdf. (Accessed April18, 2008) Clarke, Susan E; Gary L. Davis (1992), Post federal Local Economic Development strategies; Economic Development Quarterly 6 (2): 187–198. Available at http://en.wikipedia.org/wiki/Waves of Economic Development. (Accessed, January10, 2011).
[8] Desai AR, 2008. Molecular diversity and phenotyping of selected cashew genotypes of Goa, and physiological response of cv. Goa-1 to in situ moisture conservation. Thesis submitted to the University of Agricultural Sciences, Dharwad in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Horticulture. Department of Horticulture, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad-580 005.
[9] IRD, 2001. Cashew seed selection, Planting, and Production techniques. International Relief and Development Publication. The Gambia. Pp. 14-19.
[10] Martin PJ, Kasuga LJ 1995. Variation in cashew tree yield in South-East Tanzania and the implication for management of cashew smallholder farmers in Tanzania. Exp. Agric. 72:261-268.
[11] Sagar, 2007. Propagation studies in cashew nut (Anacardium occidentale L.) under mist house conditions. An M.SC. Thesis submitted to the Department of Horticulture College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad-580 005.
[12] Sharma VK, 2004. Trees and Protection of Environment. Deep and Deep Publications, 63.
[13] The Hindu, 2000. Online edition of India’s National Newspaper Thursday, December 14 2000. Source: http://hindu.com/2000/12/14/stories/0814002a.htm

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Preliminary Survey of Composition, Generation and Management of Solid Wastes in Ward 7, Soc Trang City, Soc Trang, Vietnam

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***Abstract*— *This research was to investigate the current status of solid waste generation, composition and management in Ward 7, Soc Trang City, Soc Trang Province through interviewing the households of understandings and practices with solid wastes and the placing the plastic bags for collecting the generated wastes directly at the households for compositional analysis. The results showed that organic matter accounted for 62.31%, recyclable and reusable items such as plastic accounted for 19.61%, paper occupied 14.55%, glass took 1.16 %, accounted for 0.79%, cloth occupied 1.69% and foam took 0.74%. Hazardous waste only accounted for a small proportion (0.11%). The average rate of solid waste generation was 0.91 kg/household/day and the average waste generation rate per person was 0.22 kg/person/day. Currently, the management, collection and transportation of domestic solid wastes in the ward 7 has not been guaranteed, not yet effective, causing several environmental problems; open dumping is still commonly seen in the study area due to lack of sanitary landfills. Local authority should take proper measures to effectively manage solid wastes in Ward 7, Soc Trang City, Soc Trang province.*

***Keywords*— *domestic wastes, waste collection, sorting system, generation rate, Soc Trang*.**

I. INTRODUCTION

The natural area of Soc Trang province is 160,058.69 hectares, accounting for about 4% of the Mekong Delta area and about 0.4% of the country's total natural area. Soc Trang province comprise of eight administrative units including 1 city, 2 towns and 5 districts. As of April 1, 2019, Soc Trang province's population reached 733,017 people, population density reached 480 people/km². In particular, the urban population reaches nearly 186,371 people, accounting for 25.4% of the provincial population, the rural population reaches 546,646 people, accounting for 74.6% of the population. Economic growth rate (GRDP) reached 6.5%, of which agriculture - forestry - fishery (region I): 2.77%, industry - construction (region II): 10.19%, trade - service area (region III): 6.51%. GRDP per capita is VND 45 million/person/year, equivalent to USD 1,945, up 15.63% over the same period (Soc Trang People's Committee, 2019). Soc Tran province is entering the period of industrialization - modernization of the country and the development society in order to meet the needs and interests of the people, while also leading to difficult problems such as environmental pollution is increasing. The amount of waste generated from daily activities as well as production activities of people is increasing, and the level of environmental pollution is increasingly serious in many different regions. In 2015, the whole province of Soc Trang had a solid waste generation of nearly 350 tons/day. In particular, non-hazardous solid waste was about 312.34 tons/day, hazardous solid waste was approximate 37.28 tons/day. It is expected that the amount of generated solid waste will continue to increase by 741 tons/day by 2025 (Soc Trang People's Committee, 2019). Currently, solid waste classification in the province has not been implemented at source, the collection rate is not high, in some places the collection only reaches 45%. The main treatment technology is landfill, while the landfills are overloaded, unhygienic, there is no form of leachate treatment. Previous research showed that leachate from the landfill contained heavy metals (Toufexi et al., 2013). The concentration of heavy metals in leachate depends on the composition of the waste. Heavy metals are considered hazardous waste because they can enter the food chain and increase levels in the organism. Heavy metals present in the environment around the landfill are a major threat to human and biological health because they are durable, non-biodegradable, can disperse and accumulate in the ecosystem, plants and animals, and finally to humans through consumption (Klinsawathom et al., 2017). To solve the problem of domestic waste, it is necessary to have a synchronous solution to limit the generation, classification at source, collection, transportation, disposal, science and technology and finance. The study was conducted to preliminarily investigate the composition and rate of generation of pilot solid waste in Ward 7 of Soc Trang City. The study also investigated people's knowledge about solid waste in the study area. The research results provide important information for solid waste management in ward 7, Soc Trang city.

II. MATERIALS AND METHODS

Information on the current situation of generation, collection, transportation and solid waste management in Ward 7, Soc Trang city was collected by interview the households. The questionnaire content included general information about the interviewee such as gender, age, education level, occupation; information on current situation, source separation, collection time, impacts of solid waste on environment and health, assessment of current status of solid waste management in the study area. To assess the rate of generation and composition of solid waste, thirty households in the surveyed area were selected for providing plastic bags to store all solid waste in the family. Each day the placed plastic bags were collected at 5:00 PM to classify and calculate solid waste composition and rate of generation of solid wastes. Waste separation was conducted in accordance with the guidance of the Department of Natural Resources and Environment of Ho Chi Minh City. Specifically, organic waste includes easily biodegradable waste including uneaten food, vegetables, tubers, fruits, leaves, twigs and recalcitrant including nylon bags, straws, bottles, glasses, plastic jars; inorganic wastes includes glass, bottles and jars; toxic wastes includes battery, light bulb, rubber, empty pesticide bottles; and other waste includes soil, stone, rubble. The interview data were imported into Excel spreadsheets (Microsoft Excel 2016, Microsoft, USA) to aggregate, calculate percentages and present data in the form of simple tables and charts.

Fig.1: Collected solid wastes from the households

III. RESULTS AND DISCUSSION

3.1 General information of the respondents

Males were accounted for 73.3% while females occupied 26.7%. In the surveyed area, Kinh ethnic group was 73%, Chinese ethnic group was 7% and the Khmer ethnic group was 20%.Educational level of the households was relatively diverse. The interviewees with university, college, high school, secondary school, primary school, and illiteracy accounted for 10%, 23%, 44%, 13%, 7% and 3%, respectively (Figure 2). The education level of the interviewees is an essential issue in the people's understanding of the environment. It can be concluded that the educational level of the people in Ward 7 in Soc Trang City is mostly moderately-educated.

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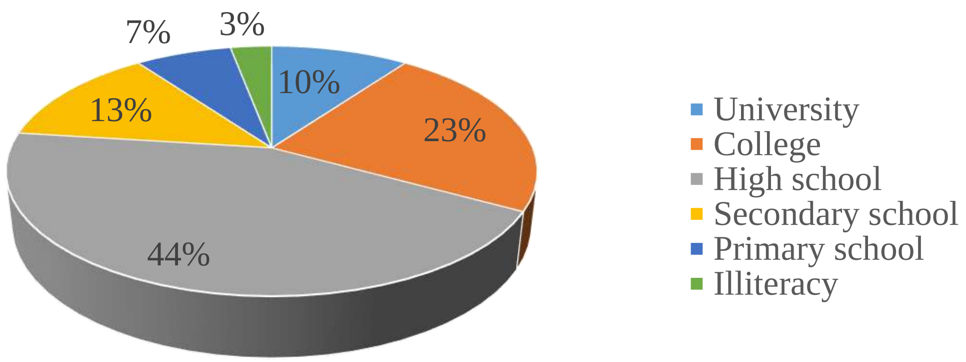

Fig.2: Educational levels in the study area

The dominant occupation in the research area was trading (accounting for 43%), government officials (accounting for 7%); housewives (accounting for 7%); workers (accounting for 20%); agricultural workers (7%), tailors (10%), drivers (3%), and construction workers (3%) (Figure 3). Occupation is one of the factors affecting the generation rate of domestic waste. In fact, the traders are also housewives in the family, along with home tailors, as they are known to stay at home so they have a big role in discharging wastes. Because they have the ability to go to the market, cook, but the business also indirectly discharges the waste into the environment such as packaging, nylon bags. The majority of people with high education would have a stable job and have a long time during the day at work so there would be less waste generation in the households. However, high-income people tend to produce more wastes due to their greater consumption capacity. In summary, occupation and education level are important factors affecting public awareness in environmental protection, especially waste management.

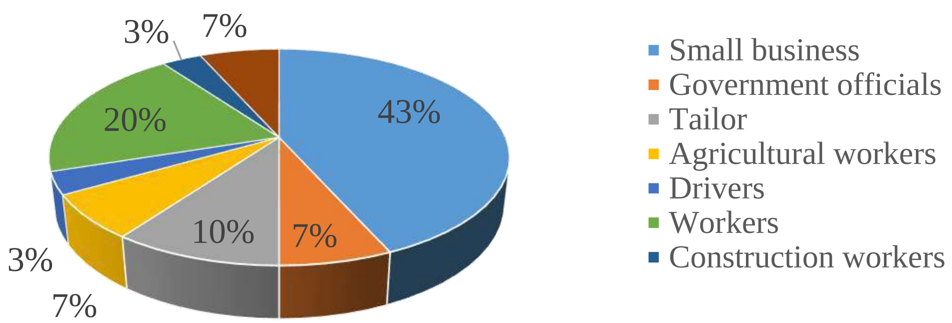

Fig.3: Occupation of the interviewed households

3.2 Current status of solid waste generation

Survey results showed that the percentage of households generating solid waste 0-0.5kg accounted for 7%, from 0.5-1kg accounted for 33%, 1-2 kg accounted for 40% and over 2kg accounted for 20%. Solid waste in ward 7, mainly generated from specific sources such as from households, business and production establishments (**Figure 4**).

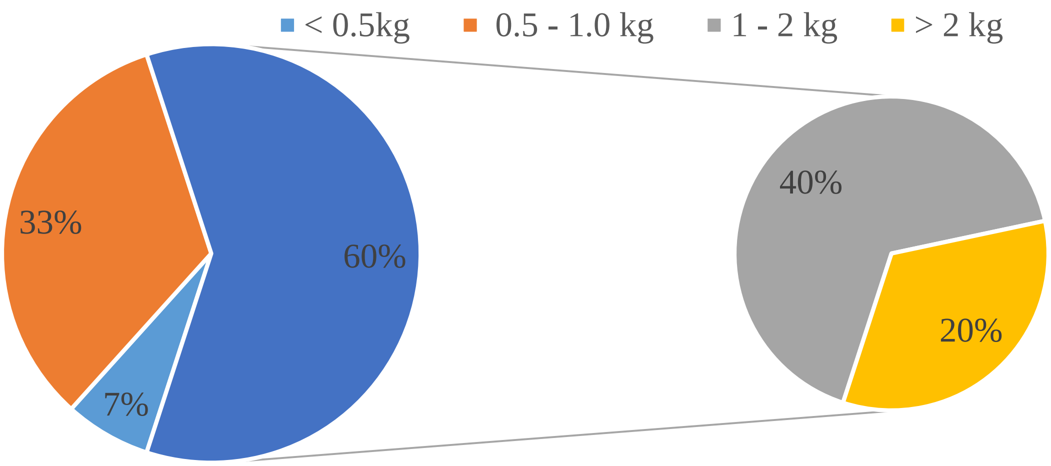

Fig.4: Waste generation in the study area

Through interviewing and observing waste before being collected, it showed that the amount of waste from production and business establishments has the largest volume because it is the area where many types of products are traded.Table 1 showed that in Ward 7 of Soc Trang city, the highest amount of waste per capita was 0.46 kg/person/day and the lowest was 0.13 kg/person/day. The amount of waste generated by each household varied widely by occupation, income level, and living and production habits of households. However, this difference is mainly due to the level of income, which results in higher living standards, eating and drinking, and household activities generating more solid wastes. On average, each household in Ward 7 discharged about 0.91 kg/household/day or 0.22 kg/person/day. Thus, the average volume of solid waste per capita of the households in the sampling area was relatively low with 0.22 kg/person/day (Table 1). Numbers of households in the survey should be increased to get more representative result.

Table1. Solid waste generation at ward 7 of Soc Trang province

No.	Household code	No. of family members	Total weight (kg/week)	Amount of solid waste per household (kg/hh/day)	Amount of solid waste per capita (kg/person/day)
1	HH1	5	10.92	1.56	0.31
2	HH2	3	9.73	1.39	0.46
3	HH3	5	4.94	0.71	0.14
4	HH4	5	5.61	0.80	0.16
5	HH5	4	4.93	0.70	0.18
6	HH6	4	3.81	0.54	0.14
7	HH7	4	6.43	0.92	0.23
8	HH8	4	7.02	1.00	0.25
9	HH9	5	4.89	0.70	0.13
10	HH10	3	5.63	0.80	0.27
11	HH11	5	4.93	0.70	0.14
12	HH12	6	5.85	0.84	0.14
13	HH13	4	8.89	1.27	0.32
14	HH14	4	5.62	0.80	0.20

15	HH15	5	6.56	0.94	0.19
Mean		4.4	6.384	0.91	0.22

3.3 Solid waste composition

From the results of the study, the composition of solid waste in the area is mainly organic matter, paper, plastic, glass, metal, cloth, foam and hazardous waste. Among the components on the biodegradable organic waste accounted for 62.31% including mostly vegetables, tubers, fruits, leaves, tree branches. This component is easy to decompose under high temperature conditions and within an appropriate time, this amount of waste will decompose and create gases, especially H_2S and CH_4 gas, causing odors to pollute the environments. In the area, this is also a disadvantage for waste collection and treatment because if it is not collected in time, the easily decomposable organic waste can cause stench and develop bacteria to pollute the environments affecting people's lives and health. In addition, degraded organic waste attracts, arises and develops mice, flies, mosquitoes, cockroaches and other germs that cause many infectious diseases to humans and domestic animals.

Plastic was the second main component accounting for 19.61% nylon bags, straws, bottles, cups, jars of which plastic bags and plastic bottles took the majority. Especially, plastic bags accounted for a large amount in waste plastic components. The plastic bags were small, thin, convenient, and cheap, so it is widely used. As a result, the amount of plastic has been increasing. However, this is a difficult to decompose component, the decomposition time of plastics in the environment is 500 years so it is one of the biggest obstacles in the treatment and management of solid wastes. The next component contributing fairly high in the waste component was paper (accounting for 14.55%). Glass components such as bottles, glasses and jars (accounting for 1.16%), metals such as cans, scrap metal, wire (accounting for 0.79%), cotton fabrics (accounting for 1.47%), foam consists of foam boxes (accounting for 0.74%) and hazardous wastes accounted for a small proportion (0.11%). Because glass and metal components were often stored by people to sell vendors. The proportion of cottons was low (Figure 5).

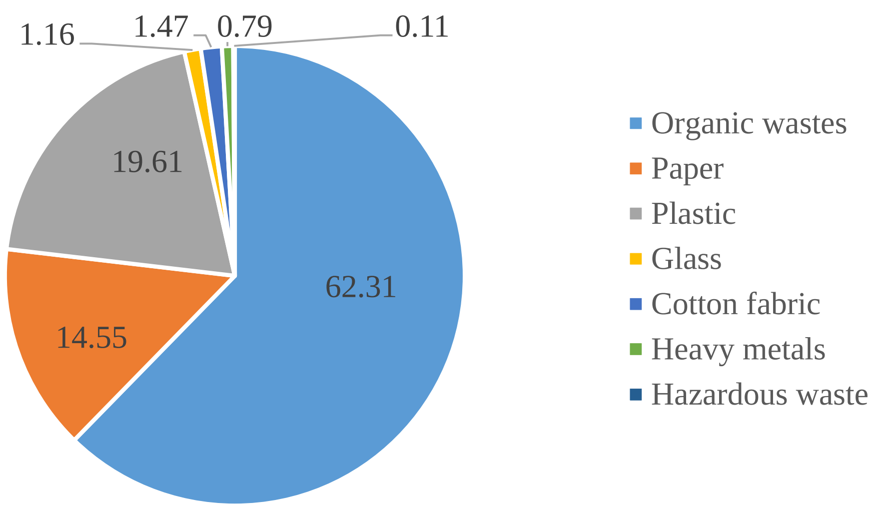

Fig.5: Composition of solid wastes in the study area

From comparative data, it can be seen that domestic wastes in the study area in Can Tho and in the study area in Soc Trang has relatively diverse components. Solid waste is generated mainly from business areas. Main components were organic matter, paper, plastic, glass, and hazardous wastes. The proportion of each type of solid waste generation in the two study areas was different. In the study area in Can Tho, there was a higher content of organic matter (72.66%) than Soc Trang (62.31%). The composition of other wastes surveyed in Can Tho which were higher than those in Soc Trang were glass (2.65%) and hazardous waste (1.39%). Paper and plastic in Soc Trang accounted for a higher proportion than those in Can Tho.

Table2.Composition of solid wastes in Soc Trang and Can Tho city

No.	Composition	Ward 7, Soc Trang (%)	Cai Khe, Can Tho (%)
1	Organic matter	62.31	72.658
2	Paper	14.55	7.242
3	Plastic	19.61	13.404
4	Glass	1.16	2.653
5	Hazardous wastes	0.11	1.392
6	Reference	This study	Previous study

3.4 Understanding of solid wastes of the households

The survey results showed that people often found out information about the environment from the mass media (30%), local governments (26%), local governments combined with mass media (20%), relatives and neighbors (10%) and relatives and neighbors combined with local authorities (7%). Through interviewing 30 households about the impact of solid wastes on health, 90% of interviewed people said that waste affects health, 10% said it does not affect. In 90% of households who were aware of the impact of solid waste, they believed that solid waste would cause digestive diseases (60%), respiratory (27%), skin diseases (13%). However, the remaining 10% said that it was not affected because they did not understand the impact of waste and the access to propaganda, possibly because of low awareness due to low education.

3.5 Management of solid wastes in the households

The interviewing results on the status of household waste classification indicated that 72% of the households had their waste classified at home, and the remaining 28% of the households said that the solid waste was not classified. This proved that the majority of households classifiedsolid waste daily in the ways that they only pick the paper, scrap metal, and plastic bottles which can be sold to vendors. As for nylon bags, people keep them for the reuse. Part of the leftovers would be reused as pet food. This classification could also help to reduce the amount of solid waste collected thus reducing environmental pollution and this habit should be encouraged extensively. However, 28% of respondents said that environmental management belongs to government officials. In reality, the households do not know how to classify solid wastes. They just take out the valuable items for future use or for money. Solide waste was collected at a fixed daily time (13:00 PM) with the frequency of 1 time/day. All respondents did not have any feedback on the waste collection scheme. 100% solid wastes of households were collected. Each household pays a monthly waste collection fee of VND 15,000 and 100% of households think it is reasonable. People agree to pay an additional collection fee to improve the environment related to domestic solid waste.

3.6 Current solid waste management of the local authority

Currently, in Soc Trang city, Soc Trang Urban Construction Joint Stock Company is the only unit collecting and treating domestic solid waste. Solid waste collection facilities include specialized vehicles, trucks and trolleys to collect domestic solid waste from households, facilities, public areas to transfer points, then solid waste will be transported to a garbage compactor (with a spray to treat odors and flies) and transported to Soc Trang solid waste treatment plant for disposal by burial method. According to the interviews with officials of Ward 7 of Soc Trang City, the garbage collection activities in the ward reached about 80% of the amount of generated solid waste. Although the collection of domestic solid wastes in the ward is focused on implementation. However, due to the low awareness of certain households, they do not collect waste but throw it away in public areas, causing loss of urban beauty and environmental pollution. In addition, some households near the countryside and garbage collection facilities have not been able to collect waste yet, so the people themselves collect and treat but not thoroughly, so the wastes are generated immediately roadside, along canals and ditches.

Fig.6: Open dumping in the study area

Local environmental protection has been focused. At present, Ward 7 Committee has assigned a task for an officers in charge of Land Administration - Environment to perform the role of advising and reporting on environmental protection in the locality, updating legal documents. In the past year, the locality has created favorable conditions for the officials to participate in training courses on environmental protection organized by the Department of Natural Resources and Environment of Soc Trang city. Environmental officers are allowed to implement legal documents such as the current Law on Environmental Protection; Decree No. 18/2015/ND-CP dated February 14, 2015 of the Government; Circular No. 27/2015/TT-BTNMT dated May 29, 2015 of the Ministry of Natural Resources and Environment. At the same time, the ward People's Committee also applies the legal policies on the sanctioning of administrative violations in the field of environmental protection such as: Decree 38/2015/ND-CP on waste and discarded material management; Decree 59/2007/ND-CP on solid substance management; Decree 155/2016/ND-CP stipulates penalties for administrative violations against regulations on environmental protection. The ward People's Committee draws up a plan for environmental protection, performs the task of protecting the environment and preserving environmental sanitation in the locality; regularly propagandize and mobilize people to develop environmental protection content, guide the inclusion of criteria for environmental protection in assessing residential areas and cultural families. However, environmental management in the locality still faces many difficulties, largely due to poor people's sense of common sanitation, and the domestic waste of some households who do not dispose of their garbage in accordance with regulations leading to environmental issues.

3.7 Recommended solutions for solid waste management

In order to overcome difficulties and exist in environmental protection in the area, in the coming time, the People's Committee of Ward 7 should implement the following solutions:

Disseminating information about newly promulgated environmental regulations, framework for sanctioning violations of environmental protection to community through meetings with the community. Using the mass media to bring educational message to the people. The use of banners and posters to show fully and briefly the contents to be propagated. Continuing to launch the movement of garbage collection and street sanitation on the occasion of World Water Day (March 22), World Environment Day (June 5), Vietnam Sea and Islands Week, the campaign to make the world cleaner, ... Local officials actively guide households in remote areas to collect and treat waste such as burial in home gardens or burning, not disposing of solid waste to canals. Launching hazardous waste collection (pesticides after use) to clean the environment. Launching the emulation movement for environmental protection in Soc Trang city according to the Plan No. 10/KH-UBND dated January 11, 2018 of the People's Committee of Soc Trang City.

Strengthening the inspection and monitoring of operations of livestock farms, thereby detecting and strictly handling violations, as well as detecting establishments causing environmental pollution. Managing and closely monitoring the exploitation of underground water, enterprises operating in exploiting underground water in the ward area. Promoting the investment in socialization of environmental protection in the ward, attracting capital from businesses and people to build gathering points, handle agricultural wastes. Dredging canals and ditches have been deposited, cleared the flow to meet the circulation needs and agricultural production of people. Sending environmental officials to participate in training courses organized by departments to improve their professional knowledge and skills. Requiring environmental officials to regularly update environmental laws and disseminate to groups, hamlets and households in the ward area.

IV. CONCLUSION

According to the survey results, the amount of solid waste generated by households generated an average of 0.91 kg/household/day and the average amount of waste generated by each person was 0.22 kg/person/day. The composition of household waste was relatively abundant, the composition of organic substances in the garbage (62.31%) was higher than the other components, followed by the components that can be recycled and reused such as plastic (19.61%), paper (14.55%), glass (1.16%), metal (0.79%), cloth (1.69%), styrofoam (0.74%), and hazardous wastes (0.11%). Most people do not know how to separate waste at source. The means of garbage collection and transportation were still simple and seriously degraded. Currently, the management, collection and transportation of domestic waste in ward 7 has not been guaranteed, not yet effective, causing many problems. The locality needs to invest more specialized collection vehicles, improve equipment, regularly supervise the collection, transportation and handling to achieve higher efficiency. Encouraging people to separate and reuse biodegradable organic matters for composting and biogas. Public waste bins are needed to limit the current littering. Local environmental managers need to strengthen the inspection, examination and supervision of the objects engaged in production and business activities that cause environmental pollution.

REFERENCES

[1] Nguyen Van Phuoc (2008), Management and treatment of solid waste: volume 2. Hanoi Construction Publishing House, 2008.

[2] Soc Trang People's Comittee (2009). Soc Trang Province Environmental Status Report 2009.

[3] Mai Vang Ngoc Bich (2018). Current status of solid waste management in Cai Khedistric, Can Tho city. Bachelor's thesis. Can Tho University.

[4] Klinsawathom, T., Songsakunrungrueng, B., and Pattanamahakul, P., 2017. Heavy metal concentration and risk assessment of soil and rice in and around an open dumpsite in Thai Lan. Enviromentasia, 10(2), 53-64.

[5] Toufexi, E., Tsarpali, V., Efthimiou, I., Vidali, M.S., Vlastos, D., Dailianis, S., 2013. Environmental and human risk assessment of landfill leachate: an integrated approach with the use of cytotoxic and genotoxic stress indices in mussel and human cells. Journal of Hazardous Materials 2013; 260: 593-601.

[6] Hang, X., Wang, H., Zhou, J., Ma, C., Du, C., and Chen, X., 2009. Risk assessment of potentially toxic element pollution in soil and rice (Oryza sativa) in a typical area of the Yangtze River delta. Environmental Pollution. 157 (8-9): 2542-49.

[7] Lakhdar, A., Scelza, R., Scotti, R., Rao, M.A., Jedidi, N., Gianfreda, L., Abdelly, C. 2010. The effect of compost and sewage sludge on soil biologic activities in salt affected soil. Revista de la ciencia del sueloy nutrición vegetal 10, 40-47.

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The impact of using the*Zingiber officinale* extract in controlling the endogenous bacterial contamination of date palm during tissue cultures.

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Abstract— *Endogenous bacterial infections are the most critical problem hampers date palm (Phoenix dactylifera) micropropagation cycle. This work aimed to evaluate the influence of the antibacterial property of ginger(Zingiber officinale) extract on suppressing the endogenous bacterial contamination of date palm tissuecultures, to avoid the continuous using of the ordinary chemical antibiotic. The prepared aqueous extract ofZingiberofficinaleat different concentrations (0.5, 1, 2, 4, 6, 8, or 10 ml/l), in comparison toantibiotic, Chloramphenicol or Tetracycline at 100 mg/l, studied onmultiplication media of Gondelah cultivar.The residual effect of all studied treatments also followed up on the rooting media. The results showed that the concentrations of ginger extract at 8 or 10 ml/l (without significant result between) came in the second rank follow the Tetracycline in inhibition the growth of the endogenous bacterial contamination of shoot clusters during the multiplication stage, but subsequently, their residual effect during the rooting stage recorded the highest significant results over the use of antibiotics in preventing bacterial growth in plantlets' cultures. Ginger extract at 8 or 10 ml/l gave the highest significant results of the vegetative growth of shoot clusters explants on multiplication media, as well as, the highest values of plantlets vegetative growth during the rooting stage. So, using the ginger extract as an organic antibacterial source for controlling the endogenous bacterial problem of the date palmensures that cultures are controlled protective, well vegetative growth, and avoiding the disadvantages of the continuously using of ordinary antibiotics.*

Keywords— ***Antibiotics, Ginger extract, Endogenous bacterial contamination, Phoenix dactylifera, Micropropagation.***

I. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a perennial tree, represents an essential fruit crop around the countries of the Middle East and North Africa. It possesses a great economic importance and has an obvious role in socio-economic life in these countries. (**El Hadrami and Al-Khayri, 2012**). Because of all constraints associated with conventional date palm propagation using seeds or offshoots, *in vitro* culture has become attractive and alternative for mass propagation of commercial cultivars. This technique allows the rapid multiplication of healthy and true- to- type date palm planting materials (**Al-Samir et al., 2015**).Microbial contamination by fungi or bacteria, is one of the most obstacles hinder the date palm tissue culture process, which can destroy almost cultures in any stage during micro propagation protocols (**Odutayo et al., 2007;Abass, 2013**). Date palm Bacterial contamination may result from the plant explants materials, the surrounding environment of the laboratory, operators' performance, or inadequate sterilization processes, resulted in reduce growth rate and increase culture mortality (**Mbah and Wakil, 2012**).Date palm micro propagated cultures are occasionally highly contaminated with, endogenous bacteria, even well-sterilized cultured explants remain with them these contaminants during the in vitro cycle (**Abahmane, 2017**). In this respect, endogenous(endophytic) bacterial contamination caused serious problems in micro propagation protocols of various plants species such as banana(**Oliveira et al., 2016**), yam (**Wakil and, Mbah, 2012**), prunes (**Quambusch et al., 2014**) strawberry (**Taghizadeh et al., 2016**) and grapevine (**Thomas et al., 2017**).Generally, these endogenous contaminants are not easily eliminated by the action of surface disinfectants, due to their location inside cell tissues. Endophytic bacterial contamination is remaining latent during in vitro growth and they expected to attack suddenly in any stage on plant cultures media (**Cassells,2012**).The great distinctions of both gram-negative and gram-positive endogenous bacteria have been explored in date palm tissues. Mainly, genus Bacillus is a dominant group, with four species (*B. subtilis*, *B. safensis*, *B. cereus* and *B. sonorensis*), Enterobacteriaceae, and other isolates that belonged to Achromobacter, Acinetobacter, Serratia and Pseudomonas species. (**Siala et al., 2016**).Different antibiotics sources i.e., Nystatin, Streptomycin Gentamicin, Amoxicillin, Chloramphenicol and tetracycline reused to restrain bacterial infection inside in vitro date palm cultures. (**Al-Kaby 2004; Al-Dosary et al., 2011; Abass et al., 2016;Abahmane, 2017**). However, antibiotics may lose their efficiency for many reasons as the bacteria resistance because of repeatedly use infrequently subcultures. Moreover, phytotoxicity or poor penetration into plant tissues were serious adverse of using antibiotics (**Mbah and Wakil, 2012**).

Plant-derived compounds illustrate an untapped source of secure, influential, and environmentally opportune antimicrobial. The essential role of some plant extracts as an antibacterial activity is referring to the existent of some bioactive compounds as secondary metabolites in the plant cells (**Taghizadeh et al., 2016**).At this point many studies determined plant extracts as an organic and safe source for anti-bacterial activity as betel (**Hoque et al., 2011**), ginger (**Kamazeri et al., 2012**), Hindi clove (**Joshi et al., 2011**), Lavender (**Hui et al., 2010**), lemon (**Kirbaslar et al., 2009**), and turmeric (**Allawi et al., 2009**).Ginger is an aromatic herb, that literature has well-reviewed, about its antimicrobial activity against many pathogenic species(**Sendanayake et al., 2017**). There are little reports about using plant extract against endophytic bacterial contamination of in vitro plant cultures.The current study mainly aimed to evaluate the effect of ginger extract as an organic antibacterial source for controlling the endogenous bacterial contamination of date palm tissue cultures in comparison to the antibiotics Chloramphenicol or Tetracycline,to reduce the disadvantages of repeating antibiotic application. Healthy and well full rooted, plantlets, are the main point to achieve successful transferring to the acclimatization stage. Aqueous extract of Ginger was tested on the in vitro established, clean shoot cultures of date palm cultivar Gondelah., at different concentrations, supplemented in basic nutrient MS media formulation for three frequently subcultures of multiplication stage and the residual effect during the rooting stage is also studied. Common antibiotics such as Chloramphenicol or Tetracycline and control treatments used as a comparative study to evaluate the antibacterial efficiency of Ginger extract.

II. MATERIALS AND METHODS

This study conducted at the biotechnology department, of the Central Lab for Date Palm Researches and Development- Agricultural Research Center, Giza, Egypt. Date palm offshoots, of Gondelah cultivar were brought from, Aswan, Governorate, to use as starting shoot tips explants. For the micropropagation protocol, sterilization and, establishment steps followed as recommended by(**El-Dawayati and Zayed, 2017**).

Plant Material: -Shoot cultures (2-3 cm length), taken at the multiplication stage during micro propagation cycle, wereused as a source of explants(each cluster consists of 3-4 shootlets). All shoot cultures were checked carefully for any existing light or heavy signs of endogenous bacterial colonies contamination.

Shoot cultures contaminated by endophytic bacteria were determined by, white or creamy colonies, with a cloudy appearance on the surface of the culture medium. **Fig (1)** and any suspicious cultures were discarded.

Fig.1: (A) Beginning of appearance of endogenous bacterial contamination associated with shoot culture of the date palm. (B) A high degree of cloudy appearanceof endogenous bacterial contamination in shoot cultureof the date palm.

The preparation of ginger extract: -

Fresh ginger rhizomes were washed with tap water, then peeled and chopped in slides, by a sterile knife. About 100 gm of fresh Ginger left at room temperature for 2-3 h., then oven-dried at 35-40ºC for 24 h. After drying the amount equal to 15 gmand190 ml of sterile water was added to the dried weight and equated to 200 ml in total, then boiled it on direct flame for 5-10minutes, and it left for 1hr. Then, filtration by sterile gauze. Extraction for the residue from the filtration was repeated twice. The liquid filtrates combined and saved in a sterile cap glass bottle, under storage conditions at 4 -6 ºC(**Sabra et al.,2014**).

Effect of ginger extract on contamination during multiplication: - Clean shoot clusters(free of any contamination source)were transferred and inoculated on fresh multiplication nutrient media in three groups as follows: - the first group was contained media without any addition of antibiotics or antibacterial agent(control treatment). The second group was contained media with the addition of ordinary antibiotics Chloramphenicol or Tetracycline at 100 mg/L for each. The third group was contained multiplication nutrient media with the addition of different concentrations of Ginger extract at (0.5, 1, 2, 4, 6, 8, or 10 ml/L). Multiplication nutrient media composed of ¾strength of MS (**Murashig and Skoog 1962**) basal salts, (MS) vitamins, 40 g sucrose, 0.4 mg/l Thimine-Hcl, 100 mg/L Myo-inositol, 0.1 mg/L biotin, 0.5 mg/L Pyridoxine, 0.5 mg/L nicotinic acid, 170 mg/L$NaH_2PO_4$and supplemented with plant growth regulator 0.05 mg/LB enzyladenine (BA) and 0.1 mg/L Naphthaleneacetic acid (NAA). Each treatment was contained 30 jars, and each jar contains 3 explants. All groups of cultured jars were incubated under the light condition with 1500 lux for 16 (hrs.) and 8(hrs.) dark at 27 ± 2ºC. The cultures were maintained by regular subcultures on fresh medium 3 times, with 8 weeks intervals. Data were taken daily about the appearance degree of endogenous bacteria in the cheeked cultures jars, contaminated cultures by endophytic bacteria were determined as mentioned above. At the end of each subculture, vegetative growth performance recorded for uncontaminated cultures jars (free of endogenous bacterial contamination), as follows: - the average of shoot number, shoot length (cm),secondary embryos number, and growth vigor degree. The data representing 'degree' were scored visually, as1= no change; 2=below average; 3 = above average; 4 = high, following the recommendation of **El-Dawayati et al., (2018).**

Effect of the residual effect of ginger extract on contamination during rooting: -At the end of multiplication stage, elongated plantlets (10 cm) were collected from all previous treatments of the three groups cultures, and transferred to rooting nutrient media which composed of the same components of multiplication nutrient medium but containing in addition of 1 g/L Activated charcoal and supplemented with 1 mg/L (NAA) and 1 mg/L Indole butyric acid (IBA) as plant growth regulators. The cultures were maintained by the regular subculture on fresh medium for tow subcultures (with 8 weeks intervals), in order to follow up the residual effect of the two types of ordinary antibiotics (Chloramphenicol and Tetracycline at 100mg/ L) compared to different concentrations of ginger extract on the appearance of endogenous bacteria and the growth development, during rooting stag. Each treatment presented in 30 jars and each jar contains 3 explants. All groups of cultured jars were incubated under the light condition, with 3000 lux, for 16 (hrs.) and 8(hrs.) dark at 27 ± 2ºC. Data were taken about the appearance degree of endogenous bacteria inthe cheeked cultures jars.The vegetative growth performance also recorded for uncontaminated cultures jars (free of endogenous bacterial contamination), as the average of shoot length(cm), root number, and root length(cm).

For all prepared media pH was adjusted to 5.7± 0.1 before adding agar at 6 g/l and medium dispensed at (40 ml/ jar) into small jars (150 ml) for multiplication media, or into (250 ml/ jars) for rooting media before autoclaving at 121ºC and 15 Ibs/in2 for 20 min. At the end of the experiments, all received healthy full rooted plantlets from all treatments transferred to the acclimatization stage.

Statistical analysis: -The analytical statics was performed in a complete randomized design with three replicates. All obtained results were determined by subjection to statistical analysis of variance, according to the method described by **Snedecor and Cochran (1982)**using the LSD test at 5%.

III. RESULTS

Obtained data for the endogenous bacterial appearance degree and the vegetative development of explant cultures during multiplication and rooting stages,under different concentrations of ginger extract at (0.5, 1, 2, 4, 6, 8, or 10 ml/L), or two ordinary antibiotics (Chloramphenicol or Tetracycline at 100 mg/ L), incorporated in cultures media of date palm shoot clusters explants, revealed as follows: -

Obviously, from data, of **Fig (2)**, untreated shoot cultures media group (control treatment) showed the highest significant value of endogenous bacteria appearance for three subcultures. Shoot cultures media group which treated with Tetracycline antibiotic for three subcultures recorded the lowest significant value for endophytic bacteria appearance. There was no significant difference between using ginger extract at 10 ml or 8 ml for controlling the endogenous bacteria appearance in shoot cultures media, which they ranked significantly after Tetracycline antibiotic treatment for suppressing endophytic bacterial contamination growth. Shoot cultures media which treated with ginger extract at 10 ml showed significantly less degree of endogenous bacteria appearance, to be more antimicrobial activity than using Chloramphenicol antibiotic. Data obtained revealed that by increasing ginger extract concentrations in shoot cultures media the inhibition of endophytic bacteria appearance increase significantly comparing to control treatment.

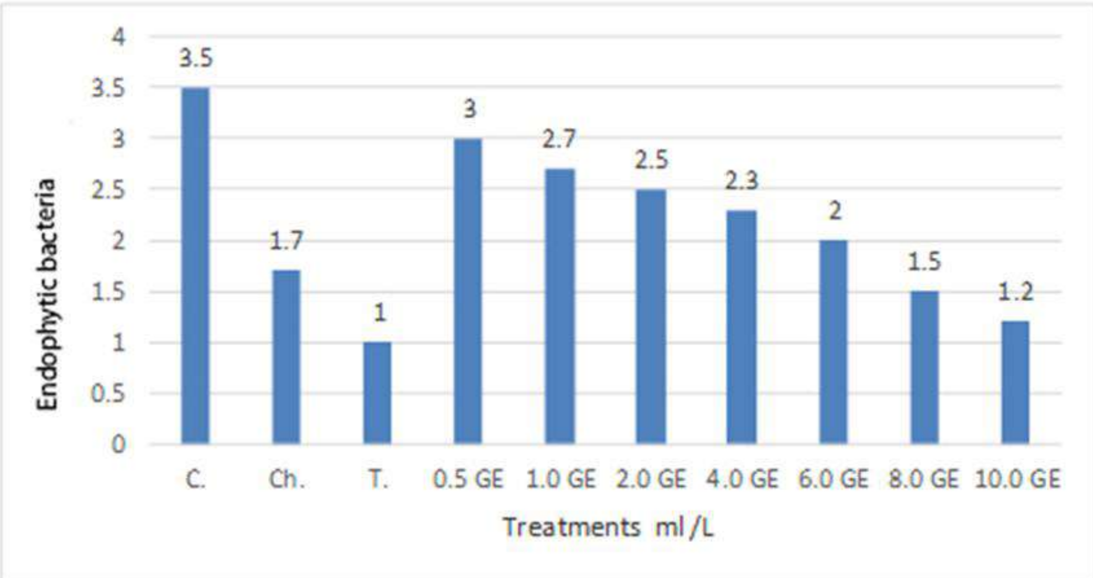

Fig.2: Effect of two types of ordinary antibiotics Chloramphenicol (Ch) or Tetracycline (T) at 100mg/ L compared to different concentrations of ginger extract (GE) on the appearance of endogenous bacteria in shoot cultures of date palm cv. Gondelah during the multiplication stage.

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Table 1.Effect of two types of ordinary antibiotics (Chloramphenicol and Tetracycline at 100mg/ L) Compared to different concentrations of ginger extract on vegetative growth of date palm cv. Gondelah during the multiplication stage.

Treatments		Avg. shoot no.	Avg. shoot length (cm)	Avg. sec.embryo no.	Growth vigor
Control		31.30	6.21	20.11	3.33
Antibiotic types 100mg/ L	Chloramphenicol	32.31	5.55	18.78	2.88
	Tetracycline	32.72	5.73	19.00	2.88
Ginger extracts	0.5ml	32.90	6.33	23.34	3.55
	1.0 ml	34.70	6.94	26.36	3.55
	2.0 ml	35.71	6.94	29.22	3.77
	4.0 ml	37.10	7.15	33.35	4.00
	6.0 ml	39.71	7.63	34.71	4.66
	8.0 ml	42.31	8.51	36.66	4.88
	10.0 ml	43.55	8.53	36.72	4.88
LSDat0.05		3.33	0.51	5.20	0.55

Observation from **Table (1)** showed that maximum values of the average of shoot number shoot length, secondary embryo number and the growth vigor degree obtained with ginger extract treatments at 8 ml and 10 ml, without significant differences between them. Where the lowest significant results of the average of shoot number of date palm shoot cultures obtained on control media treatment, culture media supplemented with both of (Chloramphenicol or Tetracycline at 100 mg/ L) antibiotics treatments and culture media supplemented with ginger extract treatment at 0.5 ml treatment, without significant differences among them. Data indicated that the average of shoot length and growth vigor degree of shoot cultures increased significantly by increasing the ginger extract concentration, from 4 ml to 10 ml, comparing to control media treatment and, both of (Chloramphenicol or Tetracycline at 100 mg/ L).The addition of ginger extract at 8ml or 10 ml to shoot cultures media, during multiplication stage, gave a great positive effect on the vegetative growth parameters, compared to the addition of ordinary antibiotics (Chloramphenicol or Tetracycline at 100 mg/ L).

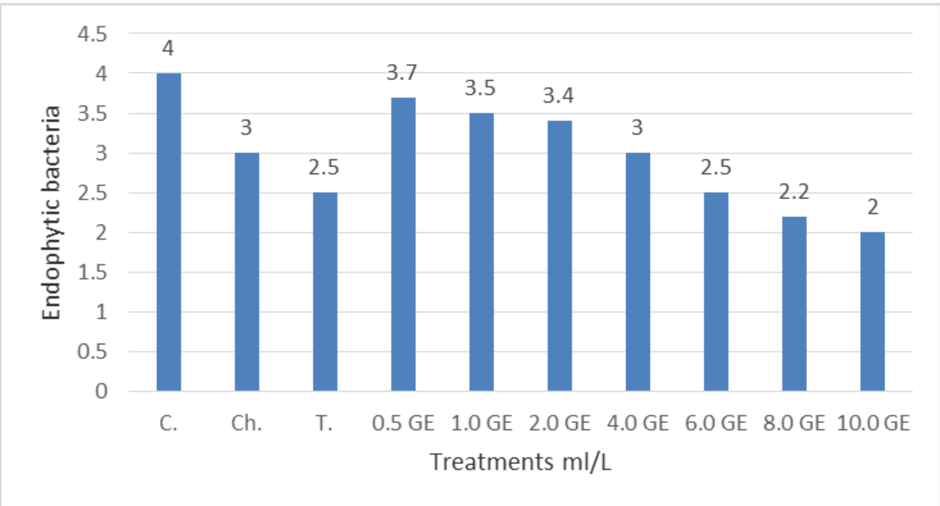

Fig.3: The residual effect of two types of ordinary antibiotics (Ch) Chloramphenicol and (T) Tetracycline at 100mg/ L) compared to different concentrations of (GE)ginger extract on endogenous bacteria appearance of date palm cv. Gondelah cultures during the rooting stage.

Data in **Fig (3)** revealed that the highest degree of endogenous bacterial contamination appearance recorded with control treatment. The lowest significant results for endophytic bacteria appearance degree on plantlets cultures achieved when these plantlets cultures developed on multiplication media, whish supplemented with high concentrations of ginger extract at 8 and 10 ml, without significant differences in between. Where the residual effect of using a common antibiotic (Chloramphenicol or Tetracycline) on controlling endophytic bacterial contamination appearance degree during the rooting stage, were ranked significantly in the second order. The residual effect of using Tetracycline treatment was more effective significantly than the residual effect of using Chloramphenicol treatment in suppressing bacterial contamination growth. On the other hand explants, cultures received from multiplication cultures supplemented with a low concentration of ginger extract at 0.5 ml, hadn't inhibited endophytic bacterial contamination as a residual effect during the rooting stage, without significant difference with control treatment result. It seemed to be that, with the increasing of ginger extract concentrations in cultures media during the multiplication stage, endophytic bacterial contamination gradually showed decreasing in bacteria appearance as residual effects during the rooting stage.

Table.2 The residual effect of two types of ordinary antibiotics (Chloramphenicol and Tetracycline at 100mg/ L) compared to different concentrations of ginger extract on vegetative growth of date palm cv. Gondelah cultures during the rooting stage.

Treatments		**Avg. plantlet length (cm)**	**Avg. root no.**	**Avg. root length(cm)**
Control		14.6	5.6	4.7
Antibiotic types 100mg/L	Chloramphenicol	14.2	5.2	4.5
	Tetracycline	14.6	5.7	4.7
Ginger extracts	0.5ml	14.8	6.6	5.2
	1.0 ml	15.0	6.9	5.5
	2.0 ml	15.5	7.6	5.5
	4.0 ml	16.3	8.3	5.5
	6.0 ml	16.6	8.3	5.7
	8.0 ml	17.5	8.6	5.9
	10.0 ml	17.7	8.0	5.9
LSDat0.05		0.52	1.90	1.65

From data in **Table (2)** obtained results showed that the highest values of plantlets length and roots number/plantlets during the rooting stage recorded after using ginger extract at 8 ml or 10 ml as an antibacterial agent to control the endophytic appearance, during multiplication stage. Received plantlets from shoot cultures treated with ordinary antibiotic Chloramphenicol, Tetracycline at 100 mg/ L or control treatment gave the lowest results in increasing of plantlets length and root number/plantlets, during the rooting stage as a residual effect. It could be noted that all plantlets received from 8 ml and 10 ml as best treatments for controlling endogenous bacterial contamination of multiplied shoots cultures, could resume their development successfully for further acclimatization stage **Fig (4)**.

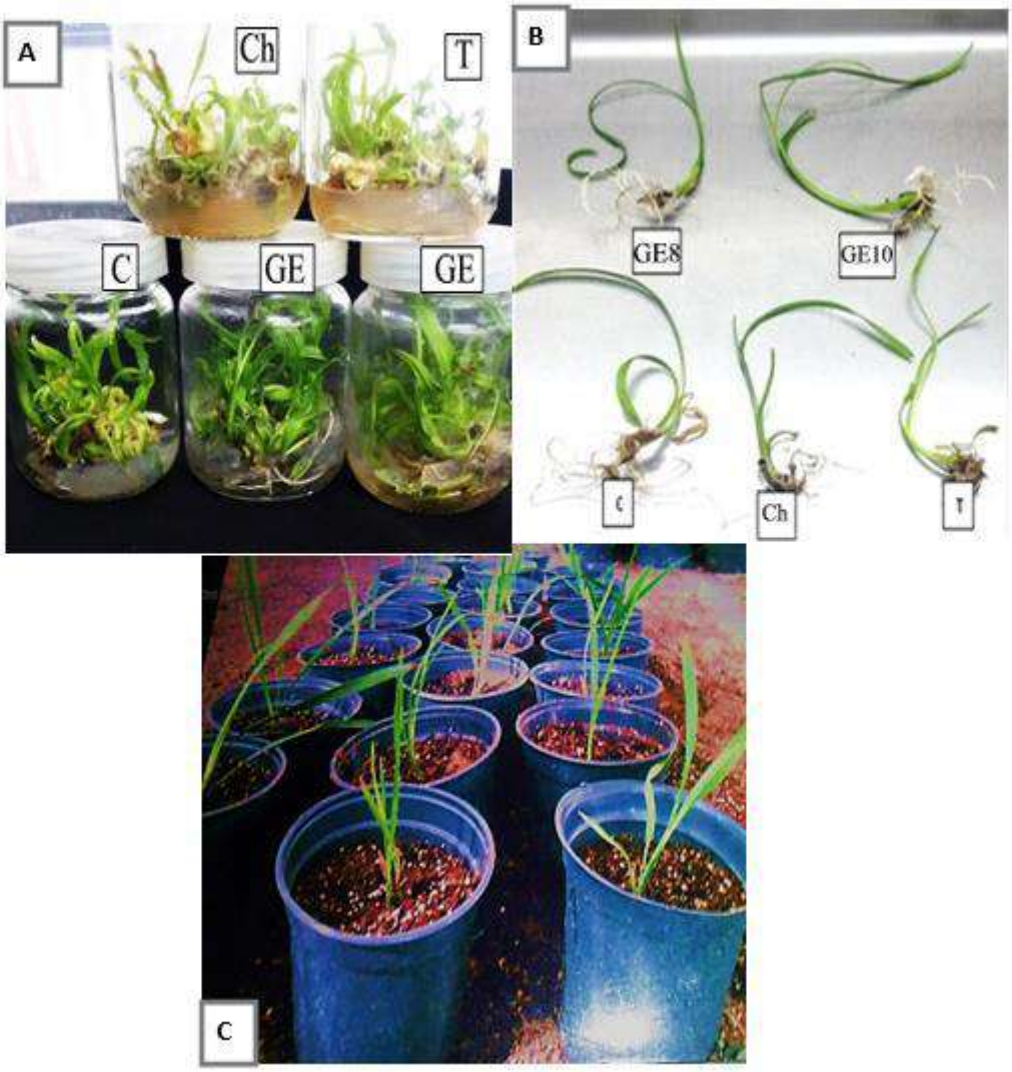

Fig.4: A) The addition of ginger extract (GE) at 8 ml or at 10 ml as antibacterial agent have a great effect on the vegetative growth parameters comparing to the addition of ordinary antibiotics (Ch) Chloramphenicol or (T)Tetracycline and (C) control, during multiplication stage of shoot cultures of date palm cv. Gondelah.B)Full intact Plantlets obtained from ginger (GE) treatments at 8 ml or 10 ml comparing to antibiotics (Ch)Chloramphenicol or(T) Tetracycline at 100 mg/ L) and (C) control treatments. C)Healthy and well-developed plantlets from ginger treatments at 8 ml or 10 ml, transferred successfully tothe acclimatization stage.

IV. DISCUSSION

Endogenous or endophytic bacterial contamination is a serious problem in date palm tissue cultures due to competition for nutrients, the release of toxins and overgrowth of plant tissue which could destroy the whole propagation process. This source of contamination in date palm tissue culture is not extensively studied spot. It could be suggested that these endophytic bacteria can remain undetected due to some conditions which are not optimal for their growth, such as the composition of the culture medium of the nutrient elements concentrations, the carbon source concentration, the pH of the growth culture medium, or the surrounded temperature (**Odutayo et al., 2007**).On the other hand, **Cassells (2012**) reported that infection maystay implicit within callus tissue for more than eight months, and reached to a year within shoot cultures, without causing any visible symptoms in contaminated cultures. When the surrounded growth conditions of cultures changed throughout subcultures transferring, endogenously bacterial contaminants, which attend in small numbers, can rapidly, increase and harm the plant cultures (**Leifert, 2000**). There are recent opinions about the capabilities of the influential natural antimicrobial compounds derived originally from plants that contain a broad set of secondary metabolites such as alkaloids, tannins, flavonoids, glycosides, quinones, which are considered beneficial as a substitution to antibiotics. (**Savoia, 2012, Compean, and Ynalvez, 2014**).Hydrophobicity is a significant feature, for plant extracts and their ingredients, which qualify them to split the lipids of the bacterial cell membrane and mitochondria, troubling the whole-cell construction, and causing more cell permeability (**Taghizadeh et al.,2016**). In this regard, antimicrobial property and phytochemical contents of ginger were reviewed by many types of research(**Ali et al., 2008; Sendanayake, 2017**).

The obtained results of this study revealed that ginger extract at 10 ml/l showed significantly less value of endophytic bacteria appearance in shoot cultures of date palm cv. Gondelah, than those treated with Chloramphenicol antibiotic. This is on the other side of **Abass et al.,(2016**) who found thatboth Gentamicin and Chloramphenicol showed, high defense performance for date palm callus culture. However, **Sabra et al., (2014**)mentioned that antimicrobial activity of ginger rhizomes extract, versus both bacterial isolates of Gram-positive and Gram-negative, refers that chemical components of extract may responsible for a disturbance of the membrane and/or genetic structure of the isolates. The antimicrobial activity of ginger may be owing to the considerable quantity of phenols as gingerol paradol, shogaols and zingerone (**Rahmani et al., 2014**).Beside also the existent of flavonoids in ginger, which found to be affective as in vitro antimicrobial substances, against an ample range of microorganisms (**Fullerton et al.,2011**).At this point, **Dina and Hussein (2017**) reported that, highest amount of total phenolic and flavonoids were detected in extract of both ginger and cinnamon. This was confirmed by **Miklasińska-Majdanik**(**2018**) who reported that,the increase in the number of hydroxyl groups presented on the phenolic ring, leading tohydroxylation property of plant extracts, that resulted in the increasing in the antimicrobial activity. These findings may explain the antimicrobial activity of ginger extract.

From our results, it could be noted that shoot cultures group treated with Tetracycline antibiotic at 100 mg/L for three subcultures recorded the lowest significant value for endophytic bacteria appearance during multiplication stage, but less and weak vegetative growth were observed. This is confirmed by **Khafagi et al., (2001**)who reported that antibiotics are currently used but are not always effective, can alter plant growth, and are costly, and resistant strains can result in extensive use. However, it has to be taken into consideration the phytotoxicity of some antibiotics(**Oliveira and Scherwinski-Pereira, 2016**).

Thus antibiotics should be restricted to very limited culture stages. Prolonged exposure to Tetracycline or Chloramphenicol at 100 mg/ L adversely affected shoot formation. The same result was obtained with shoots of *Pelargonium* cultures grown in the presence of cefotaxime antibiotic for long periodshowed a low rate of shoot formation, shorter and pale, shoots, finally caused the end of cultures by death (**Wojtania et al., 2005**).Growth value and plant shape followed up to check whether using antibiotics or ginger extracts, led to phytotoxicity on the later plantlet's growth. From resultsit could be observed that the residual effect of using Tetracycline or Chloramphenicol antibiotics treatment on controlling endophytic bacterial contamination appearance degree during rooting stage, came at the second rank significantly after using ginger treatment at 8 ml or 10 ml, which showed superiority in suppressing the endophytic bacterial growth during rooting stage as residual effect for being used as antibacterial agent during multiplication stage. This was contrary to **Cassells (2012),**who reported that bacterial growth is only repressed by antimicrobial curing and when chemical compounds are stopped to using, the bacteria colonies recapture their growth. In general ginger extract addition at 8 ml or 10 ml to date palm cultures during multiplication stage and also their residual effect during the rooting stage showed well enhancement for vegetative growth and plantlets development, this may be due to the nutrition value of Ginger rhizome contents of fats, carbohydrates, vitamins, minerals, proteins, vitamins and minerals as sodium and calcium as reported by (**Kumar et al., 2011; Sendanayake et al., 2017**).

V. CONCLUSION

Under the light of the presented study, the antibacterial potential of ginger aqueous extract was investigated as a primary study. Satisfying results obtained from studied concentrations at 8ml/L and 10 ml/L to eliminate endophytic bacterial contamination attack during the multiplication stage and their residual effects were resumed during the rooting stage. Moreover, well development and good vegetative growth of received plantlets were obtained. These results can be a diver to limit the repeated use of antibiotics. Therefore, more studies with ginger extract antibacterial potential can be carried out. Also, studies of using other plant extracts and phytochemicals sources of defined antimicrobial properties would have a great significance in date palm tissue culture, to eliminate or control microbial contamination and to restrict using antibiotics. Characterize and identify of date palm-associated bacteria which lead to more successful antibacterial therapies, intensive studies in this concern are needed. The lack of descriptive information and antibiotic susceptibilities of a large number of date palm endophytic bacteria further complicate the use of antibiotics. The ultimate goal for *in vitro* propagation is obtaining plantlets free off any microbial contamination.

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REFERENCES

[1] Abahmane L., 2017.Cultivar-dependent direct organogenesis of date palm from shoot tip explants, pp. 3-15.In J. M. Al-Khayri et al., (eds.), Date palm biotechnology protocols,vol. I.Springer, New York, USA.

[2] Abass M.H., 2013. Microbial contaminants of date palm (*Phoenix dactylifera* L.) in Iraqi tissue culture laboratories. Emirates J. Food Agr. 25: 875-882.

[3] Abass M.H., Al-Utbi S.D.,Al-Samire E.A., 2016. Morphological and biochemical impact of different decontamination agents on date palm (*Phoenix dactylifera* L.) procallus. Australian J. C. S. 10:1022-1029.

[4] Al-DosaryN.H., Al-Mussawi M.A.,Al-Taha H.A., 2011. Isolation and identification of bacterial types that cause contamination of date palm (*Phoenix dactylifera* L.) callus and studying the inhibition activities of some plant extracts and antibiotics. Basra J. Date Palm Res. 10: 68-81.

[5] Ali B.H., Blunden G., Tanira M.O.,Nemmar A., 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiberofficinale* Roscoe). Food Chem. Toxico. 46: 409-420.

[6] Al-Kaby A.M., 2004. The effect of some antibiotics and fungicides on the growth of embryogenic callus of date palm (*Phoenix dactylifera* L.). Basra J Date Palm Res. 3: 97-110.

[7] Allawi S.S., Auda J.M., Hameed H.Q.,Ali T.I., 2009. The effect of Curcuma longa (turmeric) rhizomes extracts on pathogenic bacteria in comparison with standard antibiotics. J. Biotechnol. Res. C. 3: 15-20.

[8] Al-SamirE.A., Al-Utbi S.D.,Abass M.H., 2015. Phytotoxic effect of 2,4-D and dicamba on date palm (*Phoenix dactylifera* L.) tissue cultures at the initiation stage. A.A.B. Bioflux Soc. 7:96-108.

[9] Cassells A.C., 2012. Pathogen and biological contamination management in plant tissue culture: phytopathogens, in vitro Pathogens, and in vitro pests, pp. 57-80. In: VM. Loyola-Vargas and N. Ochoa-Alejo,3thed. Plant cell culture protocols methods in molecular Biology. Springer, New York, USA.

[10] Compean, K.L.,Ynalvez, R.A., 2014. Antimicrobial activity of plant secondary metabolites: A review. Research Journal of Medicinal Plants, 8, 204-213.

[11] Dina I.,Hussein R., 2017. Phytochemical screening and nematicidalactivity of cinnamon and gingerextract against root-knot nematode (*Meloidogyne incognita*) infecting tomato. Egyptian J. Agr. 16: 63- 84.

[12] El-Dawayati M.M.,Zayed Z.E., 2017. Controlling hyperhydricity in date palm in vitro culture by reduced concentration of nitrate nutrients, 175-183. In: J.M. Al-Khayri et al., (eds.). Date palm biotechnology protocols,vol. I. Springer, New York, USA.

[13] El-Dawayati M.M., Ghazzawy H.S.,MunirM., 2018. Somatic embryogenesis enhancement of date palm cultivar Sewi using different types of polyamines and glutamine amino acid concentration under invitro solid and liquid media conditions. Int. J.Biosci. 12: 149-159.

[14] El Hadrami A., Al-Khayri J., 2012. socioeconomic and traditional importance of date palm. Emirates J. Food Agric. 24: 371-385.

[15] FullertonM.,Khatiwada J., Johnson J.U., Davis S.,Williams L.L., 2011. Determination of the antimicrobial activity of sorrel *Hibiscus sabdariffa* on *Esherichia coli* O157:H7 isolated from food, veterinary, and clinical samples. J. Med. Food. 14: 950-956.

[16] Hoque M.M., Rattila S., Shishir M.A., Bari M.L., InatsuYand Kawamoto S., 2011. Antibacterial activity of ethanol extracts of betel leaf (*Piper betle* L.) against some foodborne pathogens. BangladeshJ.Microbiol. 28: 58-63.

[17] Hui L., He L., Huan L., Xiaolan L.,Aiguo Z., 2010. Chemical composition of lavender essential oil and its antioxidant activity and inhibition against rhinitis related bacteria. African J. Microbiol. Res. 4: 309-313.

[18] Joshi B., Sah G.P., Basnet B.B., Bhatt M.R., Sharma D., Subedi K., Pandey J.,MallaR., 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants (Ocimum *sanctum*) tulsi, (*Eugenia caryophyllata*) clove, (*Achyranthesbidentata*) datiwan and *(Azadirachtaindica*) neem. J. Microbiol. Antimicrob. 3: 1-7.

[19] Kamazeri T.S., Samah O.A., Taher M.,Susanti D.,Qaralleh H., 2012. Antimicrobial activity and essential oils of *Curcumaaerugonosa*, *Curcuma mangga* and *Zingibercassumunar* from Malaysia. Asian Pac. J. Trop. Med. 5: 202-209.

[20] Khafagi I., Dewedar A., Kord M.,Mohammed E., 2001. Identification and antibiotic sensitivity of bacteria occasionally isolated from differentiated and undifferentiated cultures of Sinai medicinal plants. Egyptian J. Biol. 3: pp.67-78.

[21] Kirbaslar F.G., Tavman A., Dulger B.,Turker G., 2009. Antimicrobial activity of Turkish (*Citrus peels*) oils. Pakistanian J Bot. 41: 3207–3212.

[22] Kumar G., Karthik L.,Rao B., 2011. A review on pharmacological and phytochemical properties of Z*ingiberofficinale*(Roscoe) (Zingiberaceae). J. Phar. Res. 4: 2963-2966.

[23] Leifert C., 2000. Quality assurance systems for plant cell and tissue culture, the problem of latent persistence of bacterial pathogens and Agrobacterium – based transformation vector systems. Acta Hort. 530: 87-91.

[24] Mbah E.I.,Wakil S.M., 2012. Elimination of bacteria from in vitro yam tissue cultures using antibiotics. J. Plant Pathol. 94: 53-58.

[25] Miklasińska-Majdanik M., Kępa M., Wojtyczka, R.D., Idzik, D.,Wąsik, T.J., 2018. Phenolic compounds diminish antibiotic resistance of *Staphylococcus Aureus* clinical strains. International journal of environmental research and public health, 15, 2321.

[26] MurashigeT.,Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant. 15:473-496.

[27] Odutayo O.I., Amusa N.A., Okutade O.O.Ogunsanwo Y.R., 2007. Sources of microbial contamination in tissue culture laboratories in Southwestern Nigeria. African. J.Agr. Res.2: 67-72.

[28] Oliveira J.P.,Scherwinski-Pereira J.E., 2016. Biochemical characterization of systemic bacteria in bananas, sensitivity to antibiotics and plant phytotoxicity during shoot proliferation. Acta Sci.Agr. Mar. 38: 193-200.

[29] Rahmani A., Al shabrmi F.,Aly S., 2014. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. Int. J. Physiol. Pathoph. Pharm. 6: 125-136.

[30] Sabra M.M., Al-Masoudi L.R., Al-Gehani S.H.,Abu-Harbah A.O., 2014. Comparative laboratory study on antimicrobial effects of fresh and dry ginger (*Zingiberofficinale*), Taif, KSA. J.Env. Sci.Tox. Food Technol. 8: 114-123.

[31] Savoia D., 2012. Plant-derived antimicrobial compounds: Alternatives to antibiotics. Future Microbial. 7: 979–990.

[32] Sendanayake L.R., Sylvester TP, De Silva UH, Dissanayake DR, Daundasekera DCand Sooriyapathirana SS, 2017. Consumer preference, antibacterial activity and genetic diversity of ginger (*Zingiber officinale* Roscoe) cultivars grown in SriLanka. The J.Agr. Sci. 12: 207-221.

[33] Siala R., Chobba I.B., Vallaeys T., Triki M.A., Jrad M., Cheffi M., Ayedi I., Elleuch A., Nemsi A., Cerqueira F., Gdoura R., Drira N.,Gharsallah N., 2016. Analysis of the cultivable endophytic bacterial diversity in the date palm (*Phoenix dactylifera* L.) and evaluation of its antagonistic potential against pathogenic fusarium species that cause date palm bayoud disease. J. App. Env. Microbiol. 4: 93-104.

[34] Snedecor G.W., Cochran W.G., 1982. Statistical Methods. 7th Edition, Iowa State Univ. Press, Towa, 511.

[35] Taghizadeh M., Solgi M.,Shahrjerdi I., 2016. Various aspects of essential oils application for pathogens controlling in strawberry in vitro culture. Academia. J.Agr. Res. 4: 667-674.

[36] Thomas P., Sekhar A.C.,Shaik S.P., 2017. High taxonomic diversity of cultivation-recalcitrant endophytic bacteria in grapevine field shoots, their in vitro introduction, and unsuspected persistence. Planta. 246: 879-898.

[37] Quambusch M., Pirttilä A.N., Tejesvi M.V., Winkelmann T.,Bartsch M., 2014. Endophytic bacteria in plant tissue culture: differences between easy- and difficult-to-propagate *Prunusavium* genotypes. Tree Physiolo. 34: 524-533.

[38] Wakil S.M.,Mbah E.I., 2012. Screening antibiotics for the elimination of bacteria from in vitro yam plantlets. A.U. J. T. 16: 7-18.

[39] Wojtania A., Puławska J.,Gabryszewska E., 2005. Identification and elimination of bacterial contaminants from pelargonium tissue cultures. J. Fruit Orn. Plant Res. 13: 101-108.

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Preliminary evaluating the possible use of water-decanted sludge from seafood processing wastewater treatment to raise *Peryonyx excavatus*

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Abstract— *The study was conducted to evaluate the possibility of using sludge from processing seafood after dehydration to grow earthworm (Peryonyx excavatus). The worms (purchased from the Xuan Nong worm farm) were raised with a density of 5,000 individuals in three plastic drums with dimensions of LxWxH of 60x42x18 cm. Plastic drums contain a layer of coconut fiber in the bottom and sludge layer above. At the bottom of the plastic drums there are several drainage holes with a diameter of 5mm which could let the water out but not the worms. Soil samples were collected before and after the experiment to analyze the parameters of temperature, pH, humidity, total nitrogen (Nt), total potassium (Kt), total phosphorus (Pt). The results showed that the sludge after raising earthworms has the nutrient components increased significantly. Nt increased from 0.7% to 3.32%, Pt from 0.675% to 3.381%, and Kt from 0.046% to 0.245% compared to input sludge. E. coli was not detected and coliform was reduced to a level that is safe for human health. The use of sludge from the wastewater treatment system of the seafood processing company for raising Peryonyx excavatusbrings benefits since it both brings an additional income, and converts sludge into organic fertilizer, contributing to solving environmental problem.*

Keywords— *Sludge, seafood processing, worm, environment, coliform, nutrients, Peryonyx excavates.*

I. INTRODUCTION

Seafood exploitation and processing is one of the key economic sectors and has great potential of Ca Mau province. Up to now, the province's export turnover has reached over 1.3 billion USD. In particular, the shrimp alone is over 1.2 billion USD, up 34% over the same period, total shrimp production is over 155,000 tons, the industry has always affirmed as the leading industry in the country in terms of output, solving employment, increasing budget revenue for the province. Along with the growth of shrimp production and other aquatic products over the past time are the establishment and development of a system of seafood export processing companies and enterprises. The province currently has 32 companies and nearly 38 affiliated enterprises with a total design capacity of over 190,000 tons/year. With this number of powerful companies and enterprises, Ca Mau shrimp has been contributed to over 40 countries and territories with fastidious markets such as the US, EU, Japan, and some Western countries. With the growth and development of the seafood industry, the treatment of wastewater from existing seafood processing companies, the wastewater treatment capacity of 10,000 m^3/day, the amount of sludge after each day up to 20 tons. This amount of sludge is not treated and discharged directly into the environment, which will be favorable for the development of pathogens and environmental pollution. This type of sludge contains many nutrients and does not contain heavy metals, can be used as a source of raw materials to produce vermicompost fertilizer effectively. This study aims to assess the possibility of using the sludge after decanting water to raise earthworm (**Peryonyx excavatus**). The results of the study provide important information in the direction of the sludge management.

II. MATERIALS AND METHODS

2.1 Experimental design

Preparation of three plastic drums with dimensions of 60x4LxWxH of 60x42x18 cm with drainage holes with a diameter of 5mm and lined with plastic to prevent worms from moving out of the drums (Figure 1a) . Placed a thin layer of coconut fiber lining the bottom of the drums, and then placed sludge into the drums accounting for about 3/4 of the height of the drums. The worms (Peryonyx excavatus) were purchased from the production facility at the Xuan Nong worm farm in Can Tho city, stocked with a density of 5000 individuals with a total weight of 1500 g, evenly distributed into the three drums (1,500 gram of living worms for each drum). The worms were released into the drums by scattering it in a straight line in the middle of the drums. About 5 to 7 minutes after scattered the worms in the drums, worms should move down into the sludge. The drums were covered with the black plastic sheets (Figure 1b) to prevent the sunlight that could reach and kill worms. The moisture, pH, and temperature were measured daily. Plastic tool was used to dig and mix the sludge in the drums to take care of the worms and harvest them. Worms were harvested after 60 days of raising.

Fig.1: Preparation of plastic drums for worm raising

Fig.2: Harvesting worms in the drum 1, 2 and 3

2.2 Sample collection and analysis

Sludge samples were collected before and after the experiment to assess changes in physical, chemical and biological conditions. About 200g of sludge samples were collected on the plastic drums, then dried, pulverized and mixed to obtain the representative sample. The parameters of temperature, humidity, pH, total nitrogen (Nt), total phosphorus (Pt), total potassium (Kt), E. coli and Coliforms were analyzed for the sludge samples. Methods of collecting and analyzing the criteria were presented in Table 1.

Table 1. Parameters and methods for analysis

Parameter	Unit	Methods
pH	-	TCVN 5979:2007 (ISO 10390:2005) - pH
Moisture	%	TCVN 4048:2011 –Method for determining moisture
Temperature	^oC	TCVN 5508:2009 – Requirements on climatic conditions and measurement

		methods
Total nitrogen (N_t)	%	TCVN 8557:2010 – Fertilizers - Determination method for Nt
Total phosphorus (P_t)	%	AOAC 990.08
Total potassium (K_t)	%	AOAC 990.08
Coliforms	MPN/g	TCVN 4882:2007 (ISO 4831:2006) – Methods to detect and quantify Coliforms
E.Coli	MPN/g	TCVN 6846:2007 (ISO 7251:2005) – Method to detect and quantify E. coli

III. RESULTS AND DISCUSSION

3.1 Characteristics of sludge before the experiment

Some physical, chemical and biological properties of sludge were presented in Table 2. The neutral pH (pH = 7.3) was suitable for raising worms, with the best threshold for the worms to grow in the range of 7.0-7.5. The amount of Nt in the sludge reached a high level of 0.7%. The total potassium concentration in the sludge was low level of 0.046% which was below the nutrient level for plants. The total Pt concentration in the sludge was of 0.675%. Fishery processing sludge with E. coli and coliforms densities were within the permissible levels regulated in the Circular 41/2014 / BNNPTNT(<1000 MPN/g for E. coli; and <3000 MPN/g for coliforms.

Table 2.The characteristics of the sludge sample before the experiment

Parameters	Unit	Value
pH	-	7.3
Moisture	%	85
K_t	%	0,046
P_t	%	0,675
N_t	%	0,7
Coliforms	MNP/g	43
E. coli	MNP/g	0,74

3.2 Characteristics of sludge after the experiment

3.2.1 pH

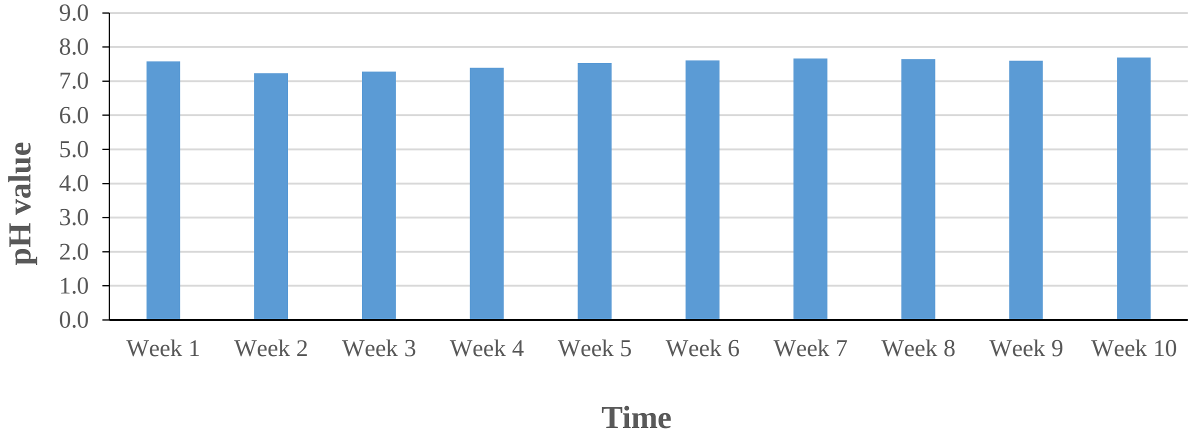

Fig.3: pH during the experiment

Figure 3 showed that the pH maintained neutral level ranged from 7.6 to 7.7. Peryonyx excavatus favors to live in a wet and stable environment, the most suitable pH is about 7.0 - 7.5, but they can withstand a fairly wide pH range of 4 - 9, if the pH is too low they will move away. Thus, the pH in the experiment meets the growth and development requirements of Peryonyx excavatus.

3.2.2 Moisture

Figure 4presented that the humidity did not change much and ranged from 76.0% to 76.7%. The appropriate humidity for Peryonyx excavatus is between 75-80%. Thus, the moisture in the experiment was kept stable during raising worms.

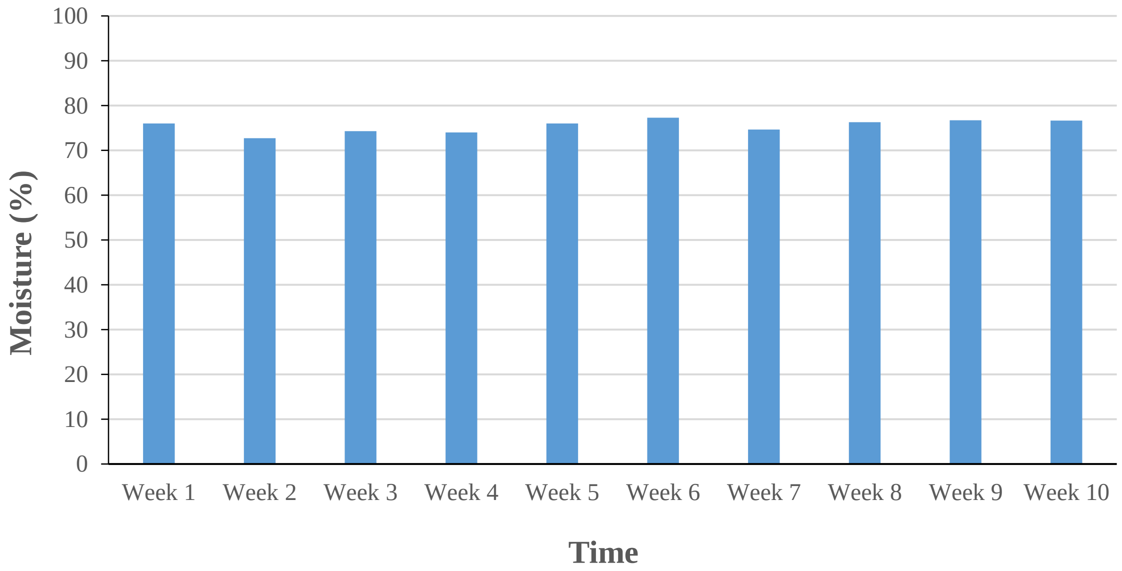

Fig.4: Moisture during the experiment

3.2.3 Temperature

The results in Figure 5indicated that the temperature varied with the weather outside, because the temperature range of raising the worms is relatively wide from 22ºC to 38ºC, the best for worms to live and grow well from 28ºC to 33ºC. The temperature results during the study were kept consistent for Peryonyx excavatus to grow well.

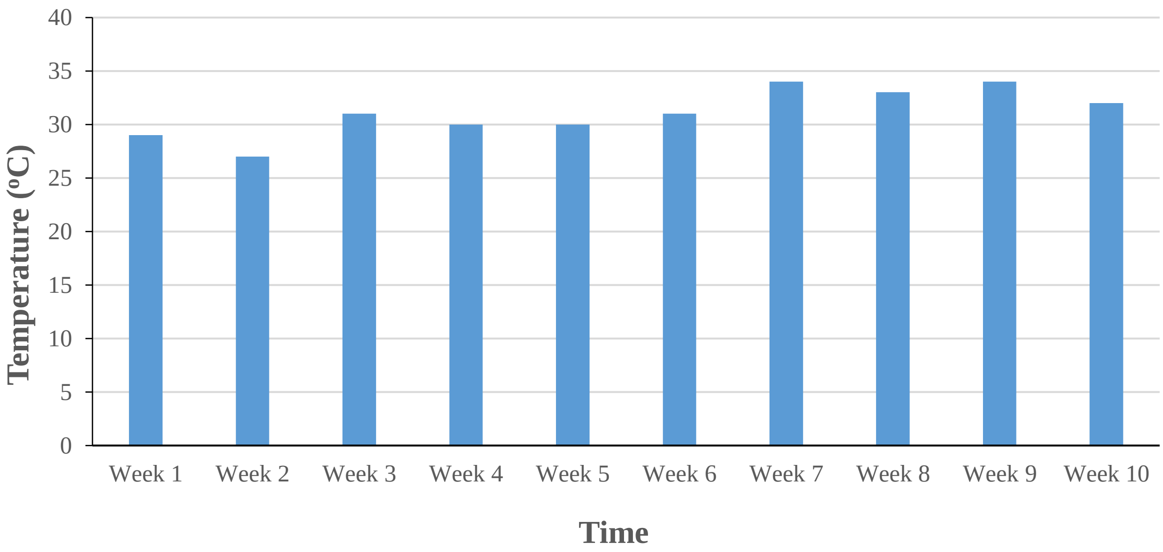

Fig.5: Temperature during the experiment

3.2.4 Properties of sludge after the experiment

The results from table 3 indicated that the potassium content in the sludge after used for raising the worm was high increasing from 0.046%to 0.245% estimated 5.32 times higher than that in the sludge before raising worm. The sludge after the experiment containedrich potassium which will be very beneficial for the use as fertilizer. Potassium is one of the macronutrient elements, plays an important role in agricultural production, contributing to increasing productivity and quality of agricultural products.Total phosphorus Pt increased sharply from 0.675% to 3.381%, 5 times higher than the input sludge. Further studies need to clarify this metabolic mechanism. The total phosphorus content increased by five times, indicating that the worms play an important role. Phosphate content in sludge after the worm is suitable for plants. Total nitrogen increased sharply from 0.70% to 3.23%, 4.6 times compared to the input sludge samples. Similar to phosphorus, nitrogen is present in the sludge after raising the worms suitable for use as fertilizer. The results of the analysis of the finished product samples showed that Coliform and E. coli decreased sharply, in which E. coli was no longer presented in the samples (Table 3).Thereby, it was found that the amount of input sludge providing feed as well as a living environment for the worms. The metabolism of the worms will release feces (Vermicas) out of the surface which is very nutritious. With the intial amounts of worms of 1.5 kg after sixty days the amounts of worms increased to 5 kg.

Table 3. Properties of sludge after the experiment

Parameters	Unit	Before the experiment	After the experiment
K_t	%	0,046	0.245
P_t	%	0,675	3.381
N_t	%	0,70	3.32
Coliforms	MNP/g	43	2.3
E. coli	MNP/g	0,74	0

3.3 Benefit of using sludge for raising Peryonyx excavatus

The intial estimate of economic benefit of growing *Peryonyx excavatus* using the sludge from the seafood processing wastewater treatment plants was described below.

In the experiment, 30 kg of water-decanted sludge was used, 1.5 kg seed worms was placed in the three drums. After 2 months of experiment from February 22, 2019 to April 22, 2019, the amount of the worms in each plastic drim increased from 1.5 kg to 5 kg.

Table 4.Simple cost-benefit analysis

Materials	**Unit**	**Cost**	**Amount**	**Money (VND)**
Input				**315,000**
Seed worm	Kg	30.000	4.5	135.000
Sludge	Kg	0	0	0
Plastic drum	piece	55.000	3	165.000
Plastic folk	piece	15.000	1	15.000
Output				**572,000**
Feeding worms	kg	35,000	15	512,000
Soil after raising worms	kg	2,000	30	60,000
Benefit				**315,000**

In addition to economic benefit, the use of sludge to feed the worms could contribute to solve the environmental problem and reduce the cost for sludge treatment. The sludge after the experiment could be used as organic fertilizers for improving soil fertile.

IV. CONCLUSION

The results showed that the sludge after raising the worms (*Peryonyx excavatus*) has the nutrient components increased significantly. Total nitrogen increased from 0.7% to 3.32%, total phosphorus from 0.675% to 3.381%, total potassium from 0.046% to 0.245% compared to input sludge. E. coli is not detected and coliform is reduced to a level that is safe for human health. The use of sludge from the wastewater treatment system for raising *Peryonyx excavatus* has many benefits because it both brings an additional income, and converts sludge into organic fertilizer, contributing to solving the environmental problem. This study should be continued to elaborate all the social-econimic and environmental aspects of raising worms using the water-decanted sludge from the seafood wastewater treatment process.

REFERENCES

[1] Vo Phu Duc (2013). Establish a process for producing compost from the sludge generated during pangasius processing. Dong Thap Science and Technology project.
[2] Tran Ngoc Hung (2009). Study on making soluble protein products from earthworm.
[3] Nguyen Lan Phuong (2011). Studying the process of producing compost from waste with the participation of earthworm.

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Surface Water Quality in Aquacultural Areas in an Giang Province, Vietnam

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***Abstract*— *This study was conducted to assess the impact of aquaculture with three fish cultivating models including net fence, cage and earth-pond to surface water quality, using water monitoring data from 2011 to 2019 provided by Department of Natural Resources and Environment, An Giang province, Vietnam. Water quality parameters included temperature, pH, dissolved oxygen (DO), total suspended solids (TSS), biochemical oxygen demand (BOD), nitrate (NO_3^--N), orthophosphate (PO_4^{3-}-P) and coliforms at 13 locations were evaluated followed the National Technical Regulation on surface water quality (QCVN 08-MT: 2015/BTNMT). Multivariate analysis methods comprising Cluster Analysis (CA) and Principal Component Analysis (PCA) were used to group sampling locations according to pollution levels and to identify main water variables influencing on surface water quality. In the aquacultural areas, DO was low while TSS, BOD, PO_4^{3-}-P and coliform were high comparing to QCVN 08-MT: 2015/BTNMT. Among the three types of aquaculture, earth-pond culture resulted in more serious environmental pollution than cage and net fence. Five sources of pollution in the studied water bodies were identified using PCA in which temperature, pH, DO, TSS, BOD, NO_3^--N, PO_4^{3-}-P and coliforms could reflect the quality of water environment affected by aquaculture. CA finding suggested that the number of monitoring points could be reduced from 13 to 9 sampling locations, thus reducing monitoring cost. Future studies should focus on investigating sources of surface water pollution in the aquacultural areas.*

***Keywords*— surface water quality, net fence, earth-pond, cage, organic pollution, microorganisms, An Giang.**

I. INTRODUCTION

An Giang is one of the localities highly potential for economic development in the Mekong Delta region. The dense rivers, canals and abundant fishery resources have generated favorable conditions for the province to increasingly develop its potential, especially its strengths in aquaculture. Approximate140 species of fish have been added during the annual flood season (Nhi, 2005). According to the Department of Agriculture and Rural Development of An Giang province, the area of aquaculture in 2013 reached 2,496 hectares, including the main cultured species of Tra catfish (*Pangasius hypophthalmus*), giant freshwater prawn (*Macrobrachium rosenbergii*), snakehead fish (*Channa striata*), basa (*Pangasius bocourti*), grouper (*Pangasius conchophilus*), he fish (*Barbonymus altus*), tilapia (*Oreochromis niloticus*). Of which, Pangasius hypophthalmus is the main fish for farming in the province with an area of 1,119 ha with a harvested fish output of 273,939 tons equal to 120% compared to 2018. In the first nine months of 2019, the socio-economic situation of the first nine months of 2019 reached 361,000 tons, increasing 4.6% (+ 15.8 thousand tons) over the same period. Of which, cultivation were 347.6 thousand tons, increased 5.8% (Pangasius hypophthalmus accounted for 287.6 thousand tons, increased 7.4%); exploitation was 13.6 thousand tons, equaling 87.8% over the same period, due to low flood water this year reducing natural aquatic resources. Currently, most aquaculture areas do not have water treatment system and cleaning the wastewater maily relies on self-purification of water.

Aquaculture is often associated with environmental pollution if it is not well managed. One of the common environmental problems is the release of excess nutrients into the environment. Thich (2008) reported that the amount of N discharged from intensive Pangasius hypophthalmus ponds was 57.3% (5.43% in water, 50.4% accumulated in sediment and 1.5% losses due to evaporation or infiltration) and the amount of P release was 70.2% (1.8% in water, 64.5% in sediment and 3.9% losses due to evaporation or infiltration). Producing one ton of catfish would discharge 25.2 kg N and 12.6 kg P into the environments. The results also showed that 48.0 - 87.3% of nitrogen (N) and 75.0 - 94.0% of phosphorus (P) of inputs in shrimp ponds were not absorbed for building up shrimp biomass but discharged into the environment through replacing water, discharging at harvest, depositing in pond bottom. It was calculated that every ton of shrimp would discharge approximate 16.8 - 157.2 kg of N and 2.3 - 45.9 kg of P into the environment depending on the food source as well as intensive level.

Several chemicals were also used in Pangasius hypophthalmus farming. According to Phu et al. (2012) investigated chemical use in catfish farming in Chau Thanh district, Dong Thap showed that there were 28 chemicals used to improve water quality, for examples BKC, Zeolite, Chlorine, Formol, Attack, Protex, Vorkon), 8 types of chemicals were added to foods (such as Vitamin C, intestinal probiotics, Sorbitol, Premix types including Yucca, Levet, Zyme Biotic) and 14 antibiotics (such as Enrofloxacin, Sufadimethoxine, Trimethoprime). Le and Munekage (2004) reported Trimethoprim, Sulfamethoxazole, Norfloxacin and Oxolinic acid in the water samples were found at 1.04; 2.39; 6.06 and 2.50 ppm, respectively and in the sediment samples at 734.61; 820,49; 2615.96; 426.31 ppm, respectively. Consequently, the antibiotics resulted in resistant strains of bacteria at most research sites. In addition to the impacts of aquaculture on surface water quality, natural activities such as erosion, stormwater runoff, production and business activities, residential and urban areas, industrial production activities also affect surface water quality (Nga, 2009; Ly and Giao, 2018; Truc, 2019).

Multivariate analysis methods including cluster analysis and principal component analysis are widely applied to assess water quality according to space and time at several monitoring locations using multiparameters (Zeinalzadeh and Rezaei, 2017). Multivariate analysis is used to assess changes in river water quality and identify sources of pollutants (Chounlamany et al., 2017). In addition, the multivariate analysis method is used to establish water monitor network and identify water quality variables that cause changes in surface water quality (Zeinalzadeh and Rezaei, 2017). This study was conducted to assess surface water quality in aquaculture areas in the form of net fence, cage and earth-pond between 2011 and 2019. The results of the study could provide scientific information on progression of water quality due to the impact of aquaculture for better management of water quality in aquacultural sector.

II. MATERIALS AND METHODS

2.1. Water quality data

The data on surface water quality in An Giang province was collected at the Department of Natural Resources and Environment of An Giang province in the period of 9 years from 2011 to 2019 at 13 locations in three types of aquacultural farming including three locations in the net fence model (TS7, TS8, TS9), three locations in the cage model (TS1, TS3, TS4) and seven locations in the earth-pond model (TS2, TS5, TS6, TS10, TS11, TS12, TS13). The sampling locations were shown in Figure 1. Water quality parameters including temperature, pH and dissolved oxygen (DO) were measured directly on site by handheld device. The water quality parameters such as biological oxygen demand (BOD), nitrogen nitrate (NO_3^--N), orthophosphate (PO_4^{3-}-P), and coliforms were collected, properly stored and transported to the laboratory for the analysis using standard methods listed in Table 1.

Table 1. Water quality parameters and analytical methods

No.	Water parameters	Unit	Analytical methods
1	Temperature	^oC	SMEWW 2550B:2012
2	pH	-	TCVN 6492:2011
3	Dissolved oxygen (DO)	mg/L	TCVN 7325:2004
4	Total suspended solids (TSS)	mg/L	SMEWW 2540D:2012
5	Biological oxygen demand (BOD_5)	mg/L	SMEWW 5210B:2012
6	Nitrate (NO_3^- as N)	mg/L	SMEWW 4500-NO_3^-.E:2012
7	Orthophosphate (PO_4^{3-} as P)	mg/L	SMEWW 4500-P.E:2012
8	Coliforms	MPN/100mL	TCVN 6187-2:1996

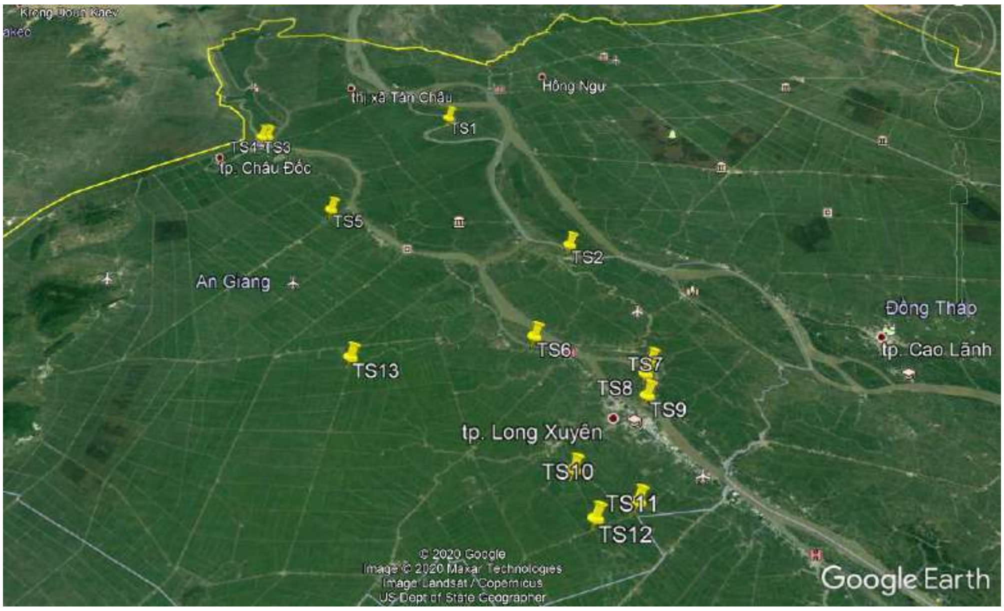

Fig.1: Locations of water quality samples (Google Earth, 2020)

2.2. Data processing

The difference in the mean value of the water quality parameters was performed by analysis of variance (ANOVA), at the significance level of 5%, Duncan test (Ahrari et al., 2015) using statistical software IBM SPSS statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). The two main methods used in the evaluation of monitoring data are Cluster Analysis (CA) and Principal Component Analysis (PCA). The cluster analysis method is applied to group water locations based on all physical, chemical and biological criteria. Sampling sites with similar pollution characteristics will be grouped into the same group, while different pollution characteristics will be grouped into another group and presented as a structural tree (Feher et al., 2016; Chounlamany et al., 2017). PCA method is used to reduce initial data variables that do not make an important contribution to data fluctuation while creating a new group of variables called principal components (PC). These PCs are not related to each other and appear in descending order of importance. The important value to consider in principal component analysis is the eigenvalue coefficient, the greater the coefficient, the greater the major contribution to explaining the variability of the original dataset. This technique is used to determine the number of sources that affect surface water quality in environmental monitoring (Feher et al., 2016). The correlation between the principal component and the primary data variables (water quality indicators) is shown by the significant correlation coefficients (Feher et al., 2016). The absolute value of the significant correlation coefficient is greater than 0.75, which means that the strong correlation between the principal components and water quality criteria, from 0.75-0.5 is the average correlation, and 0.5-0.3 is the weak correlation (Liu et al., 2003). CA and PCA are implemented using copyrighted Primer Software V5.2.9 (Plymounth, UK).

III. RESULTS AND DISCUSSION

3.1. Water quality in the fish cultivating areas

The surface water quality affected by cultivating models were presented in Tables 2-4. Water temperature at 13 sampling locations in three models were in the range of 29.8±1.3-29.9±1.0ºC, 29.4±0.7-29.6±1.0ºC, 29.0±0.7-30.3±1.1ºC in the cage, net fence and earth-pond models, respectively in the period of nine years. Previous study showed that temperature in Hau River ranged from 27.1-32.0ºC (Giao, 2020) and Mekong River ranged from 19.9 to 32.2ºC (Ongley, 2009). Water temperature at the study sites is still suitable for aquatic life according to National technical regulation on surface water quality (QCVN 08-MT: 2015/BTNMT) and Boyd (1998). The mean pH values in the two areas influenced by cage model (7.1±0.2-7.2±0.2) and net fence (7.0±0.2-7.2±0.3) were not much different over the years as well as the sampling locations, fluctuating around the neutral value. In the earth-pond area, the mean pH ranged from 6.7±0.3 to 7±0.3 which is slightly lower compared to the other models. Previous studies reported the pH values in Tien river and Hau river ranged from 7.1 to 7.2, 6.7 to 7.12 (Giao, 2020). The pH values were still suitable for the development of fish as well as aquatic animals and plants (QCVN 08-MT:

2015/BTNMT, column A1). The mean DO concentrations in the cage, net fence and earth-pond models were in the ranges of 4.8±1.0-4.9±1.0, 5.2±1.0-5.5±0.9, 3.9±0.7-5.5±1.0 mg/L, respectively (Table 2-4). There was high variation of DO in the earth-pond area compared to the cage and net fence models. However, DO at the water bodies influenced by fish cultivating areas were lower compared to QCVN 08-MT: 2015/BTNMT which could indicate influence of fish cultivation on surface water quality. Previous studies presented that DO in the rivers rarely meet the national standard. For example, DO concentration in Hau river were in the ranges of 4.8 ± 1.1 - 5.5 ± 0.7 mg L^{-1} (Lien et al., 2016), and 5.29 ± 0.33 – 5.65 ± 0.56 mg L^{-1} (Giao, 2020). DO in canals in Soc Trang province ranged from 1.7 to 6.17 mg L^{-1} (Tuan et al., 2019). The release of wastewater containing nutrients and organic matters could be the main cause of low DO concentration in the aquacultural areas (Ngoc, 2004; Te and Munekage, 2004; Thich, 2008). According to Ongley (2009), the DO concentration suitable for aquatic life is at least 5 mg/L or higher, but the DO concentration at the study sites were relatively low, showing that the water source has been organically polluted. Measures are urgently needed for better management of the surface water quality influenced by aquacultural activities.

Table 2. Surface water quality affected by cage cultivating model

Variables	TS1	TS3	TS4	*QCVN
Temp.	29.9±1.0	29.9±1.3	29.8±1.3	-
pH	7.2±0.2	7.1±0.2	7.1±0.2	6 – 8.5
DO	4.8±1.0	4.9±1.0	4.9±1.0	≥ 6 mg/L
TSS	43.2±18.8	36.1±13.9	36.8±15.8	20 mg/L
BOD	6.7±1.5	7.6±2.5	6.7±2.2	4 mg/L
NO_3^- -N	0.3±0.2	0.2±0.2	0.2±0.2	2 mg/L
PO_4^{3-} -P	0.1±0.1	0.2±0.2	0.1±0.1	0.1 mg/L
Coliforms	3.9±0.5	3.5±0.4	3.7±0.6	3.339 MPN/100mL

Table 3. Surface water quality affected by net fence cultivating model

Variables	TS7	TS8	TS9	*QCVN
Temp.	29.5±0.9	29.4±0.7	29.6±1.0	-
pH	7.0±0.3	7.0±0.2	7.2±0.3	6 – 8.5
DO	5.2±1.0	5.3±0.9	5.5±0.9	≥ 6 mg/L
TSS	37.4±12.3	44.6±16.8	34.8±10.6	20 mg/L
BOD	8.5±3.1	10.7±6.6	7.3±3.8	4 mg/L
NO_3^- -N	0.3±0.3	0.4±0.3	0.4±0.2	2 mg/L
PO_4^{3-} -P	0.3±0.3	0.2±0.2	0.1±0.0	0.1 mg/L
Coliforms	3.5±0.6	3.6±0.5	3.4±0.5	3.339 MPN/100mL

Table 4. Surface water quality affected by earth-pond cultivating model

Variables	TS2	TS5	TS6	TS10	TS11	TS12	TS13	*QCVN
Temp.	29.8±1.1	29.5±1.6	30.3±1.1	29.6±1	29.2±0.9	29±0.7	30±1.1	-
pH	6.9±0.2	6.7±0.3	6.9±0.2	7.0±0.3	6.9±0.2	6.8±0.4	6.8±0.2	6 – 8.5
DO	5.5±1.0	4.1±0.9	4.9±0.6	3.9±0.7	3.9±0.9	4.0±1.1	3.9±0.7	≥ 6 mg/L

TSS	50.0±24.1	83.1±43.6	49.3±24.2	60.3±27.5	64.8±17.0	65.2±28.6	78.7±21.1	20 mg/L
BOD	10.9±7.6	21.5±12	7.5±2.6	9.1±2.3	9.3±4.0	10.9±3.3	12.8±5.8	4 mg/L
NO_3^- -N	0.36±0.28	0.32±0.27	0.4±0.36	0.38±0.2	0.40±0.3	0.46±0.41	0.35±0.2	2 mg/L
PO_4^{3-} -P	0.21±0.17	1.68±1.01	0.23±0.12	0.23±0.1	0.32±0.2	0.38±0.11	0.43±0.46	0.1 mg/L
Coliforms	3.71±0.63	4.11±0.47	3.67±0.61b	3.91±0.6	3.72±0.6	3.72±0.56	3.87±0.53	3.339 MPN/100mL

***Note**: * QCVN is the national technical regulation on surface water quality QCVN 08-MT: 2015/BTNMT, column A1 which is used for domestic water supply, conservation of aquatic plants and animals; coliform was presented in log form.*

The mean concentrations of TSS in the water bodies influenced by fish raising in cage, net fence and earth-pond models ranged from 36.1±13.9-43.2±18.8 mg/L, from 34.8±10.6 to 44.6±16.8 mg/L, from 49.3±24.2 to 83.1±43.6 mg/L, respectively (Table 2-4). The fluctuation of the total suspended solids was relatively high through each sampling position in the period of 2011 – 2019. TSS tended to increase gradually over time, exceeding the permissible limit of QCVN 08-MT: 2015/BTNMT, column A. In the type of cage model, the highest TSS concentration was found in 2017 at the position of TS1 (Phu Tan) and the position of TS3 (Chau Doc) were accordingly 61.7±35.0 and 66.7±18.4 mg/L, exceeded QCVN 08-MT: 2015/BTNMT column A by 2.06 and 2.22 times, respectively. In the net fence cultivation model, the highest TSS concentration was found in 2017 in Long Xuyen City at the positions TS7 and TS8 with the values of TSS 63.7 ± 7.6 and 69.7 ± 13.7 mg/L, respectively exceeded QCVN 08-MT: 2015/BTNMT column A by 3.15 and 3.20 times. In the earth-pond cultivation areas, the highest TSS concentration was in 2014 at the TS5 site (Chau Phu) reaching 187 ± 105.8 mg/L, 9.35 times higher compared to QCVN 08-MT: 2015/BTNMT column A. Former studies reported that water bodies in the Vietnamese Mekong delta have been long contaminated by total suspended solids. For instances, TSS concentrations in Hau River ranged from 41.2 ± 33.7 to 89.57 ± 31.31 mg/L (Lien et al., 2016), TSS in the canals in An Giang province in the period of 2009-2016 fluctuated from 25.0 ± 11.5 to 93.7 ± 28.3 mg/L (Ly and Giao, 2018). In Soc Trang province, TSS concentrations in canals ranged from 16 to 176 mg/L (Tuan et al., 2019). In the current study, the average TSS concentrations in the earth-pond areas were more polluted than those at the cage and net fence areas. The findings revealed that aquacultural activities partly contributed to high TSS in the water bodies which could be artributed to the direct discharge or improperly treated of wastewater generated from aquacultural sites to the receiving waters.

The mean BOD concentrations were in the ranges of 6.7±1.5-7.6±2.5 mg/L, 7.3±3.8-10.7±6.6 mg/L, and 7.5±2.6-21.5±12.0 mg/L in the cage, net fence and earth-ponds models, respectively (Table 2-4). BOD at the water bodies influenced by earth-pond cultural practices was found higher than those in the water bodies influenced by cage and net fence models. Previous studies reported that BOD concentration in water bodies in An Giang province was in the range of 6.6 ± 1.2 - 8.2 ± 2.5 mg/L (Ly and Giao, 2018) which was lower than the values of BOD in the current study areas influenced by aquacultural activities. The mean BOD concentrations in the water bodies influenced by aquaculture exceeded QCVN 08-MT: 2015/BTNMT column A2, from 1.25 to 3.56 times. This result is consistent with the values of low DO and high TSS discussed in the previous sections.

NO_3^--N concentrations in the cage, net fence and earth-pond models were in the ranges of 0.20±0.20-0.30±0.20, 0.30±0.30 0.40±0.30, and 0.32±0.27-0.46±0.41 mg/L, respectively. The data indicated that NO_3^--N concentrations were highly fluctuated over the period of 2011-2019. The concentrations of NO_3^--N in the water bodies in the the earth-pond model was higher than those influenced by cage and net fence models revealed the significant impact of the earth-pond model to surface water quality. Former studies reported that NO_3^--N concentration in Hau River ranged from 0.002 to 0.395 mg/L (Lien et al., 2016), in river systems in Soc Trang province from 0.05 to 0.14 mg/L (Tuan et al., 2019). The concentration of nitrate in the current study was still in accordance with the permitted standard of QCVN 08-MT: 2015/BTNMT, column A1 (2 mg/L). According to Phu and Ut (2006), NO_3^--N concentration suitable for aquatic species ranges from 0.1 to 10 mg/L, so NO_3^--N concentrations at all sampling points over nine years in the current study were in the suitable ranges for aquatic life. However, high concentrations of NO_3^--N can potentially cause eutrophication which subsequently lead to degrade surface water quality resulting in unfavorable conditions for aquatic organisms.

Orthophosphate (PO_4^{3-} -P) concentrations ranged from 0.1±0.1 to 0.2±0.2 mg/L, 0.1±0.0 to 0.3±0.3 mg/L, and 0.21±0.17 to 1.68±1.01 mg/L at the water bodies influenced by cage, net fence and earth-pond models, respectively (Table 2-4). Concentrations of PO_4^{3-} -P in the water bodies influenced by the earth-pond model was much higher compared to those influenced by cage and net fence models. PO_4^{3-} -P agricultural areas and in Hau River ranged from 0.02 to 0.47 mg/L (Ly and Giao, 2018), in Hau River section from An Giang to Hau Giang provinces ranged from 0.04 to 0.11 mg/L (Giao, 2020), and in canals in Soc Trang province ranged from 0.05 to 0.9 mg/L (Tuan et al., 2019). The former studies and the current study revealed that PO_4^{3-}-P at the water bodies in the Vietnamese Mekong delta exceeded the QCVN 08-MT: 2015/BTNMT, column A1. The eutrophication is very likely to take place onece the PO_4^{3-}-P concentration is greater than 0.1 mg/L (Boyd and Green, 2002). This study found that surface water quality influenced by the aquacultural activities is at risk of eutrophication due to orthophosphate concentration.

The density of coliforms ranged from 3.5±0.4 to 3.9±0.5, from 3.4±0.5 to 3.6±0.5, and from 3.67±0.61 to 4.11±0.47 MPN/100 mL at the water bodies influenced by cage, net fence and earth-pond models, respectively (Table 2-4) which exceeded QCVN 08-MT: 2015/BTNMT, column A1. The density of coliforms in the water bodies influenced by earth-pond culture was higher than those at the water bodies influenced by cage and net fence cultures. The results from this study was in accordance with the previous studies that the surface water in the Mekong delta of Vietnam have been long contaminated by coliforms (Lien at al., 2016, Ly and Giao, 2018, Tuan et al., 2019, Giao, 2020).

3.2. Key parameters influencing water quality at the fishery cultivating areas

A total of eight variables of water environment parameters were included in the PCA. The key water parameters influence on surface water quality were presented in Table 5. PC1 explained 58.4% of the variation in the data obtained. The source of this pollution is mainly reflected in the pH (0.420), TSS (-0.446), and BOD (-0.400). PC1 could explain for the water quality at the study site being polluted organically due to the residues of aquatic feed and untreated sewage in the areas of earth-ponds discharged directly into the receiving water. PC2 explained 18.7% of the fluctuation of water quality through temperature (0.50) and NO_3^--N (-0.673). PC3 explained 11% of the data fluctuation, is affected by a moderate level of temperature (0.549), a weak level of DO (-0.445), and a PO_4^{3-}- P (-0,424). PC4 explained 7.4% of the data fluctuation, is affected by moderate level of temperature (0.575), DO (0.534) and weak level of NO_3^--N (0.441). PC5 explained only 2.3% of the water quality variation, affected by the weak level of DO (0.464) and the high level of coliforms (0.772), and showed that microbiological pollution at the surveyed locations. The results also showed that the change of temperature is influenced by at least three factors (PC2, PC3, and PC4), DO by three factors (PC3, PC4, and PC5) and NO_3^--N by two factors (PC2, PC4). The other indicators were only influenced by one PC. Thus, there are at least five sources of pollution affecting water quality in the water bodies that receive aquaculture wastewater. Water pollution sources could be hydrological regimes (currents, tides, and flows), erosion, aquaculture waste, and domestic waste. Eight monitoring indicators including temperature, pH, DO, TSS, BOD, NO_3^--N, PO_4^{3-}-P and coliforms are all very important for monitoring water quality in the water bodies receiving aquacultural wastewater.

Table 5. The main parameters affecting surface water quality in aquaculture area

Parameters	PC1	PC2	PC3	PC4	PC5
Temp.	0.173	**0.509**	**0.549**	**0.575**	-0.228
pH	**0.420**	0.188	-0.064	-0.309	-0.113
TSS	**-0.446**	0.007	0.201	0.062	-0.227
DO	0.356	0.095	**-0.454**	**0.534**	**0.464**
BOD	**-0.400**	0.214	-0.398	0.218	-0.097
NO_3^- -N	-0.180	**-0.673**	0.163	**0.441**	0.109
PO_4^{3-} -P	-0.375	0.297	**-0.424**	0.089	-0.225

Coliform	-0.367	0.332	0.287	-0.186	**0.772**
Eigenvalue	4.67	1.50	0.88	0.59	0.18
Variation (%)	58.4	18.7	11.0	7.4	2.3
Cum.Var (%)	58.4	77.1	88.1	95.6	97.8

3.3. Grouping water quality in the aquaculture area

The results of water quality clustering were presented in Figure 2. At the distance of 4.0, water quality in the water bodies receiving aquaculture water can be classified into three groups. Group 1 includes the site TS5; Group 2 includes the locations of TS1, TS2, TS3, TS4, TS6, TS7, TS8, TS9; and Group 3 with the positions of TS10, TS11, TS12 and TS13. From this result, it is possible to select a water sampling location based on the locations in the same group with similar water quality, so only a representative location is needed. However, in this case, due to the locations in different water bodies such as Tien River (TS1, TS2), Chau Doc River (TS3), Hau River (TS4, TS6, TS7, TS8, TS9), Xang Vinh Tre canal (TS5), Xa Doi Canal (TS10), Don Dong canal (TS11, TS12) and Tra Cu Canal (TS13), so the locations should be arranged according to the same rivers. On Tien River, it is possible to choose TS1 or TS2 because these two locations are grouped into Group 2; On the Hau River, only one sample from TS7-TS9 is required to be sampled and one of the three positions from TS7-TS9 is located in the same sub-group in Group 2. The two positions on the Don Dong channel are in the same group with Group 3, so only one is needed. Thus, from 13 sampling points can be reduced to 9 sampling points according to this study.

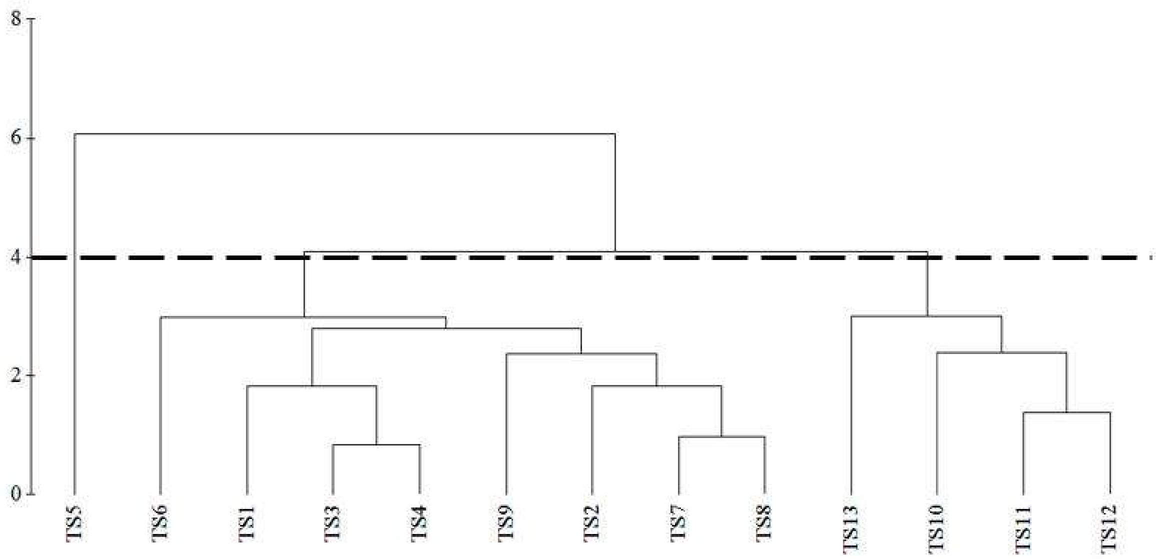

Fig.2: Grouping water quality using cluster analysis

IV. CONCLUSION

The analysis of surface water quality affected by aquaculture activities in An Giang province in the period of 2011 - 2019 showed that the water has the problems of total suspended solids, organic matters and coliforms. Among the three types of aquaculture, earth-pond culture resulted in more serious environmental pollution than cage and net fence since the water quality assessment criteria such as DO, TSS, BOD, NO_3^--N, PO_4^{3-}-P and coliforms in earth-pond culture area were much higher than those at the cage and net fence areas. PCA analysis showed that there are five main factors corresponding to five sources of pollution in the studied water bodies. The parameters of temperature, pH, DO, TSS, BOD, NO_3^--N, PO_4^{3-}-P and coliforms are very important for monitoring water quality in the area receiving aquaculture wastewater. CA analysis showed that water quality in aquaculture-affected water bodies can be classified into three groups and from this grouping, the number of monitoring points can be reduced from 13 to 9 sampling points taking into account the different water bodies. Future study should focus on investigating specific sources of pollution to effectively solve the pollution problem in the study area.

REFERENCES

[1] Ahrari, F., Eslami, N., Rajabi, O., Ghazvini, K., Barati, S. (2015). The antimicrobial sensitivity of Streptococcus mutans and Streptococcus sangius to colloidal solutions of different nanoparticles applied as mouthwashes. Dental Research Journal 12:44–49.

[2] Boyd, C.E. (1998). Water quality for pond aquaculture. Resarch and development series. No.43. International center for aquaculture and aquatic environtments Alabama quaculture experient station Auburn University.

[3] Boyd, C.E., Green, B.W. (2002). Water quality monitoring in shrimp farming areas: an example from Honduras, Shrimp Farming and the Environment. Report prepared under the World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment, Auburn, USA, pp. 29.

[4] Chounlamany, V., Tachuling, M.A. and Inoue, T. (2017). Spatial and temporal variation of water quality of a segment of Marikina River using multivariate statistical analyses, Water Science & Technology 76, 1510-1522.

[5] Feher, I,C., Zaharie, M., Oprean, I. (2016). Spatial and seasonal variation of organic pollutants in surface water using multivariate statistical techniques. Water Science and Technology, 74: 1726-1735.

[6] Giao, N.T. (2020). Evaluating current water quality monitoring system on Hau River, Mekong delta, Vietnam using multivariate statistical technique. Journal of Applied Environmental Research, 42(1):14-25.

[7] Le, T.X., and Munekage, Y. (2004). Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Vietnam. Mar. Pollut. Bull. 49: 922-929.

[8] Lien, N.T.K., Huy, L.Q,. Oanh, D.T.H., Phu, T.Q., and Ut, V.N. (2016) Water quality in mainstream and tributaries of Hau River. Can Tho University Journal of Science, 43, 68-79 (In Vietnamese).

[9] Liu, C. W., Lin, K. H., and Kuo, Y. M. (2003). Application of factor analysis in the assessment of groundwater quality in a Blackfoot disease area in Taiwan, Science of the Total Environment 313, 77–89.

[10] Ly, N.H.T., and Giao, N.T (2018). Surface water quality in canals in An Giang province, Viet Nam, from 2009 to 2016. Journal of Vietnamese Environment, 10(2): 113-119.

[11] Mekong River Commission. (2015). Lower Mekong regional water quality monitoring report. ISSN: 1683-1489. MRC Technical Paper No.51.

[12] Ministry of Natural Resources and Environment. (2015). QCVN 08-MT: 2015/BTNMT National technical regulation on surface water quality.

[13] Ngoc, L.B. (2004). Assessing the environmental quality of intensive pangasius (Pangasianodon hypopthalmus) pond in Tan Loc commune, Thot Not district, Ho Chi Minh City Can Tho. Master's thesis in Environental Management, Can Tho University. (In Vietnamese).

[14] Nam, L.H., Hoai, D.T., Trang, N.T., Ngan, N.V.C. (2017). Surveying the status of the farming area and the quality of the water resources for Pangasius farming in Thanh Binh district, Dong Thap province. Science Journal of Thu Dau Mot University. S. 32 (2017), 181 – 190. (In Vietnamese).

[15] Nga, TN. (2009). Surveying surface water quality in the water body of Long Xuyen city (Ward area: My Binh, My Phuoc, My Long, My Xuyen) to determine the pollution level of the water area. An Giang University. (In Vietnamese).

[16] Ongley. E. D. (2009). Water Quality of the Lower Mekong River. In: Campell IC (ed) The Mekong: Biophysical environment of an international river basin. Acedemic, New York.

[17] Phu, T.Q, Ut, V.N. (2006). Water quality for pond aquaculture. Can Tho University. College of Aquaculture and Fisheries. 199 pp. (In Vietnamese).

[18] Thich, CV. (2008). Water quality and nutrient accumulation in intensive pangasius ponds (Pangasianodon hypophthalmus Sauvage, 1978) in Omon district, Can Tho city. Master's thesis specialized in fisheries. Can Tho University. (In Vietnamese)

[19] Truc, D.T., Phat, P.H., Nam, N.D.G., Toan, P.V., Tri, V.P.D. (2019). The water surface quality of Tien River in the area of Tan Chau district, An Giang province. Journal of Science Can Tho University. No 55(2019), 53-60. (In Vietnamese).

[20] Tuan, D.D.A., Thu, B.A., and Trung, N.H. (2019). Assessing quality of surface water for urban water supply source for Soc Trang City. Scientific Journal of Can Tho University, No 4A (2019):61-70 (In Vietnamese).

[21] Zeinalzadeh, K., and Rezaei, E. (2017). Determining spatial and temporal changes of surface water quality using principal component analysis. Journal of Hydrology: Regional Studies 13: 1-10.

Investment Analysis of Sunflower Farming and Prospects of Raising Household income in Iramba District, Tanzania

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Abstract— *Sunflower production has a potential to play great role in poverty reduction in Tanzania. It grows well indry land like Iramba district where other crops cannot perform well. Despite this potential, poverty prevalence in Iramba district is still alarming as 52% of Iramba households are poor with an average earning of 85,125Tsh/month. This paper assessed contribution of sunflower production towards reduction of income poverty in Iramba district by using Gross Margin, Return on Investment (ROI) and poverty analysis methods. Stratified sampling technique was used to select 107 sunflower farmers for interviews. Focus Group Discussion and desk review of literature supplemented data collected through interviews.*

Findings of the study revealed that sunflower production achieved a gross profit margin of Tanzanian shillings 41,540.78/acre (18.71USD/acre) and a Return on Investment of 16% per acre. However, poverty analysis showed that the level of sunflower returns per acre had little contribution in terms of meeting the international poverty line. This is equivalent to only daily per-capita earnings of 167.63Tsh (0.07USD) that is far below income poverty line of 4,009Tsh/day (1.90 USD).

It is therefore recommended that large scale sunflower processors engage in sunflower farming through backward integration taking on board out grower schemes. The schemes will help small holder farmers gain new knowledge on best farming practices to increase productivity and gain access to reliable market for their produce. Additionally, further research on comparison of costs and benefits for monoculture onfarm/onstation sunflower production trials by using improved seeds should be conducted. The research should also investigate efficiency of mixed cropping patterns practiced by farmers to find out the contribution of each crop in the farming systems towards income poverty alleviation.

***Keywords*— Sunflower Farming, Household income, poverty reduction.**

I. INTRODUCTION

Sunflower sub-sector contributes about 32% of national edible cooking oil in Tanzania (Iringo, 2013); whose main production (61%) equivalent to 267,274 MT is grown in central corridor of Dodoma and Singida regions (RLDC-Tanzania, 2015). The rest is grown in other regions including Iringa, Ruvuma, Rukwa and Mbeya in the south corridor and Morogoro region in the East. Generally, Tanzania ranks the tenth world sunflower producer and second Africa's sunflower production accounting for 2.4% and 35% of the world and Africa's production respectively (factfish, 2010; Komba *et al.*, 2017). Production of sunflower oil seeds in Tanzania is dominated by small-scale farmers amounted to 4,000,000 households (URT, 2016) equivalent to 24 million residents which is about half of Tanzanian population. This implies that development of the sector can serve as a potential tool for improving the livelihoods and welfare of majority of the poor in the country (Zeng, 2011).

Singida region accounts for two third of all sunflower produced in the country with all of its districts engaged in sunflower production (Agricultural Census 2010/11). Meanwhile Iramba is the main sunflower producer accounting for 55.7% of the regional production whose residents (39,376 households) are engaged in the production with an average density of 1.3ha/household, and productivity of 0.7ton/ha (URT, 2008).

Sunflower's good performance in poor soils and dry conditions puts the crop in a position to be up-scaled throughout the country; particularly in marginal areas where other crops cannot perform (URT, 2016). Moreover, sunflower production has relatively higher profitability amounting to$750/ha than other crops which can be grown in semi-arid areas like maize, sorghum and millet with profitability of $300/ha, $690/ha and $260/ha respectively (Tran –SEC, 2017). Its performance in poor soils and dry conditions; and higher production profitability identifies it as a climate smart crop that can serve a great role in poverty reduction and national economic development at large.

It can be acknowledged that significant growth trends in sunflower production has been experienced in Tanzania from less than 500,000 metric tons in 2008 to about 2.9 three million metric tons in 2016 (URT, 2016; Balchin et al., 2018). Despite the growing trend of sunflower; the sector has been facing challenges that hindered smallholder farmers and stakeholders at large to deploy its benefits to its full potential. Insufficient accessibility to quality certified sunflower seeds, lack of public and private partnership, non-compliance to international food safety requirements and inadequate extension services, dependence on rain-fed agriculture, poor mechanization for cultivation using hand-hoe and dependence on smallholder farmers whose farming target the subsistence level are among challenges that have been facing agricultural sector and sunflower subsector in particular.

Various stakeholders (including Government, development partners and researchers) have devised numerous efforts to address poor yields of the crop and poverty reduction among smallholder farmers. The **Government** initiated Kilimo Kwanza in 2010 to address commercialization and modernization of Agriculture sector (including sunflower sub-sector) by improving productivity and Tradability of Agricultural products. Moreover, the government established Market Infrastructure and Value Added, Rural Finance (MIVARF) programme in 2011 to tackle limited access to financial services and poor market infrastructure in Singida region under sponsorship of International Fund for Agriculture and Development (IFAD) and the African Development Bank (Mlay, 2017). This was followed by enactment of the National Agricultural Policy 2013 (URT, 2013) that promotes production, productivity; competitiveness and profitability of agricultural sector through strengthen agricultural research services. The Policy' objectives were targeted to be achieved through research services on plant breeding, development of biotechnology techniques, promotion of various irrigation techniques and efficient utilization of agricultural mechanisation among others. Moreover, development of Sunflower Sector Development Strategy 2016-2020 (URT, 2016) for improvement of accessibility to high quality certified sunflower seeds, development of public private partnership for increasing production, enhancing business environment, assurance of compliance and adherence to international recognized food safety standards for improvement of marketability of sunflower seeds and sunflower seeds' products to international markets. Others include imposing of 10% on imports of crude Palm oil in 2016 to promote sunflower subsector in the country production and processing of locally produced sunflower seeds.

Development partners' contribution is also acknowledged through various efforts. The Rural Livelihood Development Company (RLDC) targeted to lessen high poverty prevalence in Dodoma and Singida regions. The Company aimed to improve livelihoods of people through making market systems work better for the welfare of rural subsector. This was done through establishment of Rural Livelihood Development Programme (2004-2015) aimed to improve livelihoods of smallholder producers and related enterprises in the Central Corridor of Tanzania through increased income and employment opportunities. In Phase V (2012-2015) RLDP supported contract farming focused on three crop sub-sectors of rice, cotton and sunflower (Gross et al., 2016).

Various **authors** have documented diverse knowledge on sunflower subsector. However, these studies focused on upgrading sunflower value chain (Ugulumu and Inanga, 2013; Mgeni et al., 2019); identification of the sector's potentials in the entire economy (Liberio, 2012; TEOSA, 2012; Komba et al., 2017); investigated impact of contract farming in sunflower production (Henningsen et al., 2015); and examined the institutional and policy analysis governing the sector (Tran-SEC, 2017; Balchin et al., 2018).

Despite the aforementioned efforts; the national sunflower seed production to date stands at only three million tons per annum with an average productivity of 1.2 tons/ha compared to the national production potential of 10 million tons per annum (URT, 2016) and potential yield of 3tons/ha (FAO, 2010) leading to underutilized processing plants' capacities and continued poverty prevalence in semi-arid areas like Iramba district. According to Tanzania Livelihood Baseline Profile (2015), more than half (52%) of households in Iramba district are either very poor or poor (Tanzania livelihood Baseline Profile, 2015; 2016). The remaining households (48%) fall in the middle and better-off (TLBP, 2015). The 22% (very poor) of households in the district earn an average of TZS 85,125 per month and normally own one cheap cell phone, 1-3 acres of land and manage to cultivate up to two acres only for different crops. In most times of the year these households are featured with food insecurity because during the cropping season, they normally work on both their fields and fields for the better off households. The 30% (poor) earns averagely TZS 98,375 per month. Only 14% (better off) households earn an average of TZS 380,333 per month. This paper seeks to assess contribution of sunflower production on poverty alleviation in Iramba district. Specifically, the paper intends to describe socioeconomic characteristics of households; to estimate Gross Profit Margin, Return on Investment and carries out poverty analysis based on poverty line among sunflower farming households. Information from this study is important for governments to improve policy and regulatory framework, private sector to make informed decision on investment portfolios and development partners to facilitate promotion of sunflower sub-sector.

II. METHODOLOGY

The study used cross sectional research design. A sample size of 107 was selected through multi-stage and stratified sampling techniques from four wards of Iramba district namely: Kisisiri (30), Kiomboi (30), Ulemo (17) and Urughu (30) as representatives of smallholder farmers. Primary data was collected pertaining to address objectives of the study. For social economic characteristics, variables included household demographics, education levels of household heads, farming tools, farm size and other income generating activities. For gross margin analysis, returns on investment and poverty analysis; the data collected pertained variables on sunflower yields, production cost per hectare, selling price per 65kg bags and household composition structure. These data were collected through interviews, focused group discussion and observation. Moreover, secondary information on Adult equivalent units, national basic needs poverty line, national food poverty line and international poverty line were solicited to complement the data from field work.

Data were analysed by using four methods: descriptive statistical methods, Gross Margin Analysis, Returns on Investment and Component Poverty Analysis Approach. Statistical Package for Social Science version 20 (SPSS, 20) was used for descriptive statistics that include univariate frequency distribution, bivariate analysis & measures of central tendency; while excel was used to undertake Gross margin analysis, Returns on Investment and Poverty analysis.

The GMA was done to assess costs of production incurred by sunflower farmers and revenue obtained after sale of sunflower produced; that in turn revealed gross profit earned. Gross Margin represents proportion of total sales revenue retained by a farmer after subtracting the direct costs associated with produced sunflower. The higher the percent, the more the farmer retains on each unit of money of sales to serve his other obligations. Essentially, gross margin measures how efficient household uses its raw materials and labour in producing sunflower; and indicates the household's financial health.

Gross margin (GM) was assumed to be the ratio of gross profit to the total revenue (TR). The gross profit in this case refers to the difference between Total Revenue (TR) and Operational Costs (OC). The Gross margin then is presented on equation 1

$$GM = \frac{TR-OC}{TR} \quad \text{.......} \quad (1)$$

Then, Total revenue (TR) was thought to be a product of the quantity of sunflower produced (bags) [Q_{sn}] times the price of the sunflower produced [P_{sn}] as presented in equation 2

$$TR = (Q_{sn})(P_{sn}) \quad \text{.......} \quad (2)$$

On the other hand the operational costs taken into consideration for sunflower production include: costs of hiring land (C_{ld}), costs for purchasing seeds(C_{sd}), planting costs (C_{pl}), weeding costs (C_{wd}), costs of purchasing pesticides (C_{ps}), costs of harvesting (C_{hr}), costs of fertilizers (C_{ft}), costs of extension services (C_{ext}) and transportation of the yield back home (C_{tr}) as displayed in Equation 3

$$C_{TT} = \sum(C_{ld}+C_{sd}+C_{pl}+C_{wd}+C_{ft}+C_{ps}+C_{hr}+C_{ext}+C_{tr}) \quad \text{........} \quad (3)$$

Empirically, equation (1) can also be written as displayed in equation 4

$$GM = \frac{(Qsn)(Psn)-\sum(Cld + Csd + Cpl + Cwd + Cft + Cps + Chr + Cext + Ctr)}{(Qsn)(Psn)} \quad \text{..............} \quad (4)$$

Then, as described above Returns on Investment is the ratio of gross profit (TR – TC) to the costs invested (TC). In other words, how many cents can be earned when a shilling have been invested in the sunflower production. The computation of the ROI was done using equation 5 below

$$ROI = \frac{TR-TC}{TC} \quad \text{.......} \quad (5)$$

Empirically, ROI was computed using equation 6 as follows

$$ROI = \frac{(Qsn)(Psn) - \sum(Cld + Csd + Cpl + Cwd + Cft + Cps + Chr + Cext + Ctr)}{\sum(Cld + Csd + Cpl + Cwd + Cft + Cps + Chr + Cext + Ctr)} \ldots\ldots \quad (6)$$

Then, the adjusted household size was (HHS_{adj}) was computed to reflect the costs of living per adult person used in estimation of basic needs poverty line, using OECD modified equivalency scale whereby the first household member was assigned with 1 unit, each additional adult member 0.5units and each child of less than 18 years was assigned with 0.3units.

The Per capita income ($Inc._{pc}$) was computed by dividing the monthly Gross Profit (TR - VC) from sunflower production to adjusted household size (HHS_{adj}) using equation 7 below

$$Inc._{pc} = \frac{TR - TC}{HHSadj} \ldots\ldots \quad (7)$$

Finally, contribution of sunflower production to poverty reduction (%Pov) was worked out with the percentage proportion of the monthly income per-capita ($Inc._{pc}$) divide the national basic need poverty line of Tsh 36,482 per adult equivalent stipulated by World Bank Group (2015) times 100 using equation 8

$$\%Pov = \frac{Inc.pc}{36{,}482} X\ 100 \ldots\ldots \quad (8)$$

III. RESULTS

3.1 Household socio-economic characteristics

Sunflower farmers in Iramba district also perform other income generating activities. These include: animal keeping, trade activities, employment, Beekeeping, mining, and bricks make (Table 1). Animal keeping dominated (62%) other income generating activities among respondents. The animals kept include Cattle, Goat, Sheep, Donkey, and Pig. To sunflower farmers animal keeping is very crucial as it saves three main purposes: generation of manure that is used to fertilize the farm, ploughing farm land and transporting harvested seeds from the farmland back to their homestead. These finding is similar to findings by Singida Region Agricultural Sample Census (2007/08) who revealed that majority of households (53%) were involved in both crop and livestock production.

Table 1: Income generating activities among sunflower farmers in Iramba district

Income generating Activities	Frequency	Percentage (%)
Animal keeping	67	62.62
Trade activities	31	28.97
Employed	4	3.74
Beekeeping, mining and bricks making	5	4.76
Total	**107**	**100.00**

Age of respondents:

The average age of people engaged in sunflower production ranged between 22 to 65 years with the mean age of 38.2. This was dominated (88%) with youth farmers aged between 18 and 41 years; followed by adults aged between 42 – 59 years that composed 9% and elders with at-least sixty years of age accounting for only 3%. This implies that any intervention geared improvement of the sunflower production will trickle down to the whole society as most farmers have family responsibilities. This finding is different from the finding by Liberio (2012) and Tuntufye (2013) who discovered that sunflower is more cultivated by farmers aged between 41 and 50 years in Mlali ward and Mvomero District of Morogoro region. The probable reason could be the social set up differentials in the study areas. In the central zone where Iramba District is found, people get married earlier compared to Eastern zone. Therefore, residence of Iramba become accountable for family responsibilities earlier than those of the eastern zone.

Education levels of sunflower farmers: Sunflower farmers had different education levels in the study area. Farmers with primary education dominated (82%) other education groups (Table 2). This implies that sunflower production serves marginalized group of people with lower education levels who have no alternatives to other employment opportunities. Therefore, interventions geared towards improvement of the sunflower would improve livelihood of the marginalized group of people regarding alternative employment opportunities. The findings regarding education levels are similar to that of Liberio's (2012) and Tuntufye (2013) who found that sunflower farmers are dominated by farmers with primary education accounted for 77% and 78% in Mlali ward and Mvomero district respectively.

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Table 2 Education levels

Education level	Frequency	Percentage (%)
Informal education	1	0.93
Primary education	88	82.24
Secondary education	11	10.28
Tertiary education	7	6.54
Total	**107**	**100.00**

Farming tools

Sunflower farmers employed diverse farming tools. However, these tools were dominated by ploughing that used by 95% of respondents (Table 3). Application of ploughing in the study area might explain the fact that small holder farmers cultivate relatively bigger farm size (6acres/household) compared to the most experienced of up to two acres/household elsewhere in the country on other crops. This result is different from that reported by Tuntufye (2013) that show that 88% of all sunflower farmers in Mvomero district use hand hoe. The reason behind this difference can be associated with the fact that, a big number of sunflower farmers in Iramba District are animal keepers, something that does not exist for the sunflower farmers of Mvomero District. Actually, animal production was raised to cater for three main functions (i) production of manure for fertilizing the land and (ii) ploughing land for tilling purposes and (iii) transporting harvested yields back to their homestead.

Table 3 Farming tools employed in sunflower farming

Farming tool	Frequency	Percentage (%)
Hand hoe	3	2.80
Plough	102	95.33
Tractor	2	1.87
Total	**107**	**100.00**

Sunflower farm size: Sunflower farmers in Iramba district cultivated mean farm size of 6 acres/household with the minimum farm size two acres/household and maximum farm size of 30acres/household. Moreover, most sunflower farmers (72%) cultivated farms with sizes between 1-5 acres that was followed by farmers cultivating between six and ten acres/ household (18%) (Table 4). The intervention can be done to think on how to intensify the crop through the use of improved agricultural inputs such as improved seeds and pesticides to improve productivity. The finding tallies with the findings by ASA & RLDC's (2012) who revealed that most (58%) of farmers in the central corridor grown sunflower in less than 5acres. MMA (2009) also found similar results that value chain is in its infancy with low volumes of production due to few households which on average grow less than 4 acres.

Table 4: Sunflower farm size

Acres	Frequency	Percentage (%)
1-5 acres	77	71.96
6-10 acres	19	17.76
11-15 acres	4	3.74
16-20 acres	2	1.87
21-25 acres	1	0.93
26and above acres	4	3.74
Total	**107**	**100.00**

3.2 Gross Margin and Returns on Investment

Table 5 shows that a farmer accrued revenue of 300,762Tsh/acre with a gross profit of 41,540.78Tsh/acre. Moreover, the associated Gross Margin for sunflower production was 13.8% while the return per shilling invested of 16%. The return on investment seems to be little since it is similar to the current normal banking lending rates in the country that ranges between 16% and 25% per annum. Moreover, the household raising sunflower on farm size of 6acres/year in mixed cropping pattern, was able to earn only 249,244.68 per annum from sunflower production. In this situation, the investment in sunflower production can only suffice to repay banking interest rate. Although assessment of profitability for sunflower production seem to be unviable for someone to invest, it should be noted that farmers practice mixed farming with other crops such that the operational costs of the farm can be shared among all of the crops raised, that in turn raise the profitability of sunflower production. The results from this study regarding profitability is different from findings by MMA (2009) in Tanga region who revealed the gross margin of up to 60% among smallholder farmers. Moreover, the results also differ from findings reported by Ugulumu (2008) with an average profit margin of USD 257 equivalent to 359,800 Tsh/acre in Singida region.

Table 5: Gross Margin and Returns of Sunflower Production

S/N	Cost Items	Cost (Tsh)	(%) composition
1	Land hiring costs (Tsh/acre)	25,327.33	9.8
2	Seeds cost (Tsh/acre)	32,476.67	12.5
3	Tilling costs (Tsh/acre)	48,666.67	18.8
4	Planting Costs (Tsh/acre)	12,959.33	5.0
5	Weeding Costs (Tsh/acre)	55,881.33	21.6
6	Fertilizer Costs (Tsh/acre)	6,928.67	2.7
7	Pesticides Cost (Tsh/acre)	4,336.67	1.7
8	Harvesting Cost (Tsh/acre)	38,386.00	14.8
9	Extension Services Costs (Tsh/acre)	7,55.33	2.9
10	Transport Costs (Tsh/acre)	26,707.33	10.3
11	**Total Cost per acre [1+2+3+4+5+6+7+8+9+10]**	**259,221.3**	**100**
	Revenue computation		
12	Sunflower seeds Harvested (Bags/ acre)	6.0	
13	Price of sunflower seeds (Tsh/bag)	50,127	
14	Total Revenue per acre [12*13] (Tsh)	300,762	
15	**Gross Profit [14-11] (Tsh/acre)**	**41,540.78**	
16	**Gross margin [15/14] (%)**		**13.8**
17	**Return On shilling invested [15/11] (%)**		**16**

Moreover, gross margin analysis revealed costs involved in sunflower production (Table 5). The weeding costs dominate (21.56%) costs involved in the sunflower production. This is followed by tilling, harvesting, seeds and transport costs that compose 18.77%, 14.81%, 12.53% and 10.30% respectively of the total costs incurred. These five cost components account for more than three quarters of the total costs incurred amounting to 259,221Tsh/acre. Hence whoever wants to intervene in sunflower production have to think on these cost components as key drivers of investment in that venture and hence its profitability. However, the cost of producing sunflower seeds in Iramba district is more or less similar to the costs of sunflower production in Ilonga, Morogoro that stood at 380,000/Tsh/acre (Mpangalile et al. 2008).

Through Focused Group Discussions (FGDs), it was revealed that smallholder sunflower producers normally work collectively in the farm of one farmer after another rather than hiring labours. However, these costs can be regarded as implicit costs since farmers were to work to the neighbours' farm in some days to compensate works done to his farm. This would even lower further earning from sunflower production to losses. Implicit costs incurred in the study area include costs of cultivation, costs of weeding, costs of harvesting and costs of transporting the yields to their homestead though was out of the scope of this study.

3.3 Contribution towards poverty reduction measures

Findings from poverty line analysis showed that sunflower production contributes by 13%, 19%, and 4.18% of the national monthly basic needs poverty line, national food poverty line and international poverty line of USD1.90 respectively. The adjusted household size equivalent was found to be 4.13 persons based on OECD modified equivalence scale. Thus the Annual per-capita income from sunflower production of 60,249.80Tsh (249,244.68T ÷ 4.13). This income is equivalent to per-capita monthly income of 5,029.15 (60,249.80 ÷ 12) is far below the national basic needs poverty line and food poverty line of 36,482Tsh and 26,085Tsh respectively. In fact, income from sunflower production contributes to 13% and 19% of basic needs and food needs requirements reflecting the fact that $[(5{,}029.15/36{,}482)*100]$ and $[(5{,}029.15/26{,}085)*100)]$ respectively. The daily per-capita income from sunflower production of 167.36Tsh (5,029.15 ÷ 30) equivalent to (0.76$/day) was attained. This is far below the international poverty line of $1.90 (4,009Tsh) with the exchange rate of $1 = 2,220Tsh during data collection. Hence, the 167.63Tsh (0.07USD) contributed only 4.18% to the international basic need poverty line. Although income earned from sunflower production cannot suffice farmers from poverty, the fact that sunflower production is done using mixed farming with other crops; isolation of costs pertained to only sunflower farming could yield a meaningful result since costs of production is shared among other crops. The crops grown in tandem with sunflower are mainly maize and millet. Furthermore, the households growing sunflower were also engaged in other income generating activities such as animal keeping (62%) and trading activities (29%) to buffer the income from sunflower production. It can be concluded that sunflower production alone could not suffice the basic household needs; that necessitate farmers to adopt copying strategies like practicing the mixture cropping pattern with other crops and engaging in off-farm activities.

The generated income from sunflower production had multiple uses: purchase of food, clothes, shoes and kitchen utensils. Moreover, the money earned were also used to pay school fees, raising capital for trading activities, buying cattle for ploughing building materials, mostly bicycle and motorcycle (Table 6). In this case it can be concluded that sunflower production contribute to the improvement of the livelihood in the study area. However, the campaigns towards improvement of sunflower productivity and the income from the venture is necessary for up scaling the crop production and expand the income earned from the production at large.

Table 6 Spending of the income generated from sunflower production

Income expenditure	**Frequency**	**Percentage (%)**
Buying home needs	50	46.73
Paying school fees	15	14.02
Raising capital	8	7.48
Buying cattle, building materials, transport vessels (bicycle and motorcycle).	34	31.78
Total	**107**	**100.00**

IV. CONCLUSIONAND RECOMMENDATIONS

Sunflower production in Iramba district was found to be a profitable venture with a Gross profit of 41,540.78Tsh/acre and a Gross Margin of 13.8%. Meanwhile, its return on investment stood at 16% meaning that every shilling invested in sunflower production would yield a profit of an average of 16 cents that is just recovering the normal bank lending rates in the country. This income was able to contribute by 13% and 19% of basic needs and food needs per capita poverty line respectively. Moreover, this contribution is far below the international poverty line of $1.90 whereby it contributes to 4.18% of the international poverty line.

To reduce income poverty among sunflower smallholder farmers; pro-poor efforts geared towards lowering production costs and increasing the crop yield are pre-requisites. In this case, development and adoption of high yield and high oil content cultivars needs attention; since currently farmers mostly use open-pollinated cultivars that yields less that 3tones of sunflower seeds per hectare.

Firstly, the National Agricultural Research Institutes (NARI) and other developmental partners interested in seeds breeding can serve the purpose. The hybrid seeds must take into considerations different ecological zones in Tanzania. Secondly, increased irrigation facilities for commercial producers be established with out-grower schemes having contract farming to enhance not only increased production but also reliable market for the produce. This can be coupled with growing of sunflower in wetland during the dry seasons. Moreover, proper agronomic practices must be emphasized for the new cultivars developed. In fact development of improved seed varieties should be accompanied with development of irrigation schemes under monoculture cropping patterns to test the yields of the seeds during dry season. Production of sunflower during off-season would serve processors to make more raw material available hence increasing the utilization of processing plants installed capacity. High value varieties for sunflower seeds canbe produced on farm such that farmers learn the practices.

Investment in the irrigation facilities along the semi-arid regions (such as Singida) be subsidized. Investment of commercial sunflower farming that serves as market for the sunflower seeds grown by smallholder farmers but also spread modern agronomic practices among them.

Further research could compare the costs and benefits of the on-farm sunflower production practicing monoculture with improved seeds, and mixed farming to take on board the risk mitigating strategies for farmers practicing mixed cropping pattern in the study area. The study would reveal how costs are shared among various crops grown with sunflower in the study area.

REFERENCES

[1] Balchin, N., Kweka, J. and Mendez-Para, M. (2018). Tariff setting for the development of the edible oil sector in Tanzania. 73pp. http://dev.ansaf.or.tz/wp-content/uploads/2018/04/I4ID-Tariff-setting-in-Tanzanias-edible-oil-sector_FINAL-Report_26-Feb-2018.pdf accessed on 12.05.2019

[2] Benjamin, Y.L. and Lazaro, E. (2019). Design, Fabrication and Performance Evaluation of Centrifugal Sunflower Dehuller for Small-scale Enterprises in Developing Countries. African Journal of Science, Technology,

Innovation and Development. 1-8. Doi:10.1080/20421338.2019.1573956

[3] Factfish (2010). http://www.factfish.com/statistic-country/tanzania/sunflower+seed,+yield accessed on 15.05.2019

[4] FAO (2010). Agribusiness Handbook. 39pp. www.eastagri.org

[5] Gross, L., Nickerson, E. and Masbayi, M. (2016). Contract Farming in Tanzania's Central Corridor: Lessons from the Rural Development Program, Tanzania. 37pp. https://www.helvetas.org/news_blog/publication/value_chains.cfm

[6] Henningsen, A. Mpeta, D.F., Adem, A.S., Kuzilwa, J.A. and Czekaj, T.G. (2015). A Meta-Frontier Approach for Causal Inference in Productivity Analysis: The Effect of Contract Farming on Sunflower Productivity in Tanzania. 24pp. Selected Paper prepared for presentation at the 2015 Agricultural & Applied Economics Association and Western Agricultural Economics Association Annual Meeting, San Francisco, CA, July 26-28.

[7] IJSBAR, (2013). *Tanzania's Small-Scale Sunflower Farmers*: Upgrading the Value Chain.

[8] Iringo, R. (2013). *Collaboration between Sunflower Oil Processors and RLDC for Improved Sunflower Sector in Tanzania*.

[9] Kombe, C., Mpemba, Z., Yabu, N., Kazi, M., Kibessa, B., Mwita, D., Mgangaluma, E., Mashini, S., Chaula, A., Ndunguru, E., Lugobi, M. and Mziya M. (2017). Potential of Sunflower Subsector in Tanzania. The World Bank Working Paper number 10, 2017. 34pp

[10] Liberio, J. (2012). *Factors Contributing To Adoption of Sunflower Farming Innovations* in Mlali Ward, Mvomero District, Morogoro Region – Tanzania. Unpublished Master dissertation, Sokoine University of Agriculture, Morogoro, Tanzania.

[11] LRDC, (2012). *Brief On Project Implementation Sunflower Sector*, Dodoma.

[12] Mgeni, C. P., Müller, K. and Sieber, S. (2019). Sunflower Value Chain Enhancements for the Rural Economy in Tanzania: A Village Computable General Equilibrium-CGE Approach. Sustainability 2019, 11, 75; doi:10.3390/su11010075

[13] Mlay, F. (2017). Improving Sunflower Production and Reducing Post-Harvest Losses. "Capitalization of Experiences for Greater Impact in Rural Development" project, implemented by CTA, FAO and IICA and supported by IFAD. http://experience-capitalization.cta.int. 5pp

[14] MMA (2009). Subsector / Value Chain Analysis Report for Sunflower Edible Oil for Local Market Tanga Region – Tanzania.

[15] MMA (2010). Value Chain Analysis of Sunflower for the Regions of Morogoro, Iringa, Mbeya and Rukwa.

[16] Mpangalile, J. J., Ishengoma, R. and Gillah, P. (2008), *Agribusiness Innovation in six African Countries*, The Tanzania Experience, Morogoro.

[17] RLDC (2012). The Sunflower Sector in Tanzania – *A Great Potential for Industrial Competitiveness.*

[18] RLDC, (2008). Sunflower Sector; *Market Development Strategy, an intiative by Rural Livelihood Development Company*.

[19] RLDC, (2015). Sunflower Sector; *Market Development Strategy, an intiative by Rural Livelihood Development Company.*

[20] Tanzania Livelihood Baseline Profile (2015). Iramba Midland Maize, Sorghum and Sunflower Livelihood.

[21] Tanzania Livelihood Baseline Profile (2016). Iramba Midland Maize, Sorghum and Sunflower Livelihood. Retrieved July 3, 2016 from World Wide Web http://www.kilimo.go.tz/publications/english%20docs/fslbp/T17%20FINAL.pdf

[22] TEOSA (2012). *Assessment of the Potential of Edible Oilseeds Produced in Tanzania*: The Case of Sunflower and Sesame.

[23] Trans-SEC (2017). Institutional and policy analysis of Food Value Chains in Tanzania: The case of sunflower and edible-oils. (eds) [Herrmann, R.; Nkonya E., , Brüntrup, M.' Mutabazi, K., Claude Maeda, C., Mwinuka L. and Faße, A.] http://project2.zalf.de/trans-sec/public/media/upload/product/pdf/39009624577cb122976d0e3555026413.pdf accessed on 25.04.2014

[24] Tuntufye, G. (2013). *Study on Socio-Economic Factors Affecting Smallholder Sunflower* Production in Mvomero District, Morogoro Region. Unpublished Master dissertation, Mzumbe University, Morogoro, Tanzania.

[25] Ugulumu, E. S. (2008). *Sunflower Value Chain Analysis in Tanzania.* *http://www.tzdpg.or.tz/fileadmin/_migrated/content_uploads/Sunflower_value_chain_Tanzania_1_.pdf accessed on 25.05.2019*

[26] Ugulumu, E. S. and Inanga, E. L. (2013). Tanzania's Small-Scale Sunflower Farmers: Upgrading

[27] (2013) Volume 10, No 1, pp 126-145

[28] URT (2008) [United Republic of Tanzania]. Singida Region Agriculture Sample Census – 2007/08. 222pp. http://www.instepp.umn.edu/sites/default/files/product/downloadable/Tanzania_2007-8_Vol_5m.pdf

[29] URT (2013) [United Republic of Tanzania]. The National Agricultural Policy. 43pp.

[30] URT (2016) [United Republic of Tanzania]. Sunflower Sector Development Strategy. 81pp

[31] World Bank Group (2015). Tanzania Poverty Assessment. 150pp. retrieved from http://www.worldbank.org/content/dam/Worldbank/document/Africa/Tanzania/Report/tanzania-poverty-assessment-05.2015.pdf on 21.06.2019

[32] Zeng, D.Z. (2011). The Sunflower Sector in Tanzania: A Sectoral Policy Study. https://openknowledge.worldbank.org/bitstream/handle/10986/27747/115259-WP-TanzaniaSunflowerSector-PUBLIC.pdf?sequence=1&isAllowed=y accessed on 18th March 2019.

Investigating the Effectiveness of Manual Drum Pulper on Genotypes of Robusta Coffee (*Coffea canephora* L.) for Seed Production

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***Abstract*— *Mortar and pestle has long being in used to de-pulp coffee cherries for planting either in small or in large scale farm. This method is very laborious, time consuming and is not devoid of seed damage. Therefore it becomes imperative to use mechanical pulper for both small and large scale seed production. Ripe coffee cherries were harvested during 2019 season from three different genotypes of Robusta coffee planted on the field. Two genotypes were selected from coffee germplasm and the third genotype was from agronomy plot all from cocoa research institute of Nigeria. The cherries were processed using both traditional method of de-pulping and manual drum pulper. Three operators were used to create different speed rate of 40, 75 and 90 rev/min. Equal quantity of cherries were fed into the pulper. The de-pulped coffee seeds were air dried, undamaged seed were selected for planting at pre-nursery. Data on seed emergence were collected periodically, twenty five days after sawing, forty days after sawing and sixty days after sawing. Data were subjected to analysis of variance (ANOVA) using statistical analysis system (SAS) version 9.1. The result shows best percentage de-pulping efficiency (99.91), separation efficiency (98.87) and percentage beans damage (0.2) were achieved with the machine speed range between 40-75 rev/min. The percentage seed emergence of genotype T797 (66.67), FM (66.67) and T45 (64.44) were statistical similar irrespective of de-pulping method used. Better machine performance was observed on genotype T797. Genotype FM de-pulped with manual drum pulper gave least germination percentage; lowest machine performance was observed on genotype FM. Coffee seed production with minimal affection on seed emergence, could be achieved through use of manual drum pulper to reduce time and labour required using traditional de-pulping method.*

***Keywords*— Coffee seeds production, genotypes, manual drum pulper, effectiveness, seed emergence.**

I. INTRODUCTION

Coffee is one of the agricultural products that is of economic importance and serve as a means of income to farmers (Muleta, 2007). In Nigeria, many farmers established their coffee plantation using seedlings derived from seeds. Establishment of coffee farm through vegetative propagation (clone/grafting) is being practice only by research stations for improvement in productions (Wilson 1999). Cherries are harvest when most of them are fully ripped. It is appropriate for coffee cherries to be harvested once the pulp is soft and become easy to remove. Harvesting of coffee cherry is either done by strip picking method or selective picking method. Strip picking method which is the commonest among coffee farmers involving removal of all cherries from the branches. As it is being practice, both ripe and unripe cherries are harvested together and subject the harvested fruits to further post- harvest processing. The Selective picking method of harvest allowed picking of only ripe cherries, while unripe cherries remain on the tree branches until when fully ripe.

Knowledge about coffee fruit is very important, as it consist of exocarp (pulp), mesocarp (mucilage), endocarp (parchment or hull), spermoderm (silver coat), endosperm (albumen) and embryos with tow cotyledons. Coffee cherry is a drupe containing two flat seeds which is use for propagation. Germination of the coffee seeds under field condition is defined as emergence of seedling from soil, with radical extending down ward. Cotyledons are the first seed part to emerge from the soil, showing epigeal seedling germination characteristics (Huxley, 1964). Coffee seeds are ready to germinate as soon as possible since they have no dormancy, but also lose viability easily when stored for a longer time (Coste, 1992). According to Rena *et al.*, (1986), germination of Coffee seeds is very slow; this make establishment of coffee plantation through seeds not encouraging early enough to increasing production. However, some species that have smaller seeds such as C. *racemosa* germinate within 10 days or less after sawing. Sowing period also affect germination of coffee seeds, as *C. arabica* seeds sown during warm period, begin to germinate from 50 to 60 days of sowing (Maestri and Vieira, 1961). Also germination could be delayed up to 90 days if coffee seeds are sown under low temperature as reported by (Went, 1957).

Report of Rosa *et al*., (2005) revealed that endocarp prevent early emergence of coffee seeds its removal will improve emergence of coffee seed. Studies carried out by Da-Silva *et al*., (2004), on mechanism and regulations concerning coffee seed germination, show that parchment cause the cell wall not to expand on time and prevent surrounding endosperm from being active.

A coffee cherry is processed after harvest followed by the removal of both the pulp either by dry, wet or semi-dry method (Mussatto *et al*., 2011). Mucilage is removed by fermentation, followed by washing or machines processes (Brando and Brando, 2015).Viscous nature of mucilage is of greater advantage during processing of coffee as it easy de-pulp even with little apply of pressure, expel the beans from the fruit. Selmar *et al*., (2006), reported that germination of coffee seeds start during post-harvest processing which involved removal of fruit flesh (pulp) either manually or mechanically. He further corroborated is findings through the using of germination-specific isocitrate lyase (ICL) test with b-tubulin, a maker for cell division or elongation (Selmar *et al*., 2006).

Traditionally, the exocarp (pulp) is manually removed during seed processing by farmers as it is widely practice and believed to be devoid of low seed damage. Traditional coffee processing method of de-pulping requires soaking of cherries, use of mortar and pestle or even matching or tramping on cherries to remove pulp. This is followed by separation of pulps from the beans (scooping) and drying of beans. Unfortunately, the process is very tedious with attendant, drudgery and low work rate. Personal experience and that of other processors showed that separation of pulp form the beans are specifically difficult and discouraging.

Damage is a very vital index to be considered in seed production approach. De-pulping of coffee cherries using mechanical methods to reducing labour, time and cost should be paramount for the purpose of increasing coffee production **(**Weinberg *et al.*, 2001). According to Murthy and Naidu, (2011), pulp removal of fresh berries using machine is one of the process leading to increase in coffee seed production. The use of machine to increase efficiency and large scale production with little or no drudgery has been evolved since the time immemorial. Mechanical de-pulp result in higher quality beans (Wintgens, 2012). However, the use of machine with low levels of damages to seed will depend on its design should be regarded as a necessity for effective coffee seed production.

Proper understanding of coffee seed germination will help in improvement of agricultural activities and increase production of coffee needed for international market. Work done on coffee seed germination with regards to seed processing/ production using manual drum pulper is scarce considering the economic important of coffee crop in the world market. Hence effort should be intensified toward processing of coffee seeds (*C. canephora* L.) for planting using manual drum pulper. The purpose of this study was to determine the effectiveness and efficiency of coffee seed production using hand de-pulp (pestle and mortar) and manual drum pulper. It is therefore necessary to compare different methods to ascertain whether manual drum pulper can be injurious to seed production. Findings from this study will provide basic information needed for large scale coffee seed processing and production.

II. DESCRIPTION OF THE MACHINE

The drum pulper consists of 220 mm diameter and 280 mm long perforated cylinders with a hopper of 450 mm × 300 mm and 280mm × 150mm at the top and bottom respectively. The cylinder rotated through 300mm flywheels which operate two mashing spur gears of 210 mm and 100 mm diameter respectively. De-pulping occurs through abrasion force between the cylinder and adjustable plate. Separating of beans from the pulp is achieved through a fixed 290 mm × 70 mm× 5 mm plate. The drum roughened surface revolves over coffee cherry, force between it and fixed plates make the berries to be squashed and eventually separate the beans from the pulp. The viscous nature of the beans facilitates the slippery of the seeds out of the machine through the grooves.

III. MATERIALS AND METHODS

The trial was conducted at cocoa research institute of Nigeria, Ibadan, during peak harvest of 2019 season. Ripe matured Robusta coffee cherries were harvested using selective picking method. Three C. *canephora* genotypes were used for this trial from where coffee cherries were harvested from trees growing on the field. Two of the genotypes were selected from coffee germplasm, while the third genotype was selected from agronomy plot. Harvested cherries were immediately soaked in water for 24-48 hours as suggested by Palmino (2016), to allow separation of damage cherries, removal of lighter cherries and to easy de-pulp. Two de-pulped methods were applied, hand manual de-pulp and manual drum pulper. Hand manual de-pulp was done using pestle and mortar as a local method of de-pulping that has not been modified for many years, until beans and pulp is separated. Mechanical de-pulp was done with manual drum pulper, with the help of three operators to create different speed rate as suggested by (Ademosum *et al*., 1993). The same quantity of the soaked cherries were fed into the manual drum pulper. The adjustable plate was adjusted for optimal de-pulping. Operators were allowed to reach stable machine speeds of 40 rev/min, 75 rev/min and 90 rev/min respectively before feeding the cherries into the pulper. Each speed rate was replicated three times for each genotype making twenty seven observations. The de-pulp and unde-pulp beans were separated manually after which the de-pulped coffee seeds were washed in water, to remove mucilage and air dried. The good (undamaged) seeds from manual drum pulper and manual hand de-pulp after air dried were planted on pre-nursery until there emergence.

Ten seeds were selected from each genotype, planted per plot and replicated three times on pre-nursery bed prepared with sawdust, laid in complete randomized design (CRD). All pre-nursery practices were well carried out during the experiment period without bias. Data on seed emergence were taken periodically based on sowing days; twenty five days, forty days and sixty days after sowing respectively. Data on seed count emergence were subjected to analysis of variance (ANOVA) using statistical analysis system (SAS) software (version 9.1).

The means of three speed rates represent the replicates and were used to calculate the machine efficiency, separation efficiency and percentage bean damage following the procedures of (Adeleke *at al*., 2016, and Adekanye *et al*., 2016).

$$\text{De-pulping efficiency} = \frac{\text{Depulped beans}}{\text{Total beans inputs}} \times 100$$

$$\text{Separation efficiency} = \frac{\text{Chafts removedby machine}}{\text{Total chafts}} \times 100$$

$$\text{Beans damage} = \frac{\text{Damage beans}}{\text{Total beans inputs}} \times 100$$

IV. RESULT AND DISCUSSION

There are appreciable de-pulping efficiency separation efficiency and reasonable percentage beans damage (Table 1). These are comparable to what had been reported by some other scientists. De-pulping efficiency, separation efficiency and percentage beans damage ranges from (79.73 to 99.91 %), (73.57 to 98.87 %) and (0.2 to 7.26 %) respectively. Very high de-pulping and separation efficiencies and very low beans damage indicate that this kind of machine is suitable for seed production at a very low power input. The close range in the performance remarks is also an indication of its versatile at different speeds. The close values of these parameters considering the varieties are also an indication that the machine is suitable for reasonable types of genotypes. The little differences in the value may be due to genotype or edaphic factors (Adeigbe *et al*., 2016).

The result revealed operation of the machine for this particular activity should be at 40-75 rev/min for optimum performance as these two speed range gave relatively reasonable (%) de-pulping efficiency (99.91), separation efficiency (98.87) and relatively low damage to the beans (0.2), these are key factors needed to determine the use of the manual drum pulper for coffee seed production.

Table 1: Performance of manual drum pulper at different speed rate

Genotype	40 rev/min			75 rev/min			90 rev/min		
	DEF %	SEF %	BDE %	DEF %	SEF %	BDE %	DEF %	SEF %	BDE %
FM	95.55	94.99	0.74	81.96	79.88	0.2	79.73	73.57	0.67
T45	99.91	98.87	7.26	99.33	98.72	1.90	98.42	96.79	4.79
T797	98.11	96.03	2.60	98.05	95.06	1.30	94.55	89.54	1.36

Where DEF is De-pulping Efficiency, SEF is Separation Efficiency, BDE is Beans Damage.

Performance of manual drum pulper, on genotypes as presented on Table 2. Genotype T45 and T797 were statistically similar (99.22) and (96.90) with regards to de-pulp efficiency, they both differ significantly with genotype FM (85.75). Genotype T45 was observed to be differ significantly (98.13), with other genotypes in terms of separation efficiency of seeds. Damage done to the seeds of genotype T45 (4.65) was significantly higher than on other genotypes. T797 revealed better de-pulp efficiency, moderate separation efficiency and moderate damage to the seeds when compared to other genotypes. FM shows least significant different with regards to performance of manual drum pulper. This suggests that effective performance of manual drum pulper could be genotype based. Indicating that some genotype could be viscous than the others, which is the natural means that facilitates the slippery of the seeds out of the machine through the grooves.

Table 2: Performance of manual drum pulper on genotype (%)

genotype	DEF (%)	SEF (%)	BDE (%)
T45	99.22a	98.13a	4.65a
T797	96.90a	93.54ab	1.75ab
FM	85.75b	82.81b	0.54b

Means with the same letter are not significantly different

Where DEF is De-pulping Efficiency, SEF is Separation Efficiency, BDE is Beans Damage.

The result of analysis (Table 3) shows genotype FMH, T797 and T45 were statistically similar with regard to percentage emergence of coffee seeds sixty days after sowing, but they differ significantly with others. The percentage emergence with regards to twenty-five days and forty days after sowing irrespective of genotypes were similar. This could be due to few seeds emergence within this period occasion by slow rate of seed germination. Since most of the seeds during this period were still on their dormancy period. Implying that germination of coffee seeds does not start until many days after sowing, as reported by (Maestri and Vieira, 1961). This result show that irrespective of genotype, speed rates of manual drum pulper has no significant effect on seed emergence percentage as observed among the genotypes.

Table 3: Means of Percentage germination, Sixty days after sowing, Forty days after sowing and Twenty five days after sowing.

Genotype	Speed	Twent-five days after sowing	Forty days after sowing	Sixty days after sowing	Germination percentage (%)
FM H	0.00b	0.00a	3.00a	6.67a	66.67a
T797	68.33a	0.22a	3.67a	6.67a	66.67a
T45	68.33a	0.44a	3.33a	6.44a	64.44a
T45 H	0.00b	0.00a	3.00a	5.67b	56.67b

T797H	0.00b	0.33a	2.67a	5.67b	56.67b
FM	68.33a	0.11a	3.22a	6.00b	60.00ab

Means with the same letter are not significantly different

Pearson correlation (Table 4) revealed negative correlation between speed rates of manual drum pulper and twenty-five days after sowing. There was significant correlation between germination percentage and sixty days after sowing corroborating the initial result on Table 3 that higher percentage germination were observed at sixty days after sowing.

Table 4: Pearson Correlation: Speed, twenty five days, forty days, sixty days, and germination percentage

Speed	Speed	Twenty-five days after Sowing	Forty days after Sowing	Sixty days after Sowing
Speed				
Twenty-five days after sowing	-0.050			
Forty days after sowing	0.278	0.059		
Sixty days after sowing	0.254	0.075	0.073	
Germination Percentage (%)	0.254	0.075	0.073	1.000**

Significant ** (P≤ 0.01)

V. CONCLUSION

De-pulping of coffee cherries is among steps taken in processing of coffee seeds for planting. Efforts have been made to see how effective and efficient de-pulping of cherries can be done with less stress. Percentage emergence of seeds processed with manual drum pulper and traditional method of de-pulp (pestle and mortar) did not differ significantly. This implies the effectiveness of the machine. This finding had proving that using of manual drum pulper could be effective in reducing effort and money spends on processing of coffee seed for planting. Therefore mechanical manual pulper can be useful for coffee seed production which will assist local farmers and seed producers. In cognizant of this, efforts should be intensified toward incorporating of manual drum pulper for coffee seed production.

REFERENCES

[1] Adeleke, S. A. and Ogunjobi, M. A. K. (2016). Construction of dehulling machine for improved coffee processing J. Inno. Agric., 3 (4): 1-10.

[2] Adeigbe O.O., Adewale, B. D., Muyiwa, A.A., Olasupo, F. O., Olaniyi, O. O., Adenuga, O. O., Williams, O. A., and Aliyu, O. M. (2016). Quantitative Descriptors of cashew nut Categories of in Nigeria: providing indices for superior nut selection. *J. of Agriculture and Biological science*, 11(4): 142-148.

[3] Adekanye, T. A. Osakpanwan, A. B. and Osaiybia, I. E. (2016). Evaluation of a soya beans threshing machine for small scale farmers, Agricultural Engineering International: CIGR Journal 18(2): 426-434.

[4] Ademosun O. O. (1993). Development performance evaluation of a redal-operated multi-crop cleaner *J. Agric. Engrg and Tech* 1: 27-37.

[5] Brando, C. H. J. and Brando, M. F. (2015). Methods of coffee fermentation and drying. In: Schwan, R.; Fleet, G. (Ed.). Cocoa and coffee fermentations. Boca Raton: CRC,chap.10.

[6] Coste, R, (1992). Coffee: The plant and The product. Mcmillan Press Ltd; United Kingdom.

[7] Da- Silva, E. A. A., P. E. Tootop, A. C. Van-Aelst and H. W. M. Hilhorst, (2004). Abscisic acid control embryo grow potentials and endosperm cap weakening during coffee (Coffea Arabica cv Rubi) seed germination. *Planta*, 220: 251-261.

[8] Huxley, PA (1964) Some factors which can regulate germination and influence viability of coffee seeds. *Proceedings of the International Seed Testing Association 29,* 33-60.

[9] Maestri, M and Vieira, C (1961) Nota sobre a reducao da porcentagem de germinacao de sementes de cafe por efeito do acido giberelico. *Revista Ceres* **11,** 247-249.

[10] Muleta, D. (2007). Microbial Inputs in Coffee (Coffea Arabica) Production Systems, South Western Ethopia.

Implication for Promotion of Biofertilizers and Biocontrol Agents. Doctoral Thesis, Swedish University of Agricultural Sciences.

[11] Murthy P. S. and Naidu M. M. (2011). Improvement of robusta coffee fermentation with microbial enzymes EUR. J. of Appl Sci. 3(4):130-139.

[12] Mussatto, S. I. et al. (2011). Production, composition, and application of coffee and its industrial residues. **Food and Bioprocess Technology**, New York, v. 4, n. 5, p. 661-672, July.

[13] Rena, AB, Malavolta, E, Rocha, M and Yamada, T (1986) Cultura do cafeeiro-fatores que afetam a produtividade. 447. Piracicaba; Potafos.

[14] Rosa, S.D.V.F., Brandão Junior, D.S., Von Pinho, E.V.R., Veiga, A.D. and Silva, L.H.C. (2005). Effects of different drying rates on the physiological quality of *Coffea canephora* Pierre seeds. *Brazilian Journal Plant Physiology*, **17**, 199-205.

[15] Palmino poltrnieri and frances Rossi (2016). Challenges in specially coffee processing and quality assurance . Academic Editor lescopeland challenges p. 1-22.

[16] Selmar, D. Bytof, G., Knopp, S.-E. and Breitenstein, B. (2006). Germination of Coffee Seeds and its Significance for Coffee quality. *Plant Biology,* **8**, 260-264.

[17] Went, FW (1957). The experimental control of plant growth. Ronald Press, New York.

[18] Wilson, C. K. (1999). Coffee, Cocoa and Tea; crop production science Horticulture CABI Publishing Wallingford ISBN-10 0851989195; Pp 320.

[19] Weinberg, Bennett, Alan and Bealer, Bonnie, K. (2001): The world of caffeine: the science and culture of the world's most popular drug. New York: Routledge. ISBN 0-415-92722-6.

[20] Wintgens, J. N. (2012). Coffee : Growing, processing, sustainable production ; wilay: somerseti NJ, USA.

Biofortification of wheat: Genetic and agronomic approaches and strategies to combat Iron and Zinc deficiency

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Abstract— *This study delves into the comprehensive overview of different agronomic and genetic approaches of wheat biofortification to combat iron and zinc deficiency. Secondary source of data is used during the study of the subject. Micronutrient deficiencies, particularly those arising from zinc (Zn) and iron (Fe), pose serious human health problems for billions of people worldwide and millions of children, who predominantly depend upon cereals-based diet, suffer from malnutrition.Wheat, being a chief staple food crop for most of the under-developed countries, should be given emphasized to make it enriched with nutrients and minerals as in many cases, it constitutes a low level of nutritional elements. Most of the nutrients are lost during milling. Biofortification acts as the most promising and economic strategic option to effectively increase the micronutrients in the edible portion of the crop. Agronomic and Genetic biofortification are the two approaches; however, genetic engineering is getting more concern for researches. This uses the techniques to enhance the bioavailability of nutrients and reduce the anti-nutrient compounds. Although there are many technologies to increase nutrient contents, biofortification is assumed to be the most sustainable. Different strategies for wheat biofortification are assessed in this paper for overcoming challenges seen during the process. We discuss promising ways to enhance iron and zinc content in wheat, highlight global wheat production scenario and malnutrition status, and also key challenges are accentuated.*

Keywords— ***Agronomic and genetic approaches, Biofortification, Iron, Zinc, Wheat.***

I. INTRODUCTION

Wheat (*Triticum aestivum L.*), a major agronomic crop cultivated worldwide, is a self-pollinated long day plant belonging to family Poaceae that flourishes well under arid and semi-arid regions (Belderok, 2000). It has been a chief staple food, supplying approximately 35% of the total food as consumed by the global population (Mohammadi-joo et al., 2015). Its adaptive attributes to varied climatic conditions and environmental stresses make it a remarkable crop contributing to food security in the world (Muslim et al., 2015). Most (about 95%) of the globally cultivated wheat is hexaploid, which is extensively used for the preparation of varieties of baked products and bread (Debasis and Khurana, 2001). Therefore, the composition and nutritional concentrations of the wheat crop have a substantial impact on human health. Although, being potentially enriched with essential nutrients; especially in calories, most of the wheat varieties grown today are nutrients deficient– notably Iron (Fe) and Zinc (Zn) (Welch and Graham, 2004). The hefty quantity of these minerals is wasted during the milling process resulting in the paucity of these minerals in the human diet, leading to malnutrition. Due to the malnutrition caused by cereal-based diet, nearly about 2 billion population of the world, particularly in Asia and the African region, has suffered (Grew, 2018). As the global population is increasing alarmingly, the condition will be even more serious than expected in the near future if no urgent remedial strategies are implemented. Many scientists, researchers, and field-related experts have been working to find the way and techniques of improving nutrient contents in under-nutritive wheat varieties. Though a number of strategies have been made, they aren't cost-effective and sustainable for combating malnutrition (White and Broadely, 2009; Gomez-Galera et al, 2010; Hurrell et al, 2010). Effective approaches to solving the problem are supplementation, dietary diversification, fortification, agronomic biofortification.

Biofortification is the idea of breeding crops to enhance their nutritional value in an economic and sustainable manner (De Valenca et al, 2017). This can be carried out either through conventional selective breeding or through genetic engineering. Cereal crops like wheat, due to some barriers in potential uptake of soil nutrients, are usually mineral-deficient for which fortification is the must (Fageria and Baligar, 2008). Also, the continuous applications of weak fertilizers that are poor in mineral concentrations have negatively impacted the nutrient availability of wheat (Fageria et al, 2002). Considering this fact, biofortification acts as a feasible way of delivering micronutrients to populations who have inadequate access to diverse diets (Bouis and Saltzman, 2017; Garg et al, 2018). In wheat, it can be done through different approaches; Agronomic approach through direct foliar or soil application of fertilizers and Genomic approach which include genomic section, Marker Assisted Selection (MAS), and Quantitative Trait Loci (QTL) mapping. Owing to a large number of wild wheat relatives still unexploited, the genetic improvement of wheat can highly be achieved in the future focusing on breeding programs (Ahmadi et al, 2018; Dempewolf et al, 2017). Ordinarily, qualitative traits of wheat are governed by a single gene and quantitative by several genes (Breseghello and Coelho, 2013; Cui et al, 2015; Moose and Mumm, 2008). Through conventional breeding, breeding of qualitative trait is easier than that of quantitative trait. Advanced development in the realm of technology provides us new opportunities that can integrate natural variation, genomic achievements, and agronomic applications for improvement of Fe and Zn content in wheat grains.

1.1 Objectives of the study

The general aims of this study are to recommend promising approaches of biofortification to improve iron and zinc concentrations, including other minerals in wheat crops. Therefore, the review focus on how the increase in wheat nutrient concentrations, Fe and Zn, be achieved effectively and efficiently through an integrated agronomic and genomic approaches of biofortification. The specific objectives are to:

a. Describe the status of malnutrition and wheat production throughout the world.
b. Elaborate effective biofortification techniques to increase nutrients in a sustainable manner.
c. Figure out the future challenges in the biofortification of wheat.

II. RESEARCH METHODOLOGY

This review completely uses secondary sources of information. Pieces of Literature were collected from different Journal articles, Agricultural institutes, other sources like FAO, CIMMYT, and relevant reports were studied and the major findings were summarized. Also, suggestions from related professors and officers were considered in the paper.

III. DISCUSSION

1. Global status of wheat production and malnutrition

In 2020, global wheat production is estimated to reach approximately 765.41 million metric tons which will be the highest production to date. Since a few years, production has constantly increased. It might be due to improvement in the agronomical and genetic practices, development of stress-tolerant high yielding varieties, judicious use of bio-fertilizers, increased interests of researchers and scientists to new varietal development, and raise consumer's demand for wheat.

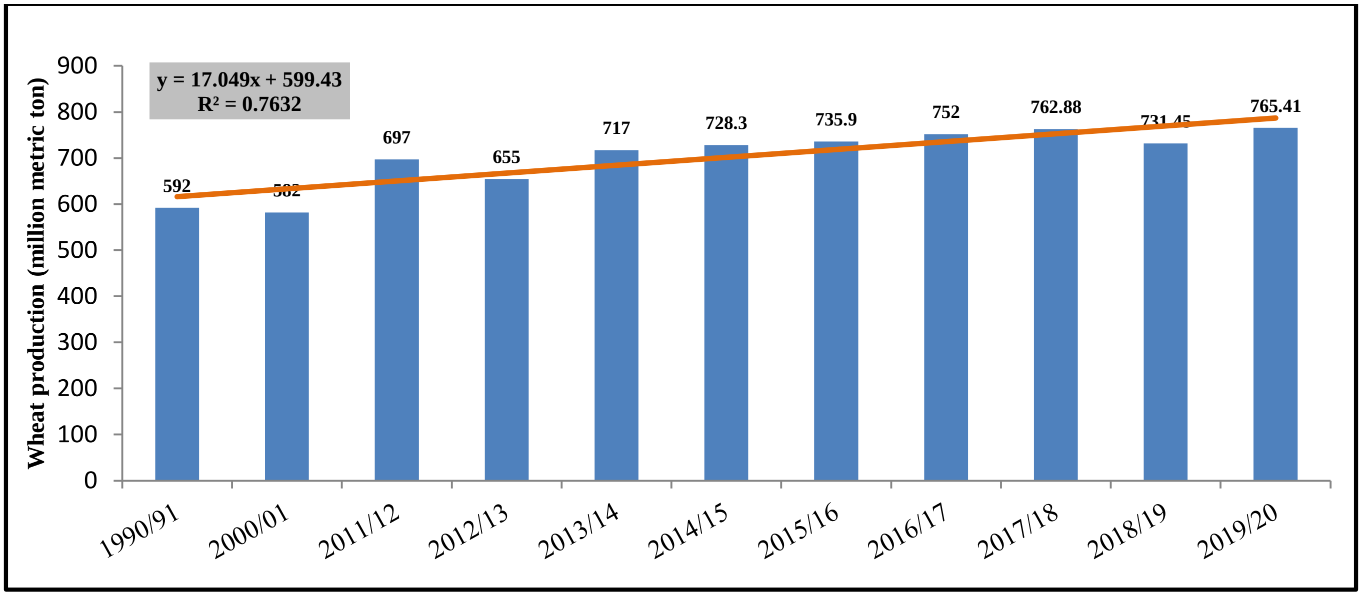

Fig.1: Global status of wheat production from 1900-2019 Source: Statista (2020)

Figure 1 shows that the global wheat production in 1990 was just almost 592 million metric tons which increased and reached 731.45 million metric tons in 2018. There was a decreased in production in 2000, 2012, and 2018 due to several factors such as natural calamities, disease outbreak, lack of interests of growers, and so on. Different organizations like FAO, CIMMYT, etc. have emphasized on quality yield with high production in different parts of the wheat-growing countries.

Within every county in the world, poor people are mostly suffered from malnutrition. Figure 2 shows the rate of malnutrition in different regions and sub-regions in 2018. Oceania is highly affected by very high malnutrition rate (38.1%). The only developed sub-region with overweight data is North America (2.3%). The average global malnutrition rate is found to be 22.2%. African and South Asian countries are still unsuccessful in providing sufficient diets to the suffered people due to a high level of poverty and unemployment. Most of the people have suffered from Fe and Zn deficiency in their diet, especially in cereal-based foods.

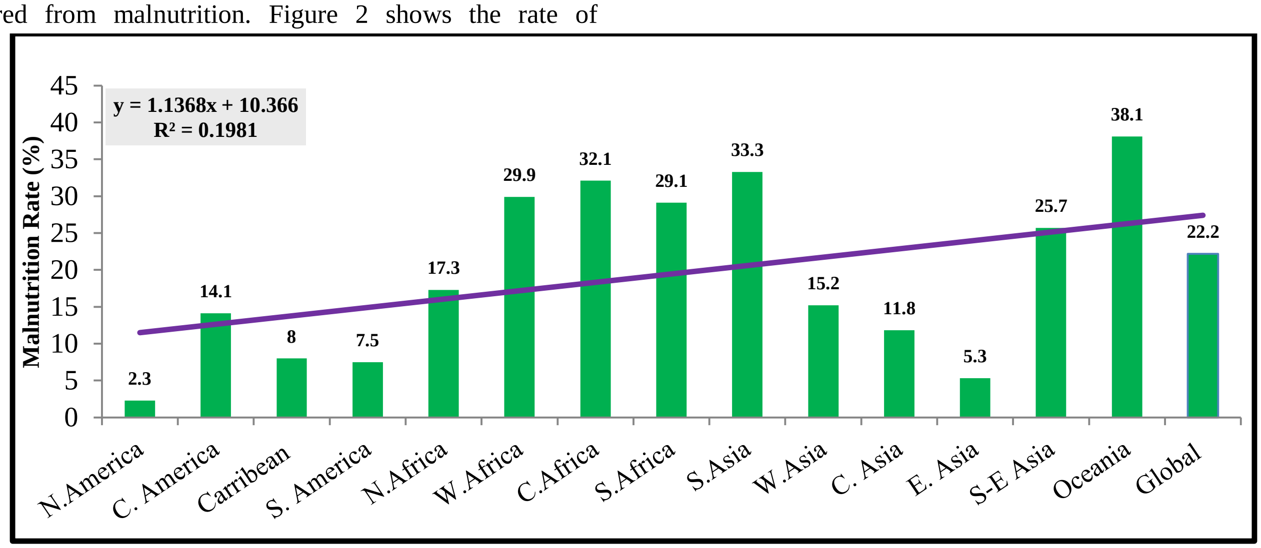

Fig.2: Global status of malnutrition in different regions Source: UNICEF (2018)

2. Biofortification for enhancing Fe & Zn content

Every human being requires essential minerals and micronutrients to enhance metabolism, which humans obtain from their diet. Wheat, like many other staple kinds of cereals, holds suboptimal levels of the essential micronutrients, particularly iron and zinc. Hidden hunger is emerging as a major challenge for the majority of the developing countries as it has become a common public health problem for poor people. Inadequacy of micronutrients results in stunted growth in children, decline in immunity, and work efficiency in adults, in particular women, and impairments in physical development. Iron and zinc have been considered as the most crucial among micronutrients. Its deficiencies causes serious human health hazards such as malnutrition, distorted growth, decreased immunity, increased susceptibility to infections and diseases, and many others (Tulchincky,2010).The potentiality of wheat in reducing micronutrient related malnutrition can be improved through direct (nutrition-specific) interventions, which include nutrient supplementation, dietary diversification, post-harvest food fortification, etc. and indirect (nutrition-sensitive) interventions, which includes biofortification (Ruel and Alderman,2013).Although the wheat crop is usually fortified during processing, an effective and more sustainable solution is biofortification, which needs developing new varieties of wheat with inherently higher iron and zinc concentrations in their grains (Bouis et al; 2011). Genetic biofortification (plant breeding) and agronomic biofortification (application of fertilizer) are two common means of biofortification which were supposed to be cost-effective to the dietary problems (Mara and Petra,2012;White and Broadely,2009;Cakmak,2008). Figure 3 displays a comprehensive overview of different sorts of biofortification for enhancing Fe and Zn content in wheat.

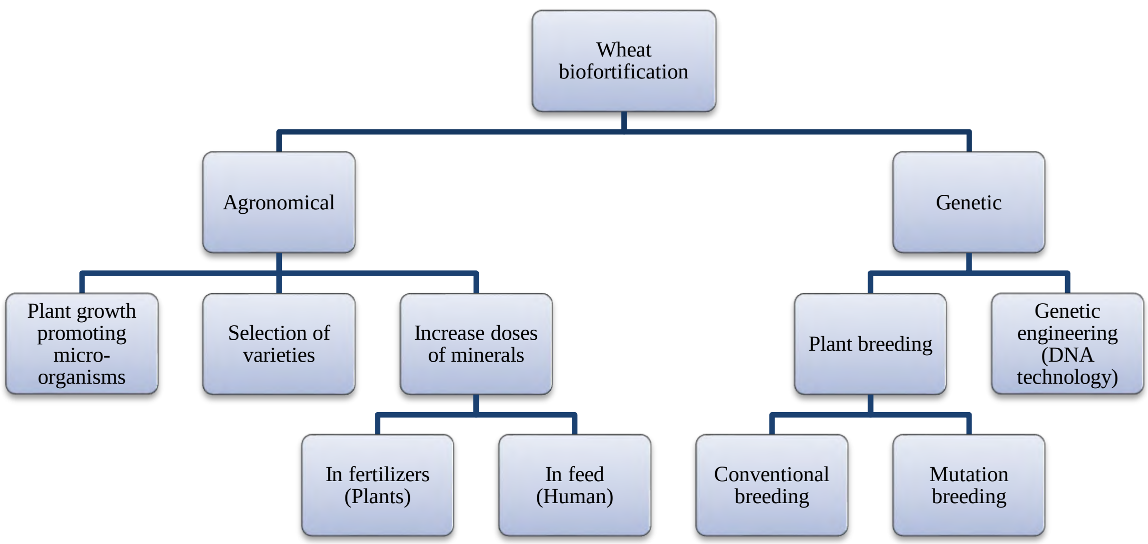

Fig.3: Classification of biofortification in wheat

2.1 Agronomic biofortification:

Most of the cultivated soil, notably which used for wheat and other cereals in the developing countries like Nepal, has a substantial number of chemical and physical constraints that lessen the plant-availability of Fe and Zn. Under such conditions, agronomic biofortification can be adopted either by applying micronutrient fertilizer to the soil or foliar application directly to the leaves of the crop(De Valenca et al,2017). Since antiquity, farmers have used mineral fertilizers to improve the health status of the food crops. A similar way of fertilizer application can also be applied upto a certain extent to enhance mineral accumulation by crop so as to increase nutrient contents (Rengel et al,1999).The various methods of fertilizer application may influence the grain yield and concentrations of Fe and Zn in the crop distinctly. Knowledge of different forms of fertilizers and the timing of soil and foliar application of these minerals is crucial for increasing nutrient content in grain (Velu et al,2013). Micronutrient follows a pathway from soil to crop and then food and finally into the human body. (De Valenca et al, 2017) stated several factors affecting the success of agronomic biofortification, which primarily depends upon;

bioavailability of micronutrients in the soil for plant uptake (soil to crop), translocation of mineral within plant and re-translocation to the harvested food (crop to food), bioavailability of nutrient to human in food and physiological state of human body to absorb and utilize the nutrient (food to human). Soil organic matter content, soil pH, and soil aeration, interaction with other elements, soil moisture content, and the variety of crops determine the extent of bioavailability of soil nutrient to crop (Alloway,2009). Although the application of nutrients through soil is common, the foliar application is considered to be more effective and economical (Cakmak et al, 2010; Zou et al,2010;Peleg et al,2007;Li et al,2016).Soil application of nutrient fertilizer is carried out on based on the soil test whereas; foliar application is done based on the plant tissue test or visual foliar symptom. Agronomic biofortification is popular for the solution to the short term problem as compared to the breeding approach (Cakmak,2008).However, it has several inconveniences in regards to efficiency, sustainability, and economic aspects. The nutrients may be stored in leaves but not in seeds and fruits. Most often, even if crops accumulate nutrients effectively through the soil, the nutrient may not be bioavailable to the crop due to some drawbacks (Frossad et al,2000).Fertilizers must be applied regularly so, this method is unsustainable. The expensive cost of fertilizers is another drawback. As compared to Genetic biofortification, agronomic biofortification is less acceptable on the basis of economics and environmental sustainability (Singh et al,2016). Therefore, agronomic biofortification with Fe and Zn fertilizers, particularly foliar applications, performs well for wheat and provide edible parts of crop plants with sufficient nutrients to combat the global Fe and Zn malnutrition problem. The foliar and soil application of fertilizers in wheat is shown in figure 4.

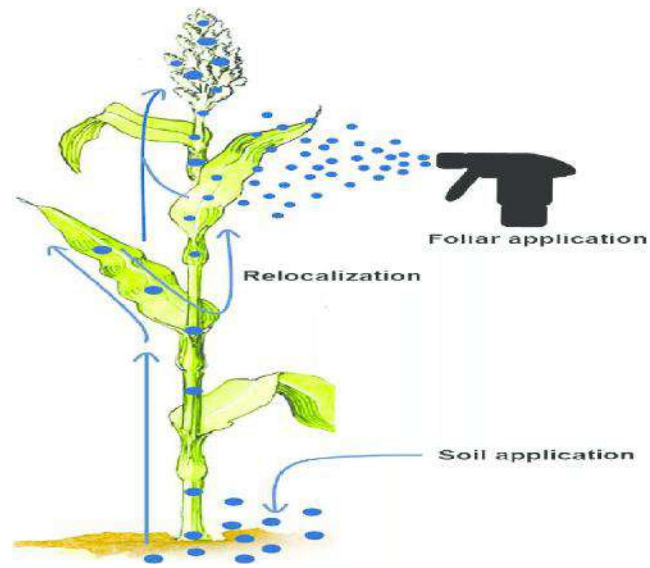

Fig.4: Agronomic biofortification (Foliar and soil application) in wheat Source: De valenca et al. (2017)

2.2 Genetic Biofortification:

Both conventional plant breeding and recombinant DNA technology (genetic engineering) are applied to increase the bioavailability and the concentration of nutrients in crops– known as Genetic biofortification. It can be achieved through marker-assisted breeding, gene discovery, or classical breeding strategy so as to exploit and characterize genetic variation for nutrient content in grain (Grusak,2002). Efficient genetic biofortification enhances the nutrient uptake by plants, increases nutrient translocation to grain, enhances sequestration of nutrients in the endosperm, decreases anti-nutrient compounds, and increases the bioavailability of nutrients(Mulualem,2015). Plant breeders or breeding institutions select and breed nutritious cultivar of wheat crop rich in Fe and Zn concentration and other substances that promote the bioavailability of Fe and Zn. (Velu et al,2013). Variability of the minerals is limited in modern-day cultivar of wheat. Nevertheless, adequate variability has been found to be harbor by the crop of wild relatives for nutrient content improvement (White and Broadely, 2009). To date, a number of researches have been performed in modern and old wheat cultivars, landraces, and wild germ plasma to investigate the variation in the grain zinc and iron amount. (Rawat et al, 2009) carried out similar research in wheat and he concluded that wild relatives were found to harbor 3-4 fold higher grain zinc and iron content than that of modern cultivars. Very close positive correlation was observed in various germplasms of wild, modern, and spelt wheat which indicates that physiological and genetic factors involved in Fe and Zn deposition in the seed are too similar (Cakmak et al,2004; Morgounov et al,2007;Gomez-Becerra et al,2010b). Most of the wheat-growers have been adopting wild relatives to transfer genes for yield and quality improvement as well as biotic and abiotic stress tolerance in cultivated varieties. Besides, conventional and modern breeding approaches can be adopted to transfer useful gene from wild relatives for grain zinc and iron content (Chhuenja et al,2008). Genetic engineering has been practiced to access genes from any desired source and introduces them to the crop directly. It has no taxonomical constraints and even artificially prepared gene can be used.But, limited gene is applicable for plant breeding that can be extracted from sexually compatible plant (Singh et al,2016). In recent years, a remarkable advancement in genetic biofortification has been made to reduce iron and zinc deficiencies that provide a sustainable diet-based solution to complement other interventions (Ludwig and Slamet-Wedin,2019).Investment for mineral improvement is necessary only at the research and development (R & D) stage which is the main advantage of genetic engineering and plant breeding (Singh et al,2016). The genetic biofortification approach is worthwhile and known to have practical values (Saini et al,2020).

2.2.1 Plant breeding approaches

Modern wheat breeding programs, in the last 50 years, have targeted to increase the total yield and productivity by selecting desirable plant height, plant resistance to diseases, and increased harvest index and biomass among all other traits (Ortiz et al,2007).Through plant breeding strategies, substantial genetic variations form the basis for crop improvement (Ortiz-Monasterio et al,2007). Along with focused on crop yield improvement, the nutritional composition in the grain is equally important to feed the world's growing population. Bioavailable Fe and Zn in the seed and grains of the staple food crop are as low as 5% and 25% respectively. Accordingly, micronutrient concentrations and their bioavailability in wheat should be emphasized in the breeding programs. The record of the past shreds of evidence showed that Fe and Zn contents in grain are quantitatively inherited traits (Trethowan et al,2005;Trethowan et al,2007). Quantitative traits aren't easier as qualitative to breed through conventional breeding approaches. An exception to some widely grown cultivars, some genotypes show significantly higher Fe and Zn concentration; e.g.: wild species, landraces, and lines from a pre-breeding program at CIMMYT (Monasterio and Gresham,2000).CIMMYT hasgiven priority on wheat breeding by transferring genes that govern increased nutrient from *Triticum aestivum spp spelta*and *Triticum turgidumspp dicoccon* based synthetics to high yielding wheat varieties(Velu et al,2013). Owing to the fact that colored wheat (black, purple, and blue) has a high concentration of phenolics, it is used in several breeding programs in different countries and many varieties of it have already been released (Garg et al,2018;Shao et al,2011;Sharma et al,2018). In this regard, the breeding programs can be considered as a product pathway driven by the potential effect of research and nutrition at the core of any biofortification (Pleiffer and MC Clafferty, 2007). The targeted regions and population are identified and steps for developing biofortified crops are proceeds which largely based on; bioavailability or bio conservation of ingested nutrient, micronutrient retention after storage, processing and cooking, and requirements of micronutrient in the population (Cakmak et al, 2010; Pleiffer and MC Clafferty, 2007). Identification of high mineral content varieties has been accelerated by breeders utilizing molecular biology techniques like Marker Assisted Selection(MAS) and Quantitative Trait Locus (QTL) maps by accounting differences in properties of soil such as pH, organic composition that may interfere with uptake and accumulation of minerals (Saini et al,2020).

2.2.2 Conventional plant breeding

Grain yields of wheat have increased continually for a few decades. Variety improvement has been responsible for more of the yield increase for a crop. Conventional plant breeding has been adapting on for hundreds of years and is still commonly used today by wheat growers in many parts of the world. It aims to develop plant having genes that help in uptake and accumulation of bioavailable nutrients by changing the genotype of the targeted crop. Once the initial research and development are completed, the benefits from these nutritionally enhanced crops will be sustainable with further little investment (Gomez-Galera et al, 2010), which can be considered as its advantage. It endorses scientists to make a significant improvements in the grain quality, nutritional content, and agronomic attributes of major subsistence wheat crops (Singh et al,2016). Several works have already been performed in conventional breeding to manipulate mineral concentration variability found in different germplasms (Qaim et al,2007).Different methods of selection are applied to develop improved genotypes (Gupta et al,2010). These methods vary not only forvegetative propagated crops but also for self-pollinated and cross-pollinated crops. Mass selection and pure line selection are used to select an improved line for self-pollinated crop from the variability existing in available germplasm. Contrarily, population improvement methods, including recurrent selection are important for cross-pollinated species (Gupta et al,2010).Genetic engineering could be applied to meet sufficient improvement, seeing that all crops don't have such genetic potential so as to meet the desired nutrient level.Conventional breeding can only use the genetic variability that has already observed and available in the improved crop, or occasionally in the wild varieties having the ability to cross with wild relatives. Mostly, by crossing to a distant relatives and thus transferring traits into commercial cultivars nutrient deficiency can be overcome. However, in some cases, breeding for specific traits would be inconvenient using conventional means and the efforts involve and time scale could be quiet unrealistic. Although being used in many cases and many parts, conventional breeding still has many withdraws. It is a slow process, labor-intensive, and often destructive and inaccurate with a high chance of committing errors as they are based either on traditional phenotyping methods or on visual assessment methods (Mwandaingoni et al,2017).

2.2.3 Mutation Breeding Approaches

Mutations have been used successfully in several crops, including wheat for breeding important agronomical traits. It has been used widely to produce grain varieties with improved quality of higher yield and other traits in developed and developing countries (Singh et al, 2016). The enhancement of grain yield and yield components of wheat through the application of mutagens drives towards the improvement of new cultivars with improved traits. The genetic bases of inherited traits can potentially broaden qualitatively and quantitatively by mutation induction through the heritable variation (Mwandaingoni et al, 2017). Mutation breeding, on elite cultivated germplasm of various crops, has previously been applied which creates superior and well-adapted variants (Shu et al, 2012). Mutation breeding develops directly or indirectly thousands of registered crop varieties enlisted in the United Nation's Food and Agriculture Organization (FAO)'s and Mutant Variety Database (MVD) (Mwandaingoni et al, 2017). It makes the perfect use of greater genetic variability by inducing mutation with irradiation or chemical treatments. Both physical and chemical mutagens can be used. Physical mutagens include UV rays, electromagnetic, and corpuscular radiation (Mba, 2013). To detect genomic mutation, such as the targeting induced local lesions in genomes (TILLING), these are coupled with efficient genomics tools (Chen et al, 2014). Thus, among mutant generations, breeder's tasks are to evaluate and screen for desirable phenotypes, and through this approach useful genetic variation is created and could be used for improving adaptability in existing germplasm (Mwandaingoni et al, 2017).

2.2.4 Genetic engineering

To fulfill the nutrient demand of an increasing population, wheat has been genetically engineered by the direct manipulation of its genome using biotechnology. Genetic modification is suggested to be an exquisite approach for obtaining high micronutrient concentrations. Also, a genetically modified organism (GMO) has a high potential for increasing agricultural productivity because of stable expression and fast development of GMO traits. Genetic engineering,compared to conventional plant breeding, needa fewer breeding generation to attain a new variety (Tewodros,2015). Since a single gene can be introduced in the target plant, genetic engineering is more precise. The genes could be extracted from any source, including microbes and animals. It works to improve the mineral mobilization efficiency in soil, reduce the level of anti-nutritional substances, and increases the level of nutrition enhancing useful compounds like inulin (Zhu et al, 2007). Genetic engineering provides a greater approach as it transfers a specific genes of desired traits from a source organism directly into the living DNA of target organisms. Once a useful gene has been identified from the source organism, it is attached to the prompter gene and marker and then inserted into the targeted organism using a carrier. Plants produced as a result of genetic engineering are transgenic orgenetically modified organisms. But GMO'S are unattainable for researchers and unaffordable for farmers as patentable inventions or patented are associated with them (Pardey et al,2000). This method facilitates control of various agronomic and quality traits by direct gene transfer into the targeted wheat crops.

3. Strategies for biofortification

Fe and Zn play a very significant role in plant growth, plant yield, nutrition, and soil fertility. Different institutions and organizations have been involved in increasing the nutrient contents of the produced wheat. Some of the strategies are briefly described below.

Application of fertilizers and foliar spray for raising Fe and Zn concentration:

Application of fertilizers like iron ferrous sulphate (FeSO4) and zinc sulphate (ZnSO4) to the wheat can raise the concentration of Fe and Zn in the growing grain. However, it is not a sustainable solution (Cakmak, 2008). Foliar application of these micronutrients is most reliable strategy (Cakmak et al., 2010a), which increase the concentration of starchy endosperm (Zhang et al., 2010). Use of seeds with high Zn contents, accompanied with foliar application of fertilizers is an effective strategy to enhance Fe and Zn concentration (Velu et al., 2013).

Germ plasm screening for increasing Fe and Zn concentration:

CIMMYT (International Maize and Wheat Improvement Centre) gene bank have been screened for Fe and Zn variation to more than 3000 germplasm accessions, including hexaploid, tetraploid and diploid sources (Monasterio and Graham 2000). Material with the highest Fe and Zn concentrations are progenitors of modern hexaploid wheat like einkorn wheat and wild emmer wheat and landraces (Cakmak et al., 2000; Ortiz-Monasterio et al., 2007).

Transgenic strategy:

Transgenic approach is most reliable and less cost program for increasing Fe and Zn nutritional status as compare to agronomic and breeding approaches (Malik et al., 2016). Researchers used different markers linked to loci to find the gene responsible to determine the variation of micronutrients. Different studies show that ZIP (Zinc transporter protein) family has a role in increasing Zn and Fe concentration (Schachtman and Barker 1999; Eide 2006).

Decreased Phosphorus and increased Nitrogen content in soil:

It is considered that there is a negative correlation between Phosphorus and both Fe and Zn uptake. In wheat grain approximately 75% of the total Phosphorus is stored as phytic acid, particularly in germ and aleurone layers (Lott and Spitzer, 1980). Recent studies show that Nitrogen nutrients status of plant also has positive effects on root uptake and shoot transport, retranslocation from vegetative tissues into seed and seed allocation of Fe and Zn (Aciksoz et al., 2011a; Kutman et al., 2010; Erenoglu et al., 2011). Increasing soil Nitrogen or foliar application was highly effective in improving root uptake and shoots and grain accumulation of Fe and Zn which was shown from wheat experiment (Aciksoz et al., 2011a, Kutman et al., 2011). When plant suffer from Fe and Zn deficiency, they release Zn-Fe mobilizing compounds from roots, which is called phytosiderophores which is promoted by improving N nutritional status (Aciksoz et al., 2011b).

Decreased glutenin content and plant height:

Significant negative correlations have been observed between glutenin content and Zn and Fe concentration (Gomez- Becerra et al., 2010a). Strong negative correlation occurred between Fe and plant height and glutenin content indicating that plant with lower glutenin content and shorter height favor higher grain Fe concentration.

Inoculation of plant growth promoting Rhizobacteria and Cyanobacteria:

Plant Growth Promoting Rhizobacteria (PGPR) comprises useful bacteria that colonizes plant roots and induce plant growth via various mechanisms. Application of PGPRs for Biofortification can be thus, considered as a possible option which along with breeding varieties, can induce increased micronutrient concentration in wheat (White P.J and Broadley M.R.,2009).

Increased in nicotianamine content:

Nicotianamine is an essential to maintainmetals homeostasis in plant. It can bind various metals including ferric and ferrous depending upon pH of soil. Regarding Fe, nicotianamine function is to confirm solubility of Fe in the cell so that, it can be utilize by different part of cell (Riaz et al., 2017). Various studies suggested that positive effect of nicotianamine on Fe uptake and its accumulation in seed (Douchkov et al., 2001; Douchkov et al., 2005).

4. Challenges ahead in biofortification

Up until now, the genotype and environmental interaction with respect to the yield of grain and nutrient concentrations have not been precisely understood. Many research programs for the enhancement of nutrient use efficiency have been restrained by expensive and laborious phenotyping. Moreover, the bioavailability of nutrients is another important factor in determining the grain quality. Changing climate situations may further amplify the problem.

- Biofortication faces challenges with high a cost of development (Bouis et al., 2011 and Nestel et al., 2006). In advance, the achievable breeding level of different nutrients is essential to be determined, which is a complex process and involves the determination of the adoption level by farmers, quantity of food products made from the crop consumed, post-harvest and preparation and cooking losses, the bioavailability of the nutrients and nutrients requirements. Thus, the target breeding level should be sure that there is a useful impact on the nutritional status of the recipient (Taylor and Taylor, 2012).

- For widely available of the released biofortified crop, it would take about a decade (Bouis et al., 2011). When the crops are biofortified through the genetic transformation process, there occur additional political and regulatory issues that have to be addressed (Birner et al., 2007).
- There is a lack of incentives and motivations to the farmers for growing improved crops,and consumers, themselves, are unaware to find quality food products from biofortified crops.
- During the manufacture of the biofortified crop, all the research teams should work together to produce an effective end product with the desired nutritional property. Sometimes, high micronutrient and vitamin has negative impact on color and flavor of end product due to which consumer rejects the product. Thus, there should be a better acceptable and good cooking quality for good adaption of biofortified crops. Also, the more acceptable yield level and persistence to biotic and abiotic stress of these biofortified crop variety. There is no better strategy supporting large-scale prospective studies on the effect of iron biofortified crop and their effective role adopted on decreasing out Anaemia (Iron deficiency diseases) and also improving better health (Hussain et al., 2010).

IV. CONCLUSION

Biofortification is a reliable, most economic, and feasible approach of delivering micronutrient to theunder-nutrient population of crops. Biofortified crop exhibits increased mineral concentration in their edible portion with better uptake of mineral from the soil, improved translocation of minerals to grain from leaves, and enhanced mineral sequestration to endosperm. There is promising and substantial genetic diversity in wild relatives of wheat, having useful and wide genetic variation in grain Fe and Zn content. This genetic variability can be utilized to increase both the concentration and bioavailability of Fe and Zn in modern wheat cultivars through conventional and modern breeding approaches. Compared to genetic approaches, agronomic Biofortification represents a short term solution to the problems, meanwhile, Genetic biofortification of staple crop, like wheat, is potentially sustainable and cost-effective. The agricultural science has been extremely developed by recent advancements in the mutant catalog, genomic resources, and transgenic strategies, and many breeding progresses. However, there are many challenges to carry out Biofortification approaches successfully. Even after the development of Biofortified crop varieties, various socio-economic and socio-political challenges are to be addressed to popularize their cultivation by farmers and their consumption by the end-user. Despite these challenges, scientists and researchers have been working now to make remarkable improvements of nutrient concentration in wheat and produce new wheat varieties. Thus, multi-tire coordination between researchers, farmers, and consumers (end-user) will play a key role in overcoming hidden hunger. The study concludes that biofortification of wheat can predominantly help in reducing malnutrition problems of the world and help in grain yield of higher quality.

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REFERENCES

[1] Aciksoz, S.B., Yazici, A., Ozturk, L., Cakmak, I. (2011a). Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizer. Plant soil, 349, 215-225. DOI: 10.1007/s11104-011-0863-2

[2] Aciksoz, S.B., Ozturk, I., Gokmen, O.O., Roemheld, V., Cakmak, I. (2011b). Effect of nitrogen on root release of phytosiderophores and root uptake of Fe (III) - Phytosiderophore in Fe-deficient wheat plants. Physiol. plant, 142, 287-296.

[3] Ahmadi J., Pour- Aboughadareh A., Ourang S.F., Mehrabi A., Sidduqe K H M. (2018) Wild Relatives of Wheat: Ageliops Triticum accessions disclose differential anti-oxidative and physiological responses toward water stresses. Asta Physiological Plant, 40, 1-14

[4] B.J Alloway (2009). Soil Factors associated with zinc deficiency in crops and humans. Environment Geo Chemistry Health, 31, 537-548

[5] Belderok, B. (2000). Bread making quality of wheat: A century of Breeding in Europe. Kulwer Academic Publisher Belderok, Netherlands, Pp;34

[6] Birner, R., Kone, S.A, Linarce,N., and Resnick,D (2007). Biofortified food and crops in west Africa; Mali and Burkina Faso AgBioForum 10(3) : 192
[7] Bouis H.E., Saltzman, A (2017). Improving nutrition through Biofortication: A Review of evidences from Harvest Plus., 2003 through 2016. Global Food Security, 12, 49-58. DOI: https://doi.org/10.1016/j.gfs.2017.01.009
[8] Bouis.H.E., Hotz.C., MC. Clafferty B., Meenakshi, J.V & Pfeiffer., W.H (2011). Biofortication: A new tool to reduce micronutrient malnutrition. Food Nutrition Bull; 32: 31-40. DOI: 10.1177/15648265110321S105
[9] Breseghello F., Coelho A.S.G (2013). Traditional and Modern Plant Breeding Methods with Examples in Rice (Oryza sativa L)., J Agric Food Chemistry., 61,8277-8286
[10] Cakmak, Ismail. (2008) Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil, 302, 1–17. DOI: https://doi.org/10.1007/s11104-007-9466-3
[11] Cakmak I., Pleiffer W.H & Mc Clafferty B (2010a) Review; Biofortication of durum wheat with zinc and iron. AACC International Inc., Cereal Chemistry 87(1), 10-20. DOI:10.1094 / CCHEM-87-1-0010
[12] Cakmak I., Torum A., Millet E., Feldman M., Fahima T., Korol A., Nevo E., Braun H.J., Ozkan H., (2004). Triticum Dicoccoides: An important genetic resource for increasing zinc and iron concentration in modern wheat. Soil Science, Plant Nutrition. 50, 1047-1054
[13] Cakmak L., Kalaya M., Kaya Y., Torun AA., Aydin N., Wang Y., Arisoy Z., Erdem H., Yazici A., & Gokmen O. (2010). Biofortication & Localization of Zinc in Wheat Grain. Jagric Food Chain. 58, 9092-9102. DOI: 10.1021/jf101197h
[14] Cakmak, I., Ozkan, H., Braun, H.J., Welch, R.M., Romheleld, V. (2000). Zinc and iron concentrations in seeds of wild, primitive and modern wheat. Food Nutr. Bull, 21, 401-403.
[15] Chen L., L Hao., M.A.Parry., A.L.Phillips & Y.G.Hu (2014) Progress in tilling as a tool for functional genomics and improvement of crops., Journal of integrative plant Biology 56: 425-443
[16] Chhuneja P., Kaur S., Goeal R.K., Aghaee M., Sarbarreb M., Prasbar & H S Dhaliwal (2008). Transfer of leaf rust and stripe rust resistance from Aegliops Umbelluta Zhuk to Bread Wheat (Triticum aestivum). Genetic Resources Crop Evolution, 55, 849
[17] Cui Y., Zhong F., Xu J., Li Z., Xu S., (2015) Mapping quantitative trait loci in selected breeding populations: A Segregation distortion approach., 115, 538-546
[18] De Valenca A W., Bake A., Brouwe l.D., Giller K.E. (2017) Agronomic Biofortication of crops to fight hidden hunger in sub-saharan Africa. Global Food Security,12, 8-14. DOI: https://doi.org/10.1016/j.gfs.2016.12.001
[19] Debasis,P., and Khrrana, P. (2001). Wheat Biotechnology: A mini-review. Electronic J, Biotech., 4(2), 74-102, DOI: 10.4067/S0717-34582001000200007
[20] Dempewolf H., Baute G., Anderson J., Killian B., Smith C., Guarion L., (2017). Past and future uses of wild Relative in Crop Breeding. Crop science, 57, 1070-1082. DOI: https://doi.org/10.2135/cropsci2016.10.0885
[21] Douchkov D., Hell R., Stephan UW, and Baumle in H.(2001). Increased iron efficiency in transgenic plants due to ectopic expression of nicotianamine synthase. Plant Nutr. 92:54-55.
[22] Douchkov D., Gryczka C., Stephan U.W., Hell R., and Baumlein H.(2005). Ectopic expression of nicotianamine synthetase genes results in improved iron accumulation and increased nickel tolerance in transgenic tobacco plant cell. Envir.28: 365-374.
[23] Eide DJ (2006). Zinc transporters and the cellular trafficking of zinc. Biochim Biophys Acta, 1763, 711-722. DOI: https://doi.org/10.1016/j.bbamcr.2006.03.005
[24] Erenoglu, E.B., Kutman, U.B., Ceylan, Y., Yildiz, B., Cakmak, I. (2011). Improved nitrogen nutrition enhances root uptake, root to shoot translocation and remobilisation of zinc (65Zn) in wheat. New phytol. 189(2), 438-48. DOI: 10.1111/j.1469-8137.2010.03488.x
[25] Fageria Nk. & Baligar VC. (2008) Ameliorating soil acidity of tropical oxisols by liming for crop production. Adv Agron, 99: 345-399. DOI: 0.1016/S0065-2113(08)00407-0
[26] Fageria Nk, Baligar VC, Clark R B (2002). Micronutrients in crop production. Adv Agron 77, 85-268. DOI: https://doi.org/10.1016/S0065-2113(02)77015-6
[27] Frossad E., Bucher M., Machler F., Mozafar A., Hurrell R., (2000). Potential for increasing the content and bioavailability of Fe, Zn & Ca in plants for human nutrition. J.Sci Food Agric, 80, 861-879. DOI: https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<861::AID-JSFA601>3.0.CO;2-P
[28] G. Velu, I.Ortiz-Monasterio, I.cakmak, Y.Hao, R.P Singh (2013). Biofortication strategies to increase grain Zinc& Iron concentration in Wheat. Journal of cereal science, 59(3), 365-372. DOI: https://doi.org/10.1016/j.jcs.2013.09.001
[29] Garg M., Sharma N., Sharma S., Kapoor P., Kumar A., Chandari V., Arora P. (2018). Biofortified Crops generated by Breeding agronomy & Transgenic Approaches are improving lives of millions of people around the world. Front Nutr, 5, 12. DOI: 10.3389/fnut.2018.00012
[30] Gomez- Becerra, H.F. Erdem., H. Yazici., A.Tutus, Y., Torun B., Ozturk L., Cakmak I.(2010 b). Grain concentration of protein and mineral nutrients in a large collection of spelt wheat grown under different environments. Journal Cereal Science, 52(3), 342-349. DOI:https://doi.org/10.1016/j.jcs.2010.05.003
[31] Gomez-Becerra, H.F. Yazici, A., Ozturk, L., Budak, H., Peleg, Z., Morgounov, A., Fahima, T., Saranga, Y., Cakmak, I. (2010a). Genetic variation and environmental stability of grain mineral nutrient concentrations in Triticum dicoccoides under five environments. Euphytica, 171, 39-52.
[32] Gomez-Galera S., Rojas E., Sudhakar D., Zhu C.F., Pelacho A.M., Capell T., etal (2010) Critical evaluation of strategies

for mineral fortification of staple food crops., Transgenic Resources 19 165-180 10.1007/s, 11248-009-9311-y
[33] Grew,R (2018). Food in global History. Routledge, NewYork, NY USA. DOI: https://doi.org/10.4324/9780429500411
[34] Grusak, M. (2002). Enhancing mineral content in plant food products. J.Am Cell Nutrition, 21(3), 1785-1835. DOI: 10.1080/07315724.2002.10719263
[35] Gupta P.K., Kumar J., Mir RR& Kumar A. (2010). Marker-Assisted selection as a component of Conventional plant breeding in wheat, 33(145). DOI: https://doi.org/10.1111/j.1439-0523.2009.01758.x
[36] Hurrell R., Ranum P., de Pee S., Biehinger R., Holthen L., Johnson Q., etal (2010) Revised recommendations for iron fortification of wheat flour and an evaluation of the expected impact of current national wheat flour fortification. Programs Food Nutrition Bull, 31, S7-S21. DOI: 10.1177/15648265100311S102
[37] Hussain S., Maqsood A M & Rahmatullah (2010). Increasing grain zinc and yield of wheat for the developing world: A Review. Emir. J. Food Agric, 22 (5): 326-339. http://ffa.uaeu.ac.ae/ejfa.shtml
[38] Kutman, U.B., Yildiz, B., Ozturk, L., Cakrnak, I. (2010). Biofortification of durum wheat with zinc through soil and foliar application of nitrogen. Cereal chem, 87, 1-9
[39] Kutman, U.B., Yildiz, B., Cakmak, I. (2011). Effect of nitrogen on uptake, remobilisation and partitioning of zinc and iron throughout the development of durum wheat. Plant soil, 342, 149-164.
[40] Li M., Wang S., Tian X., Li S., Chen Y., Jia Z., Liu K., Zhao A. (2016)., Zinc and iron concentration in grain milling fractions through combined foliar application of Zn and macronutrients. Field Crops Res, 187, 135-141
[41] Lott, J.N.A., Spitzer, E. (1980). X-ray analysis studies of elements stored in protein body globid crystals of triticum grains. Plant physiology, 66(3), 494-499. DOI: 10.1104/pp.66.3.494
[42] Ludwig Y. and Slamet-Wedin I.H., (2019). Genetic Biofortication to enrich rice and wheat grain Iron: From Genes to Product.Frontiersin Plant Science. 10, 833 DOI: 10.3389/ fplas. 2019.00833
[43] Malik, M., Pandey, S., Tripati, K., Yagoob, U., Kaul T. (2016). Biofortification strategies for enhancing grain zinc and iron levels in wheat. Microbiology, Biochemistry and Molecular Biology, 2, 7-10.
[44] Mara, C & Petra. B. (2012). Strategies for Iron Biofortication of Crop Plants. Food quality source in Technology, 2, 953-978. DOI: 10.5772/34583
[45] Mba C, (2013). Induced mutations unleash the potential of plant gentic resources for food and agriculture. Agronomy Journal, 3(1), 200-231. DOI: https://doi.org/10.3390/agronomy3010200
[46] Mohammadi-joo S., Mirasi A., Saediaboeshaghi R., and Amiri M., (2015) Evaluation of bread wheat (Triticum aestivum L.) genotypes based on resistance indices under field conditions. Intl I Biosci 6 (2): 331-337. DOI: 10.12692/ijb/6.2.331-337
[47] Monasterio I. and Gresham R.D.(2000)Breeding for trace minerals in wheat, Food nutrition Bull, 21(4), 393-396. DOI: https://doi.org/10.1177/156482650002100409
[48] Moose S.P., Mumm R.H (2008) Molecular plant breeding as the foundation for 21st century crop improvement. 147, 969-977. DOI: https://doi.org/10.1104/pp.108.118232
[49] Morgounov, A., Gomez-Becerra, H.F., Abugalieva. A., Dzhunusova M., Yessimbekova M., Muminjanous H., Zelenskiy Y., Ozturk I., Cakamak I., (2007) Iron and zinc grain density in common wheat grown in central Asia. Europhytica 155, 193-103. DOI: 10.1007/s10681-006-9321-2
[50] Mulualem, T (2015) Application of biofortication through Plant breeding to improve the value of staple crops. Blomed Biotechnol, 3,11-19
[51] Muslim Q., Xuechun W., Abdul Hamed B., Iftekhar Ahmed B., Muhammad A., Muhammad I and Muhammad S. (2015) The impact of drought on phenotypic characters of 5 advance wheat genotypes., Pure and Applied Biology, 7(2), 635-642
[52] Mwandaingoni L., Figlan S., Shimelis H., Mondal S., & Tsilo T.J (2017). Genetic resources and breeding methodologies for improving drought tolerance in wheat. Journal of crop improvement, 31(5), 648-672. DOI: https://doi.org/10.1080/15427528.2017.1345816
[53] Nestel,P., Boius, J.V., Meenakshi, J.V, and Pfeiffer,W. (2006) Biofortication of stable food crops. J.Nutr., 136(4): 1064-1067. DOI: DOI: 10.1093/jn/136.4.1064
[54] Ortiz R., Trethowan R.M., Orite ferrara G., Iwanaga M., Doods J.H., Crouch J.H., Crossa J., Braun H.J., (2007) High Yield potential, Shuttle breeding and anew international Wheat Improvement Strategy. Euphytica, 157, 365-384. DOI: https://doi.org/10.1007/s10681-007-9375-9
[55] Ortiz-Monasterio I., Palacios-Rojas N., Meng E., Pixley K., Trethowan R., Pena R.J., (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. Journal of Cereal Science, 46(3), 293-307. DOI: https://doi.org/10.1016/j.jcs.2007.06.005
[56] Pardey P G., Wright B D., & Nottenburg C., (2000). Are intellectual property rights shifting agricultural bio-technology in developing countries? International Food Policy Research Institute (IFPRI), Washington DC, https://www.ifpri.org/publication/are-intellectual-property-rights-stifling-agricultural-biotechnology-developing, 13-19
[57] Peleg Z., Aranga Y., Yazici A., Fahima T., Oztruk L., Cakmak L., (2007). Grain zinc iron & protein concentration & Zinc efficiency in wild emmer wheat under contrasting irrigation regimes. Plant soil. 306(1-2), 57-67. DOI: 10.1007/s11104-007-9417-z
[58] Pleiffer, W.H., & M C Claffererty, B (2007) Biofortification: Breeding micronutrient dense crops,Breeding major food staples M.S.kang & P.M Priyadarshan. Eds Blackwell publishing Oxford, 61-91

[59] Qaim M., Stein AJ.& Meenakshi J V. (2007)Economics of Biofortication. Agr Economics-Blackwell B7 (s1), 119-133. DOI: 10.1111/j.1574-0862.2007.00239.x
[60] Rawat N., V.K. Tiwari, P. Chhuneja and H.S Phaliwal, (2009). Evolution and utilization of Aegilops & Wild Triticum species for enhancing iron and zinc content in wheat. Genetic Resources Crop Evolution. 56(1), 53-64. DOI: DOI: 10.1007/s10722-008-9344-8
[61] Rengel Z, Batten GD, Crowley DE (1999). Agronomic approaches for improving the micronutrient density in edible portion of field crop. Felid crop Res 60, 27-40. DOI: https://doi.org/10.1016/S0378-4290(98)00131-2
[62] Riaz A., Huda NL. Abbas A., Raza S (2017). Biofortification of wheat with iron. International journal of advances in Scientific Research; 3(07): 69-76.
[63] Ruel M.T., Alderman.H. (2013). Nutrition-sensitive interventions and Programmes: How can they help to accelerate progress in improving material and child nutrition? Maternal and child nutrition study group., Lancet., 382(2013), 536-551
[64] Saini D.K., Devi.P., and Kaushik.P. Advances in genomic interventions for wheat biofortification. A review (2020); Agronomy 2020.10,62.
[65] Schachtman, D.P., Barker, S.J. (1999). Molecular approaches for increasing the micronutrient density in edible portions of food crops. Field crop Res., 60, 81-92. file:///C:/Users/Dell/Downloads/Molecular_approaches_for_increasing_the.pdf
[66] Shao Y., Jin L., Zhang G., Lu,Y; Shen,Y, Bao J. (2011) Association mapping of grain colour, phenolic content flavonoid content and antioxidant capacity in deshelled rice.Theoryand Applied Genetics, 122(5), 1005-1016. DOI: 10.1007/s00122-010-1505-4
[67] Sharma S., Chunduri V., Kumar A., Kumar R., Khare P., Kondepudi K.K., Bishnoi M., Garg M., (2018) Anthocyanin Bio-fortified coloured wheat : nutritional and functional characterization, Plos One, 13, e0194367. DOI: https://doi.org/10.1371/journal.pone.0194367
[68] Shu Q., B.P.Forster., H.Nakagawa, & H.Nakagawa (2012). Plant mutation breeding and biotechnology,FAO, CABI. http://www.fao.org/3/a-i2388e.pdf
[69] Singh.U., Prahara, C.S.,Chaturvedi, S.K., and Bohra, A. (2016) Biofortication: Introduction, Approaches, limitation and challenges. Springer India; 34. DOI: https://doi.org/10.1007/978-81-322-2716-8_1
[70] Statistica (2020). Global wheat production from 2011/2012 to 2019/2020 (in million metric tons). https://www.statista.com/statistics/267268/production-of-wheat-worldwide-since-1990/
[71] Taylor, J, and Taylor, J.R.N, (2012) Cereal Biofortication: Strategies, Challenges, and Benefit, Cereal Food World, 57, 165-169. https://www.cerealsgrains.org/publications/plexus/cfw/pastissues/2012/Documents/CFW-57-4-0165.pdf
[72] Tewodros Mululemi (2015). Application of Bio-fortification through plant breeding to improve the value of staple crops. Biomedical and Biotechnology, 3(1), 11-19. DOI: 10.12691/bb-3-1-3
[73] Trethowan R.M., Reynolds M.P., Ortiz-Monasterior., (2005). Adapting wheat cultivars to resource conserving farming practices and human nutritional needs. An Applied Biology, 146,404-419. DOI: https://doi.org/10.1111/j.1744-7348.2005.040137.x
[74] Trethowan R.M., Reynolds M.P., Ortiz-Monasterior., Ortiz R. (2007). The genetic basis of green revolution in wheat production. Plant Breed REV-28, 39-58. DOI: https://doi.org/10.1002/9780470168028.ch2
[75] Tulchincky, T.H. (2010) Micronutrient Deficiency Conditions: Global Health Issues. Public Health Rev 32, 243–255. DOI: https://doi.org/10.1007/BF03391600
[76] UNICEF (2018). Current Trends in Malnutrition in Children Worldwide. Report from UNICEF, WHO and World Bank Group. https://students4kids.org/en/blog/current-trends-in-malnutrition-in-children-worldwide-report-from-unicef-who-and-world-bank-group/
[77] Welch R.M., Graham R.D (2004) Breeding for Micronutrients in staple food crops from a human nutrition perspective. J.Exp Bot. 55 353-364. DOI: 10.1093/jxb/erh064
[78] White P.J & Broadely M.R,(2009). Biofortication of crops with seven mineral elements often lacking in human diets-iron, zinc, copper, calcium, magnesium, selenium & iodine. New Phytologist, 182, 49-84. DOI: https://doi.org/10.1111/j.1469-8137.2008.02738.x
[79] Zhang, Y., Song, Q., Yan, J. et al. (2010). Mineral element concentrations in grains of Chinese wheat cultivars. Euphytica, 174, 303–313. DOI: https://doi.org/10.1007/s10681-009-0082-6
[80] Zhu C., Naqvi S., Gomez-Galera S., Pelacho A.M., Capell T., Christon P (2007). Transgenic strategies for the nutritional enhancement of plants. Trends Plant Science, 12(12), 548-565. DOI: https://doi.org/10.1016/j.tplants.2007.09.007
[81] Zou C.Q., Zhang Y.Q., Rashid A., Ram H., Savasil E., Arisoy R.Z., Ortiz-Monasterio L., Simunji S.,, Wang Z.H., Sohu V., etal (2010) Biofortication of Wheat with Zinc through Zinc fertilization in seven countries. Plant soil, 361, 119-130. DOI: https://doi.org/10.1007/s11104-012-1369-2

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Use of McFarland Standards and Spectrophotometry for Yarrowia Lipolytica QU69 cell counting

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Abstract**—***New researches on microorganisms capable of synthesizing different carbon sources have been made to fulfill the growing use of biotechnology to obtain products with economic value and the search for reducing the environmental impact caused by inadequate waste disposal. The yeast Yarrowia lipolytica has stood out for its ability to grow in hydrophobic environments and has been used in bioconversion processes to produce various industrial products of interest. McFarland standards and Neubauer chamber are the two most common methodologies employed to count viable cells, but they were originally made to count bacterial cells and blood cells, furthermore, those methodologies can be quite subjective. In order to optimize yeast cell count for use in bioprocesses, McFarland standard associated with spectrophotometry was used to estimate the amount of strain Yarrowia lipolytica QU69 cells present in a suspension. It proved to be a reliable, accurate and reproducible method, and it could be applied in routine analysis and classroom experiments.*

Keywords**—*****hemocytometer, turbidity, bioconversion, absorbance.***

I. EXPERIMENTAL AND RESULTS

Only high purity reagents and solvents without any prior purification were employed. *Yarrowia lipolytica* strain QU69 was kindly provided by Professor Patrícia Valente (Department of Microbiology, Immunology and Parasitology - UFRGS - Brasil - RS). Cell counting was previously performed in a Neubauer chamber, Kasvi, Ref. OG200 with an Olympus CX21FS1 optical microscope. The yeast maximum absorption spectrum was determined using a Thermo Multiskan GO microplate spectrophotometer. McFarland scale was prepared in a Fume Hood and the yeast inoculum in a Bioseg 12 Class II type A1 Biosafety cabinet.

II. PREPARATION OF MCFARLAND STANDARDS

First, solutions of 1% (w/v) Barium Chloride ($BaCl_2$) ($BaCl_2 \cdot 2H_2O$) and 1% (v/v) Sulfuric Acid (H_2SO_4) with constant stirring were prepared.

Then, 11 assay tubes of the same size were sequentially numbered, and 1% barium chloride solution and 1% sulfuric acid solution were added according to Table 1. The final scale is shown in Figure 2.

Yarrowia lipolytica QU69 was isolated in GYP [5]. Agar after incubation at 28°C for 48 h. After that, some cells were transferred to test tubes containing 10 mL of sterile saline until turbidity was adjusted to meet McFarland standards.

Yeast cell counting was performed in a Neubauer chamber, prior to being transferred to a 96-well plate for optical density readings at 500 nm using a microplate reader, as well as the McFarland standards. Considering that the precipitate could easily sediment, it was standardized that before the reading the microplate would be agitated for thirty seconds so that there was no sedimentation and possible alteration in the results.

Table 1. Correlation between turbidity, bacteria, and yeast cell counting

Tube	$BaCl_2$ 1% V (mL)	H_2SO_4 1% V (mL)	[Bacteria] ($x10^8$)	Abs (500 nm)	[Yeast] ($x10^6$)
0.5	0.05	9.95	1,5	0.104	1.2
1	0.1	9.9	3	0.159	2.6
2	0.2	9.8	6	0.242	5.0
3	0.3	9.7	9	0.375	6.7
4	0.4	9.6	12	0.401	8.5
5	0.5	9.5	15	0.499	9.4
6	0.6	9.4	18	0.580	11.2
7	0.7	9.3	21	0.661	13.2
8	0.8	9.2	24	0.742	14.9
9	0.9	9.1	27	0.823	16.1
10	1,0	9.0	30	0.904	16.4

Fig.1: McFarland standard.

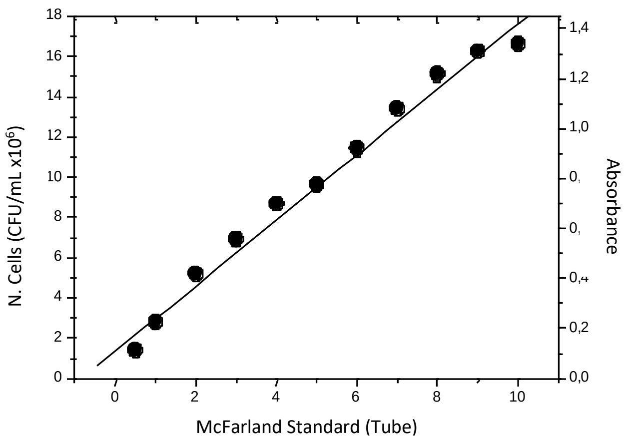

Fig.2: Correlation between Yarrowia lipolytica QU69 cell counting and the absorbance achieved by the McFarland suspensions

Figure 2 shows that the cell count reached a standard of 10^6 CFU/mL, differently from the original McFarland standard corresponding to 10^8 CFU/mL. This is due to the yeast cell size, much bigger (20 to 50 µm) than bacteria, from 1 and 10 µm [6]. Therefore, fewer yeast cells are required to reach the intended turbidity of McFarland scale.

The tested methodology presents advantages due to its speed, simplicity and greater accuracy in comparison to the colony counting or the naked eye comparing technique.

The absorbance reading can be used to estimate the number of *Y. lipolytica* QU 69 cells present in suspensions that will be used in bioprocesses. Hence, the quantification of cells becomes more precise, optimizing the time spent in Neubauer chambers and reducing the error that may occur when employing visual comparison with the McFarland standard alone.

III. CONCLUSION

The present methodology allows two jobs, presents speed and efficiency, making the results more precise.

Confirms that the scale of use of the method in question for yeasts is around 10^6.

It presents greater reliability of the results, considering the absorbance reading and the visual interplay of the turbidity of the medium, presents potential to be used in research routines

IV. DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

[1] Vendruscolo, R. F.; Ribeiro, C.S.; Espósito, E.; Ninow, J.L; Bras. *Eng. Agríc. Ambiental*, 2009, 13, n.4, p.487–493.

[2] McFarland, J. The nephelometer: Na instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. In: Campbell, H. D. et al.

[3] Oliveira, R. A. G.; Takadachi, M. M.; Nonoyma, K.; Barretto, O. *J. Bras. Patol. Med. Lab.* 2003, 39, 2.

[4] McFarland, J. The nephelometer: Na instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. In: Campbell, H. D. et al. (Eds.*)*. Methods in immunology. New York: Benjamin, 1970. p.435-437.

[5] PEREIRA-DIAS, S et al. Characterization of yeast flora isolated from an artisanal Portuguese ewes' cheese**.** International Journal Of Food Microbiology, [s.l.], v. 60, n. 1, p.55-63, set. 2000. Elsevier BV. http://dx.doi.org/10.1016/s0168-1605(00)00323-8.

[6] Vaccari, Liana *et al*. Advances In Colloid And Interface Science, 2017. 247, p.561-572.

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Development new rice varieties in the coastlines of Mekong Delta, Vietnam

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***Abstract*— *To increase productivity and improve quality, new rice varieties need to be salinity tolerance and resistant to many kinds of insects, diseases, and high levels of nutrients, resistant to disadvantageous conditions, and promises to reduce using pesticides, chemical fertilizers. Great efforts have been focused on germplasm research to discover genes resistant to disease and insect, efficient in using salinity level with good grain quality and productivity. With the development of climate-resilient varieties, scaling up or dissemination of seeds is done by province, following the locality's is Winter-Spring (DongXuan Season) and Summer – (Wet season). For instance, in the case of Winter-Spring crop, the plan of Mekong delta is to expand some lines such as HATRI 190, HATRI 192, HATRI 170were developed that can yield4-5 tons ha^{-1} under salt stress of 10.0 to 12.0 dS m^{-1}, and are being out-scaled. Normally, this expansion initiative requires inclusion of key rice varieties, that is, local or extra varieties and promising varieties, all of which will be included in the plan for developing the rice sector for each province.*

***Keywords*— rice varieties, improve quality, salinity tolerance.**

I. INTRODUCTION

Rice is a major export commodity of Vietnam and a source of livelihood of smallholder farmers, particularly in the Mekong Delta, which contributes more than fifty percent of the country's total rice production. Millions of farmers in the Delta are living below poverty level as their farming is affected by occurrences of drought, flooding, and salinity from saltwater intrusion. An estimated 1,867,910 hectares of the total rice crop area is affected by salinity(Buu et al 2004).*High Agricultural Technology Research Institute for Mekong delta*(HATRI) recognizes the importance of developing modern climate-resilient rice varieties as well as associated management practices that respond to the challenges of climatic variability in such stress-prone areas. In the case of Vietnam, a rice variety that will be seed-multiplied, first needs to undergo evaluation trials at the HATRI. Moreover, an equally daunting task is bringing the new varieties to the farmers' fields where productivity is still not at optimal level due and where there are institutional constraints related to extension activities. Country protocol for varietal release and dissemination requires that provincial officers and farmers will have to be invited to assess the performance of the varieties in the field. Upon initial assessment and acceptance, farmers and local staff in the districts and provinces will evaluate the variety this time for deployment and multiplication (Lang et al 2016a). This is an ideal strategy to bring rice varieties to the local farmers. The MARD, IFAD-CURE, and other funding institutions, which share the same goal of raising productivity in the salinity-prone rice areas in the Mekong Delta, supported the development, validation (Lang et al 2015), and eventual dissemination of climate-smart varieties. The key in achieving such goal involves partnerships and participatory approaches for a need-based strategy of developing varieties and eventual delivery to farmers from 2017-2018.The estimated rice production for the three rice growing seasons 2015 correlates well with data at the district level collected from the province statistics offices with R2s of 0.93 for the Winter-Spring, 0.86 for the Summer-Autumn and 0.87 for the Autumn-Winter season(Kersten Clauss et al 2018) Our research activities in the Mekong Delta focus on the development of proper technologies for **Cultivating climate-resilient rice varieties in the coastlines of Mekong Deltat** enhancing and stabilizing farm level productivity and for improving farmers livelihoods. This is being achieved through the development of rice varieties with tolerance to prevailing abiotic stresses, adoption of proper soil, water for higher and stable productivity of these varieties .These efforts are being supported in part, by funds provided by the Program on salinity for climated change through Tay Nam Bo project.

II. MATERIAL AND METHODS

Some lines 24 lines obtained from the High Agricultural Technology Research Institute for Mekong delta, Vietnam (HATRI).

Phenotype analysis

A field experiment was transplanted to an irrigated lowland field in a randomized complete block design in three replications in the field of HATRI at BinhThuy, Can Tho. 24 lines with their parents were used to evaluate agronomic characteristics and salinity detection through sensory test and genotypic analysis using SSR markers in lab of HATRI. Data on important agronomic traits like plant height, panicle length, filled grains/panicle, unfiled grain/panicle, 1000-grain weight, harvest index and yield were recorded.

Ten randomly selected plants of each genotype were used for agronomic data analysis. Data on plant height (cm), number of effective tillers/plants, panicle length (cm), number of filled grains/panicle, 1000-grain weight (g), days to maturity and grain yield/plant (g) were recorded and subjected to statistical analyses using SAS software. After harvesting, the seeds of each genotype were dehulled for evaluation of the grain quality and aroma. The grains were classified into different types based on their dimension according to Dela Cruz and Khush.1989 . Ten seeds of each cultivar removed rice hull and mashed by hand. Take rice powder of each cultivar and put on each experimental tube or petri dish. Add 5ml KOH 1.7% into each petri dish and cover. Store the samples in room temperature for 30 minutes. The samples were scored, corresponding to absence of aroma, slight aroma, moderate aroma and strong aroma, respectively.

Technology evaluation and adaptation. While varieties have already been developed that should be adapted to the local conditions, it is still necessary to evaluate their performance to meet national varietal release requirements. As these varieties perform completely differently undersality conditions than current varieties, comprehensive management practices must be developed so that farmers can fully benefit from them. This will involve (a) initiating testing programs of new varieties in various locations in all province deemed to be susceptible to salinity; (b) conducting comparisons of differing sources of salinitytolerance and combinations of sources to determine which are best suited for different salinity affected areas (different levelstress); (c) conducting of field trials (on-farm and on-station) to refine best management practices for each location; and (d) obtaining feedback results from field and agronomic experiments into varietal development and gene identification programs.

Data Analysis

The agro-morphological data were initially analyzed through examining variance to verify genetic variation in the traits measured. The few traits with insignificant genetic variation, based on the F-test, were not considered for further analyses

III. RESULTS AND DISCUSSION

Breeding for salinity tolerance in rice

The results show that some lines such as at 20 days after transplanting. At 35 days after transplanting, the highest variety was Evaluation of breeding lines from with local check varieties namely Pokkali and OXY 10 is conducted regularly in both wet season 2018.These lines are evaluated in farmers' fields as well as on station at HATRI. Different traits were assessed, including crop duration, plant height, panicle/ filling and sterility/ plants, quality traits as well as salinity tolerance. Lines that are early maturing (90-110 days) semi dwarf (90-110 cm) with medium number of panicles/hill (7-15 panicles) and high number of grains/panicle (77-199 grains) were selected for further testing in rice-rice cropping pattern when their salinity tolerance is high (survival after 20-25 day salinity at EC = 8 DS/m) and duration of about 98 d, similar to OXy 10. They were these lines will be suitable for salitniy affected(Table 1)

Table 1. Yield and yield components of rice varieties tested at HATRI

No.	Lines	Panicles/ plants (no.)	Filled grains/ Panicle (no.)	unFilled grains/ Panicle (no.)	P 1000 grains *(g)*	yield (g/10 plants)	% Survival in sality EC-8DS/m
1	IR28 (Checked)	18 c	198 d	12 l	26.7 cde	75 m	5.2 v
2	Pokkali (checked)	16 de	148 n	14.2 j	26.8 cd	56.5 t	78.8 i
3	HATRI 402	17 cd	77 x	14.2 j	27.6 b	99 g	45.8 mn
4	HATRI503	16 de	169 i	14.5 ij	26 g	95 i	45.6 n
5	HATRI506	15 ef	210 c	16.8 f	26.8 cd	93 j	47.8 k
6	**HATRI 507**	18 c	**230 a**	16.9 f	27 c	**94 i**	42.3 s
7	HATRI 506	12 g	120 t	20.3 b	26.5 def	96 h	45.9 m
8	HATRI 302	13 fg	145 o	18.9 c	26.8 cd	95 i	46.8 l
9	HATRI477	14 ef	187 f	17.5 e	26.9 c	99 g	44.8 p
10	HATRI 468	15 ef	176 h	16.8 f	26.4 ef	153 a	44.5 r
11	**HATRI 1**	**12 g**	**189 e**	**18.9 c**	**26.8 cd**	**124 c**	**68.9 j**
12	HATRI 170	10 h	163 j	18.4 d	26.8 cd	114 e	95.3 e
13	HATRI190	12 g	152 m	17.5 e	26.5 def	121 d	92.4 g
14	HATRI192	13 fg	148 n	16.8 f	26.7 cde	112 f	95.8 d
15	HATRI 194	14 ef	199 d	20.3 b	26.5 def	49 u	97.5 c
16	**HATRI60**	12 g	185 g	21.3 a	26.4 ef	86 k	**99.8 a**
17	HATRI62	14 ef	175 h	20.1 b	26.5 def	112 f	99.5 a
18	**HATRI61**	15 ef	126 r	12.3 kl	26.3 f	135 b	**99.8 a**
19	HATRI144	15 ef	142 p	**10.2 n**	26.8 cd	99 g	74.2 i
20	HATRI188	9 h	158 k	10.5 n	26.8 cd	86 k	79.3 i
21	HATRI195	9 h	135 q	15.4 g	26.9 c	68 o	99.7 a
22	**HATRI 50**	7 i	147 n	14.2 j	**28.7 a**	67 o	45.2 o
23	HATRI2	4 j	156 l	11.2 m	24.2 h	65 p	0 y
24	HATRI3	13 fg	112 v	10.5 n	26.8 cd	65 p	77.5 i

New lines provided a substantial enhancement in the level of tolerance of all the lines varieties. Only 11 lines (HATRI 1, HATRI 170, HATRI 190, HATRI 192, HATRI 194, HATRI 60, HATRI 62, HATRI 61, HATRI 144, HATRI 188, HATRI 195) carried 90-99% survival salinity condition in the field.

Use of DNA markers to accelerate progress in breeding for salt tolerance:

Identification of molecular markers associated with quantitative trait loci (QTLs) linked with useful agronomic or adaptive traits will help speed the progress in breeding once developed, because these DNA markers will become effective tools for selection. Moreover, positional cloning using DNA markers will make it possible to isolate agronomically useful genes, which can also be used in breeding across species via transgenic approaches.The study used restriction fragment length polymorphism (RFLP) and simple sequence length polymorphism (SSLP) markers to saturate the segment of chromosome 1 containing a major salt tolerance gene controlling the Na+/K+ ratio. RFLP and SSLP analyses were conducted to construct linkage maps based on an F8 recombinant inbred line (RIL) mapping population(Bonilla et al 2002) We developed several mapping populations using salt tolerant and sensitive genotypes and used them for mapping of QTLs associated with salinity tolerance during seedling stage(Lang et al., 2001, Lang et al 2015). Two QTLs with relatively large effects were identified, one on chromosome 1 (linked to marker 18EC) and the second on chromosome 8 (linked to marker 12EC). Microsatellite markers closely linked to these loci were identified such as RM223 associated with the QTL on Chromosome 8. This marker was further evaluated for its effectiveness in breeding using a set of 24 improved varieties, including tolerant (Pokkali) and sensitive (IR28) checks. These cultivars were genotyped at this marker and then phenotyped for salinity tolerance at 12 dS m^{-1} in culture solution (Yoshida et al., 1976) using visual SES scores. The results indicated an accuracy of more than 95% in identifying tolerant cultivars using this marker (Table 2). These results indicated the usefulness of this marker in parental surveys and in identifying tolerant lines from segregating populations; however, further tests are needed to confirm its effectiveness in different genetic backgrounds. More efforts are needed to develop closely linked markers to these two QTLs to be used for their routine introgression into popular varieties and elite breeding lines.

Table 2. Comparison between the phenotype and genotype of 24 varieties under salt stress of 12 dS m^{-1}. Lines were genotyped using markers specific for Saltol locus on chromosome 1 and phenotyped under salt stress in hydroponics

Number	varieties	Genotype	phenotype	Note
1	IR28(Checked S)	S¥	S	S
2	PokkaliChecked for R)	T	T	T
3	HATRI 402	S	S	S
4	HATRI503	S	S	S
5	HATRI506	S	S	S
6	**HATRI 507**	**S**	**S**	S
7	HATRI 506	**S**	**S**	S
8	HATRI 302	S	S	S
9	HATRI477	S	S	S
10	HATRI 468	S	S	S
11	**HATRI 1**	**T**	**T**	**T**
12	HATRI 170	**T**	**T**	**T**
13	HATRI190	T	T	T
14	HATRI192	T	T	T
15	HATRI 194	**T**	**T**	T
16	**HATRI60**	**T**	**T**	T
17	HATRI62	**T**	**T**	T
18	**HATRI61**	**T**	**T**	T
19	HATRI144	T	S	S
20	HATRI188	**T**	**T**	T
21	HATRI195	**T**	**T**	T

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22	**HATRI 50**	S	S	S
23	HATRI2	S	s	s
24	Oxy 10(Checked)	T/S	T	Not clear

¥T: tolerance and S: sensitive. £Oxy 10andPokkali were tested in the field and seeds provided to farmers in several provinces for further testing.

Advancing rice research

Confronting the huge challenges of developing varieties in the less-favorable rice areas necessitates partnerships and strategies that capitalize on and foster synergism among related projects in Vietnam. Having strong financial support from the government and other external programs, Vietnam has been successful in scientific and development work leading to releases of a significant number of climate-resilient varieties and suitable crop management practices. The well-focused development agenda of the national government has been a driving force in raising the level of productivity in difficult rice ecosystems. Moreover, HATRI institutions has also been instrumental in catalyzing support, resources and capacities that is now creating impact on Vietnam's rice productivity at the farmers' fields, and producing surplus for local and export markets.

The project has been breeding for salinity using MAS, building up on the prior knowledge and system of rice breeding at the (HATRI) institute. In collaboration with other Vietnam institutions, andRice Climate for salinity project, HATRI has developed several climate-resilient rice varieties such as grain quality and salinity-tolerant varieties by intro gressing *Saltol* and quality QTLs into elite genotypes. For its expansion efforts, partners distributed seeds of salinity -tolerant elite lines for field evaluation in farmers' fields. Beginning in 2017, HATRI aim (1) to breed and develop new rice varieties (2-3 varieties) suitable for export standards and adapted to the conditions in the Mekong Delta, salinity -tolerant, early- maturing, high-yielding, and with good quality, as well as with resistance to major insects/pests; (2) and to build up technical procedures for cultivation of new rice varieties in salinity tolerance of the Delta.

With HATRI 's involvement in Rice Climate activities, at least fourteen (14) varieties have been supported that have now been doing **releases** (Table 3). Most of these stress-tolerant varieties can provide, on average, a yield advantage of 1.0- 1.5 tons per hectare compared to the farmers' popular varieties. The average yield of a popular variety in the mid-1990s was only 3.0 tons/ha (Buu et al 2017). But with the usage of new rice varieties that have been released over the years, the average yield has been increasing and has reached 5.5 tons/ha in 2017 for salinity-tolerant varieties, and about 6.0 ton/ha for salinity -tolerant varieties. The released varieties that have been developed through marker-assisted selection (MAS) are TLG 1 (both for salinity- and submergence-prone areas), TLG 1, and HATRI 170; and for salinity, HATRI60, HATRI 61, HATRI 62. The other varieties in the table have been developed through conventional breeding methods that required at least a decade–worth of work. Results of adaptation trials were used to pass the rigorous evaluation process. For instance, shows the comparative yield performance of three varieties included in trials in 2017-2018, which indicated the good performance of salinity -tolerant varieties, particularly HATRI 10, HATRI 60, TLG1. This eventually became part of the bases for farmers' selection and continued adoption in affected environments to this today.

Yield testing on rice varieties

To select the rice varieties with high yield potential, insect pest resistance for condition in Mekong delta. To find out the rice varieties with desirable traits for hybridization in rice improvement programs.

Including lines rice varieties with Pokkali, OXY 10 for checked, growing dry seasons 2017-2018. Yield testing experiments were laid out in completely randomized block designs with 3 replications in BaTri's experimental field. Statistical analysis was cited (Gomez and Gomez, 1982). Data records on agronomic characters, yield and yield components were also guided as SES (IRRI, 1996). Insect, disease screening experiments were laid out in single replication. Evaluation of insect and disease reaction at seedling stage (IRRI, 1996). Agronomic characters were presented in table 3.

Table 3: Yield and yield components of linesforsalinity in rice at 2017-2018at Ba Triwith salinity EC= 10 DS/m

Lines	Duration (days)	Height (cm)	Panicle/m^2	Yield (tons/ha)
HATRI 1	100	107 ***b***	303.66 ***ab***	4.473 ***bcde***
HATRI 190	95	109.33 ***b***	266.66 ***bc***	5.13 ***ab***
HATRI 170	96	107.33 ***b***	242 ***c***	5.16 ***ab***
HATRI 194	100	108 ***b***	274 ***bc***	4.726 ***abcde***
HATRI62	105	108.33 ***b***	284 ***abc***	4.95 ***abcd***
HATRI 188	98	95.66 ***d***	284.33 ***abc***	3.84 ***cde***
HATRI144	98	115 ***a***	272.66 ***bc***	4.69 ***abcde***
HATRI192	95	106.66 ***b***	290.33 ***abc***	5.84 ***a***
TLG1	94	98.67 ***cd***	293.67 ***abc***	4.35 ***bcde***
HATRI 195	100	101.67 ***c***	338.33 ***a***	4.32 ***bcde***
POKKALI	115	129.67 e	318.67 ***ab***	3.66 ***e***
OXY 10 (Checked)	105	101.33 ***c***	285.33 ***abc***	3.8 ***de***
HATRI475	95	99.33 ***cd***	287.67 ***abc***	4.07 ***bcde***
HATRI60	95	107.33 ***b***	320 ***ab***	5.09 ***abc***
CV	***0***	***2.16***	***10.16***	***14.26***

It is indicated that almost of rice varieties in experiment are less than 100 days duration. Plant height ranged in 120 cm for Pokkali.

Grain yield and yield components of 16lines rice and were presented in table 4.It was indicated that HATRI 90 and HATRI 192 gave highest yield in experiment (5.13 - 5.16 tons/ha) and be higher thanOxy 10 (checked Rice famers)

The success of new varieties is assured through eventual testing and selection in target sites in partnership with farmers and under their own management to guarantee relevance and adoption. Special emphasis is placed on crop establishment because the early stages of seedling growth are extremely sensitive to salt stress (Moradi et al., 2003;Ismail et al., 2007. Lang et al 2018). This is achieved through combined use of salt tolerant genotypes, coupled with proper nursery management and seedling handling that ensures maximum survival of transplanted seedlings.

Combination of traits for multiple stresses. One of the difficulties in rice areas severely affected by variability in climatic conditions is the occurrence of multiple stresses over a cropping season. To address this concern and with the advances in molecular biology particularly using MAS, the breeding program of HATRI used pyramiding technique to combine genes responsible for tolerance to a combination of stresses. For instance, in 2017, in developing high-yielding rice varieties tolerant to salinity and quality total of ten (10) single and multiple crosses were done for combining sality and quality rice into the high-yielding genetic background. Genetic diversity is emphasized as a success factor in breeding, as well as in understanding the relationship between genotype and phenotype. Strategic development of the varieties focus on the following: (a) adaptation to effects of sality, both at the seedling stage and flowering stage, (b) improved yield to exceed 5-6 tons/ha, and (c) providing the basis of genetic diversity.

Emphasis on grain quality. Grain quality is considered an important trait contributing to farmers' preference or selection of a variety. This is also taken into consideration in breeding works to know which traits are most popular among traders. For instance, to develop long and medium grain rice cultivars, 20 crosses were made in 2017. The F2, BC1, BC2, BC3 seeds were planted at HATRI in the wet season and dry season. Samples of seed from each harvested plant were sent to the Riceland quality lab for grain quality evaluation at HATRI and selected crosses were evaluated using MAS for amylose content, aroma and blast resistance. The best yielding lines selected from these experiments were advanced to the HATRI. Twenty advanced long grain lines were also tested in the 6 provinces for trials, indicating that long grain rice cultivars could be ready for release in the near future.

Table 4. Most in-demand stress-tolerant varieties and their characteristics

Genotype	Maturity (days)	Origin	1000 grain wt. (g)	Amylose (%)	Cooked rice quality	BPH	Blast	BLB (score)	Yield (ton/ha)	Remarks by farmers
Salinity-tolerant and good quality varieties										
OM4900	95-100	Origin: C53/ Jasmine 85/	28.8	16.8	Glutinous, and aromatic rice, "World's longest cooking rice"; excellent for cooking, requires less water	Tolerant	Tolerant	3 -- 5	7-8	Excellent (Salinity-tolerant, high yield, aroma) no lodging
HATRI 10	95-100		27.5	19.6	Glutinous, soft, excellent for cooking, elongation trait, requires less water	Tolerant	Tolerant	5	6-8.5	Good (Salinity-tolerant) KienGiang, Bac Lieu, and TraVinh (5% of areas grow this variety)
TPG1	93 - 85	hybrid of M362/A S996	25.6	22	Glutinous	Tolerant	Tolerant	3 -- 5	6-8	Very good (Salinity-tolerant, high yielding)
HATRI 170	95-100		26	22	Glutinous, hard	Tolerant	Tolerant	3 -- 5	5-7	Very good (Salinity-tolerant, high yielding)
TLG1	95-100	Origin: OMCS2 012/ Pokkali	27.3	21.5	Glutinous	Tolerant	Tolerant	3 -- 5	5-7	Excellent (Salinity-tolerant, high yielding); Bac Lieu, KienGiang and TraVinh; farmers liked the variety that >408 ha in 2009 and increased to 1,496 ha in the Mekong Delta in 2010.
HATRI144	90-95		27.5	21.86	Glutinous	Tolerant	Tolerant	3	5-7	Good (high yielding), tolerant to salinity, alkalinity, iron and boron toxicity, and phosphorous and zinc deficiency;

										suitable in irrigated and rainfed lowland areas; popularly grown in salinity- and alkaline rice areas such as KienGiang, TraVinh and Long An
HATRI 61	95-105		27.5	23.5	Glutinous	Tolerant	Tolerant	3 -- 5	5-7	Good (Salinity-tolerant, high yielding) tolerant to salinity, alkalinity, iron and boron toxicity, and phosphorous and zinc deficiency; suitable in irrigated and rainfed lowland areas
HATRI 20	95-105		26.8	23	Glutinous	Tolerant	Tolerant	5	5-7.5	Very good (Salinity-tolerant, high yielding)
HATRI192	95-100		25.6	21	Glutinous and scent	Tolerant	Tolerant	3 -- 5	6-7	Good (drought, high yielding)
HATRI 144	105-110		26.5	24.5	Glutinous	No data	No data	3 -- 5	3-4	Excellent (drought-tolerant)
HATRI 181	95-100		28.8	16.8	Glutinous and scent	Tolerant	Tolerant	3 -- 5	7-8	Very good (drought -tolerant, high yielding, aromatic)
HATR60	95-100		27.5	22	Glutinous	Tolerant	Tolerant	5	7-8.5	Very good (sality-tolerant, droughthigh yielding)

Evaluation of salinity stress varieties in farmers' fields

Performance stability is one of the most important properties of a genotype to be released as a variety to ensure wide adoption. To ensure this, we tested 12 indica rice verities at 7 different locations during the wet season 2018 and dry seasons of 2018-2019, using a randomized block design with three replications in each case. Duration, grain yield (t ha^{-1} were presented in Table 4, 5. The experiment was conducted in 7 provinces, Can tho (checked),TraVinh, Ca Mau, SocTrang,Long An, Ben Tre and Bac

Lieu. The highest grain yield across the 7 sites was obtained from HATRI 190, TLG 1 during dry seasons. Most of the varieties showed excellent stability index. An understanding of environmental and genotypic causes and GxE interaction is important at all stages of plant breeding, for both selection based on specific traits or on yield (Yan and Hunt, 1998; IRRI, 1997)

Table 5.The Yield of 12 lines in riceat Mekong delta dry season 2018-2019

Numbers	Lines	Mekong delta						Can Tho	mean
		Bac Lieu	Ba Tri (Ben Tre	Ca Mau	Long An	TraVinh	SocTrang		
1	HATRI 1	7.55	7.81	7.68	7.00	6.17	7.52	7.90	**7.37ab**
2	HATRI 190	7.80	7.77	7.79	6.9	7.87	7.78	8.10	**7.71a**
3	HATRI 170	7.28	7.28	7.29	7.51	7.75	7.56	7.80	**7.49ab**
4	HATRI 475	7.52	7.84	7.53	7.05	6.84	7.67	7.10	**7.36ab**
5	HATRI62	7.39	7.01	7.21	7.16	7.53	7.78	6.50	**7.22ab**
6	HATRI 60	7.44	7.24	6.71	7.63	7.97	6.25	7.65	**7.27ab**
7	HATRI 188	7.55	7.43	7.89	7.20	7.58	7.93	6.42	**7.42ab**
8	HATRI144	7.07	7.16	7.26	7.52	7.31	7.57	7.12	**7.28ab**
9	HATRI192	7.12	7.45	7.61	7.27	6.62	7.58	7.47	**7.30ab**
10	TLG1	7.64	7.85	7.12	7.59	7.32	7.73	7.93	**7.59ab**
11	HATRI194	7.37	7.48	6.47	7.48	7.72	7.04	7.30	**7.26ab**
12	Oxy 10 (check)	6.52	7.29	6.9	7.23	7.08	6.84	6.97	**6.97b**
	EMS	**0.272**	**0.17**	**0.16**	**0.13**	**0.12**	**0.16**	**0.14**	
	Mean	**6.43**	**6.63**	**6.12**	**5.79**	**5.81**	**5.68**	**6.93**	
	(I_j)	**0.24**	**0.44**	**-0.07**	**-0.39**	**-0.38**	**-0.50**	**0.74**	

Table 6.The yield of 12 lines in rice at Mekong delta on 20018 wet season

	Mekong							
Lines	**BacLieu**	**Ba Tri**	**Ca Mau**	**Long An**	**TraVinh**	**SocTrang**	**Can Tho**	
HATRI 1	5.62ab	**5.54**ab	**5.86ab**	**5.70**ab	**6.00a**	**5.40**ab	5.69ab	
HATRI 190	5.80 ab	5.70 ab	5.40ab	5.30 ab	5.00 ab	4.50b ab	5.28ab	
HATRI 170	5.88 ab	5.33 ab	5.70 ab	4.90b	5.20 ab	4.85b	5.06ab	
HATRI 475	5.70 ab	5.10 ab	5.20 ab	5.00 ab	5.40 ab	5.20 ab	4.86ab	
HATRI62	5.40 ab	4.90b	5.00b	4.80b ab	5.10 ab	5.15 ab	5.76ab	
HATRI 60	5.46 ab	5.15 ab	5.30 ab	5.80 ab	5.15 ab	5.00 ab	4.93ab	
HATRI 188	5.46 ab	5.90 ab	6.20a	5.75 ab	5.80 ab	6.00a	5.85a	
HATRI144	6.20a	6.00a	5.90 ab	5.30 ab	6.10a	6.00a	6.33a	
HATRI192	4.84b	5.80 ab	5.55 ab	5.20 ab	5.35 ab	5.50 ab	5.93ab	
TLG1	5.62 ab	5.85 ab	5.70 ab	5.40 ab	5.10 ab	5.20 ab	5.48ab	
HATRI194	5.50 ab	5.70 ab	5.60 ab	5.10 ab	5.30 ab	5.00 ab	5.37ab	
Oxy 10 (check)	4.67b	4.85b	4.55b	4.80b	4.85b	4.77b	4.75b	
CV%	**14.59**	**15.37**	**14.67**	**17.05**	**15.41**	**14.57**	**11.24**	
LSD 0.05	**0.88**	**0.75**	**0.71**	**0.79**	**0.73**	**0.67**	**0.53**	
Mean	5.51	5.48	5.49	5.25	5.36	5.24	5.44	

Potential application site

For the salinity-tolerant varieties, scaling up can be done by region, where the stresses have been determined and estimated. In different provinces, based on results of multi-location trials, the following are the potential expansion areas for dissemination and farmers' use of the now popular varieties (Table 7).

Table 7. Potential areas for expansion of salinity-tolerant varieties.

Province (District)	Yield Potential (tons/ha/cropping)	Cropping intensity (/year)	Level of Salinity (indicate level of EC)	Recommended varieties
Bạc Lieu (HoaBinhTown)	5-6 tons/ ha	3	EC = 8-10 ds/m	Region of 3 crops: 2 for salinity and another crop HATRI170, HATRI 144
Tra Vinh (Cau Ke district)	6-7 tons under normal condition; under saline soil, yield is lower at 4-5 tons/ha	2	EC = 8-10 ds/m	HATRI 20, HATRI 190, HATRI 192
SocTrang	6-6.2 tons	2 varieties + another crop	EC = 10-12 ds/m	HATRI170
Ben Tre	5-6 ton/ ha	2 cropping, since areas are severely affected by salinity	EC = 12-15 ds/m	HATRI170, HATRI 190, HATRI 192, HATRI 475
Ca Mau (Tran van Thoi District)	3-5 tons/ha (yield is very low caused by salt, sulfate soil, and drought)	1 crop	EC = 12-15 ds/m	HATRI 170, HATRI 144
Kiengiang	6-7 tons/ha	2 crops	EC = 12-15 ds/m	HATRI60, HATRI 62

IV. DISCUSSION

Salt- affected soils of the Mekong delta are highly degraded, with a complex of abiotic stresses including salinity, acid sulfate, toxic levels of aluminum and iron and deficiency in some nutrients such as P and K. To enhance and sustain productivity of these soils, we adopt an integrated approach involving the development of adapted high yielding and salt tolerant varieties developed via novel breeding methods, proper management of resources and introduction of effective cropping patterns that cam meet farmers' needs and market demands(CGIAR .2016).

In different provinces, based on results of multi-location trials, the following are the potential expansion areas for dissemination and farmers' use of the now popular varieties (Table 7).The strong advocates of technology are still the leaders of the locality, who are normally the president of the commune and director of province of the agriculture agency.Farmers observe the trials or their neighbors' field and exchange of information among themselves. If the local community does not accept or refuses to accept the technology or rice variety, the varieties cannot be applied and disseminated.While it depends on the province, the farmers typically make the decision to adopt or refuse to grow the new rice varieties. And because it is difficult to convince farmers to grow a particular variety, sometimes unexpected difficulties in the management delay or hamper the dissemination such that the new rice varieties do not reach all farmers. Messengers from farmers also significantly decide on the adoption of newly released rice varieties.

V. CONCLUSIONS AND FUTURE PROSPECTS

Development of salt tolerance varieties is generally considered the most effective entry point for improving productivity of salt affected soils, and it is also the cheapest option for farmers. Through the use of innovative breeding strategies involving conventional and modern tools, together with effective phenotyping techniques, good progress was made in developing salt tolerant varieties with broad adaptation to the conditions of the Mekong Delta. Some varieties such as HATRI 190, HATRI 170, HATRI 192were developed that can yield4-5 tons ha^{-1} under 8.0 to 10.0 dS m^{-1},and are being out-scaled. The success of new varieties is assured through eventual testing and selection in target sites in partnership with farmers and under their own management to guarantee relevance and adoption. This is achieved through combined use of salt tolerant genotypes, coupled with proper nursery management and seedling handling that ensures maximum survival of transplanted seedlings. .

Future efforts should focus on further collection and evaluation of local germplasm to identify landraces with greater tolerance of salt stress, as sources of new genes or alleles for breeding. Additional breeding efforts such as mutation breeding and identification of soma clonal variants should continue to develop better pre-breeding material. To benefit from the new and exciting developments in the field of genomics, additional resources and efforts should be directed towards identification of QTLs and genes underlying tolerance to the multiple stresses experienced in these problem soils of Mekong Delta, for their subsequent integration into modern varieties and elite breeding lines through marker aided breeding. Special efforts should also be placed on training of young scientists to prepare a new generation that can effectively tackle these problems in a team approach.

In the future, stronger linkages and exchange of knowledge through project networks and meetings will also give considerable impacts on capacity strengthening, as well as meeting with scientists, extension workers, civil society organizations in affected areas to plan on the distribution of seed and other adaptation activities to assure farmer acceptance of seed, and determine the quantity of seed required for distribution. Consultation and meetings with people from different sectors involved in the project will also address the development of seed multiplication plan for different varieties needed for different areas identifying the most likely partners to participate in the finalization of seed development and distribution; and identifying training needs of these in terms of technology adaptation, evaluation, and dissemination.

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REFERENCES

[1] Bui Chi Buuand Nguyen thi Lang2004: Improve variety at Mekongdelta to 2010. Science &technology.Volume8: 1041-1043) .

[2] Bonilla P.S ;Jan Dvorak;David James Mackill, K. deal ;Glenn B. Gregorio.2002.RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (Oryza sativa L.) using recombinant inbred lines.hilippine Agricultural Scientist 65(1):68-76

[3] Chi Buu Bui, Thi Lang Nguyen. 2017. *New rice varieties adapted to climate change in the Mekong River Delta of Vietnam.* Life Science/ Agriculture. Vietnam Journal of Science and Technology; vol.59; No.2: 30-33 .

[4] CGIAR. Assessment Report: The drought and salinity intrusion in the Mekong River Delta of Vietnam. Hanoi, Vietnam: CGIAR Research Program on Climate Change, Agriculture and Food Security, 2016

[5] De La Normita CRUZ, Ish KUMAR, Rajendra P. KAUSHIK, Gurdev S. KHUSH.1989 Effect of Temperature During Grain Development on Stabilrty of Cooking Quality Components in Rice. Japanese Journal of breeding ,Volume 39 Issue 3 Pages 299-306

[6] Hasegawa, P.M., R.A.J. Versan, K. Zhu and H.J. Bonhert. 2000. Plant cellular and molecularresponses to high salinity. *Ann. Rev. Plant Physiol. and Plant Mol. Biol.*, 51: 463-499.

[7] Kersten Clauss;Marco Ottinger;Patrick Leinenkugel;Claudia Kuenzer.2018. Estimating rice production in the Mekong Delta, Vietnam, utilizing time series of Sentinel-1 SAR data.International Journal of Applied Earth Observation and Geoinformation 2018(73):574-585

[8] Ismail A.M., Heuer S., Thomson M.J., Wissuwa M. 2007. Genetic and genomic approaches to develop rice germplasm for problem soils. Plant MolBiol (DOI: 10.1007/s11103-007-9215-2)

[9] IRRI, 1999. Experimental Design and data analysis for agricultural research. Volume 2.277 paper

[10] IRRI, 2007.Standard evalution system for submergence in rice, international rice research insitute, Los Banos, Philippines.

[11] Lang NT. 2002. Protocol for basic of biotechnology. Agricultural Publisher, Ho chi Minh City, Vietnam

[12] Lang NT, S Yanagihara, BC Buu. 2001. A microsatellite marker for a gene conferring salt tolerance on rice at the vegetative and reproductive stages. SABRAO 33 (1):1-10

[13] Lang,NT. 1999. QTL mapping for salt tolerance in rice.final report. Japan fellowship.

[14] Nguyen thi Lang, Nguyen trongPhuoc, Pham thithu Ha, Tran BaoToan,Bui Chi Buu, Russell Reinke, Abdelbagi M, Ismail. Reiner. Wassmann. 2015. *Selection of promising lines with combination of drought and submergence tolerance traits in F7 generation through molecular marker in rice.* Omon rice No.20. P:1-8

[15] Nguyen Thi Lang, Pham Cong Tru, Nguyen Van Hieu, Nguyen TrongPhuoc, Tran BaoToan, Tran Minh Tai, Bui Chi Buu. 2016a. *Rice seed production suspected of climate change in the Mekong Delta.* International Workshop on Crop Science. the second time. No. 8. Pages: 859-875

[16] Nguyen thiLang, Nguyen TrongPhuoc.2018. Development of new varieties Such as: BL 12. Paper 34

[17] Yan, W., and L.A. Hunt, 1998. Genotype by environment interaction and crop yield. Plant breed. Rev. 16:135-178

[18] Yoshida, S., Forno, D.A., Cock, J.H., Gomez, K.A., 1976. Laboratory manual forphysiological studies of rice 3rd ed. Manila (Philippines); Int. Rice Res. Institute.

[19] Zheng K., N. Huang, J. Bennett, GS. Khush. 1995. PCR-based marker assisted selection in rice breeding. IRRI discussion paper series No. 12. International Rice Research Institute, P. O. Box 933, Manila, Philippines.

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Relationship between boreholes productivity and major fractures extracted from satellite images of katiola region

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Abstract**—** *The hard rock systems of crystalline basement are now much more under consideration as they may actually constitute viable groundwater resources. The area of katiola is located in the North-Center part of Côte d'Ivoire and belongs to Paleoproterozoic domain, where fractures constitute preferential of flow and accumulation groundwater. This study aims to establish the relationship between the wells efficiency and the structural lineaments detected. The map of major fractures was carried using a 50 x 50 cm radar image processing. A total of 323 major fractures were carried out with a dominant direction N90-100. The flow map was surimposed to those of major fractures to show the relationship between the wells efficiency and the structural lineaments detected. Several boreholes with low flows ($0 < Q < 2.5$ m^3/h) are not superimposed on the major structural lineaments detected and seem have to be implanted using simple geomorphological characteristics. Whoever, more of wells with high flow ($Q \geq 5$ m^3/h) are match and overlap with open and interconnected fractures. There is an influence of distance between boreholes and major accidents on the productivity of the works. This result show that fractures arrangement and interconnection degree take an important role in storage and conduct properties of hard rock systems in Katiola area. This approach may be used as an indirect mean of fracture network validation which can be used during the future drilling campaigns.*

Keywords**— *Côte d'Ivoire, fractures, Katiola, relationship, satellite images, well efficiency.***

I. INTRODUCTION

Hydrogeological knowledge of crystalline reservoirs is essential on a global scale, both for the supply of drinking water, particularly for developing countries where a large part of the water resources are concentrated in crystalline contexts (Inde, Afrique, Asie...) (Roques, 2013). Crystalline basement aquifers consisting of plutonic rocks (granite) and metamorphic rocks (shales) are present on all continents (Figure 1). They are typically composed of fractured crystalline bedrock and a partially altered encasing material (Wyns et *al*., 2004). These fractured basement hydrosystems are known for their structural complexity and the associated resources reflect this complexity. The study of crystalline and crystallophellian aquifer systems in West Africa is fundamental and essential for the determination of groundwater catchment areas, their management and their protection (Bassolé et *al*., 2001; Biémi, 1992; Dewandel et *al*., 2006; Kouamé 1999, 2010; Jourda, 2005; Lasm et *al*., 2012 ; Maréchal et *al*. 2006). It is the geometric organization of the fracture network that determines solute transfers in fractured basement environment (Kouamé, 2005). In Côte d'Ivoire, most of the water resources are confined in the fractured basement reservoirs, which represent more than 97.5% of the country's geological context. Several studies here and elsewhere have highlighted the good correlation between the productivity of wells and the discontinuities detected by the lineaments (Fernandes and Rudolph, 2001; Jourda 2005; Kouamé et *al*., 2010; Koïta et *al*., 2010; Larsson 1972; Naik et *al*., 2001, Neves and Morales, 2007; Savané, 1997). Indeed, knowledge of the exact relationship between fluids and fractures is essential both for storage safety and for optimizing production in cracked reservoirs. In the Katiola area, drinking water supply is provided mainly through boreholes capturing these aquifers from cracks in the Paleproterozoic basement (Gnamba, 2014). Despite the government's efforts to meet the sustainable water needs of the ever-growing population, many challenges remain to be met, as the rate of water supply in the region is between 50 and 60%. In addition, many drillings carried out during the drilling compaigns were negative (air lift flow at the end of drilling less than 1 .

m^3/h) or dry up. In the region, statistical studies have highlighted some parameters influencing drilling productivity, as well as rocks with the best hydrogeological potential (Gnamba et *al*., 2014). However, further investigations are needed to understand the complexity of these hydrosystems in a fractured basement environment. The study of the relationships between boreholes and fractures may thus be interesting to explain the hydraulic role played by the different tectonic accidents directions (Biémi, 1997). This is the context of this study which aim is to establish the link between drilling productivity and discontinuities detected as major lineaments in the Katiola area. The architecture of this article presents three parts that address respectively: i) the geographical and hydrogeological context of the study area; ii) the data used and the methods implemented; iii) the results of the study the major accidents influence on the productivity of the boreholes and their discussion

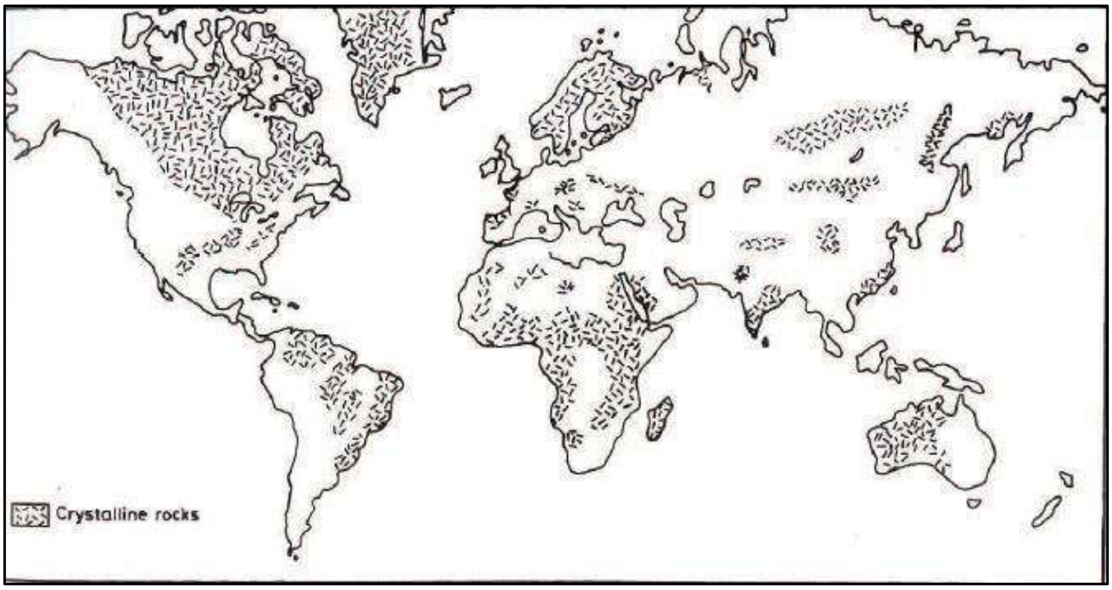

Fig. 1. Distribution map of crystalline basement aquifers (Roques, 2013)

II. STUDY AREA

2.1. Geographical context

The study area (Katiola) is located in the Centre-North of Côte d'Ivoire about 50 km from Bouaké. Katiola is the capital of the Hambol region and is between longitudes 4°75 and 5°75 West and latitudes 7°95 and 9°45 North (Figure 2). It covers an area of 9452 km2 or about 3% of the total area of the territory. The most recent census of 2014 shows an estimated population of 429 977 (RGPH, 2014). The climate context of the region derives from the interface between Sudanese and Guinean climate. Annual precipitation is estimated at about 1200 mm per year. The average air temperature varies very little (between 23 and 27°C). The daily amplitude is quite low in the rainy season (7°C) but it becomes important during harmattan period when the lowest night temperatures occur (absolute minimum temperatures 15°C). The vegetation cover is dominated by savannah, however on the southern margins of the region, below the latitude of the city of Katiola, there are mesophilic forest formations. Islets of dry dense forests, gallery and/or rock forests coexist with specific Sudanese formations; tree and shrub savannas, clear wooded savannahs, and grassy savannahs. The region is drained by two main rivers and their tributaries which constitute natural boundaries of the department, the Bandama and its tributaries (Bou, Naramou, Nabion, etc) in the West and the N'Zi and its tributaries (Loho, Kiohan, etc) in the East.

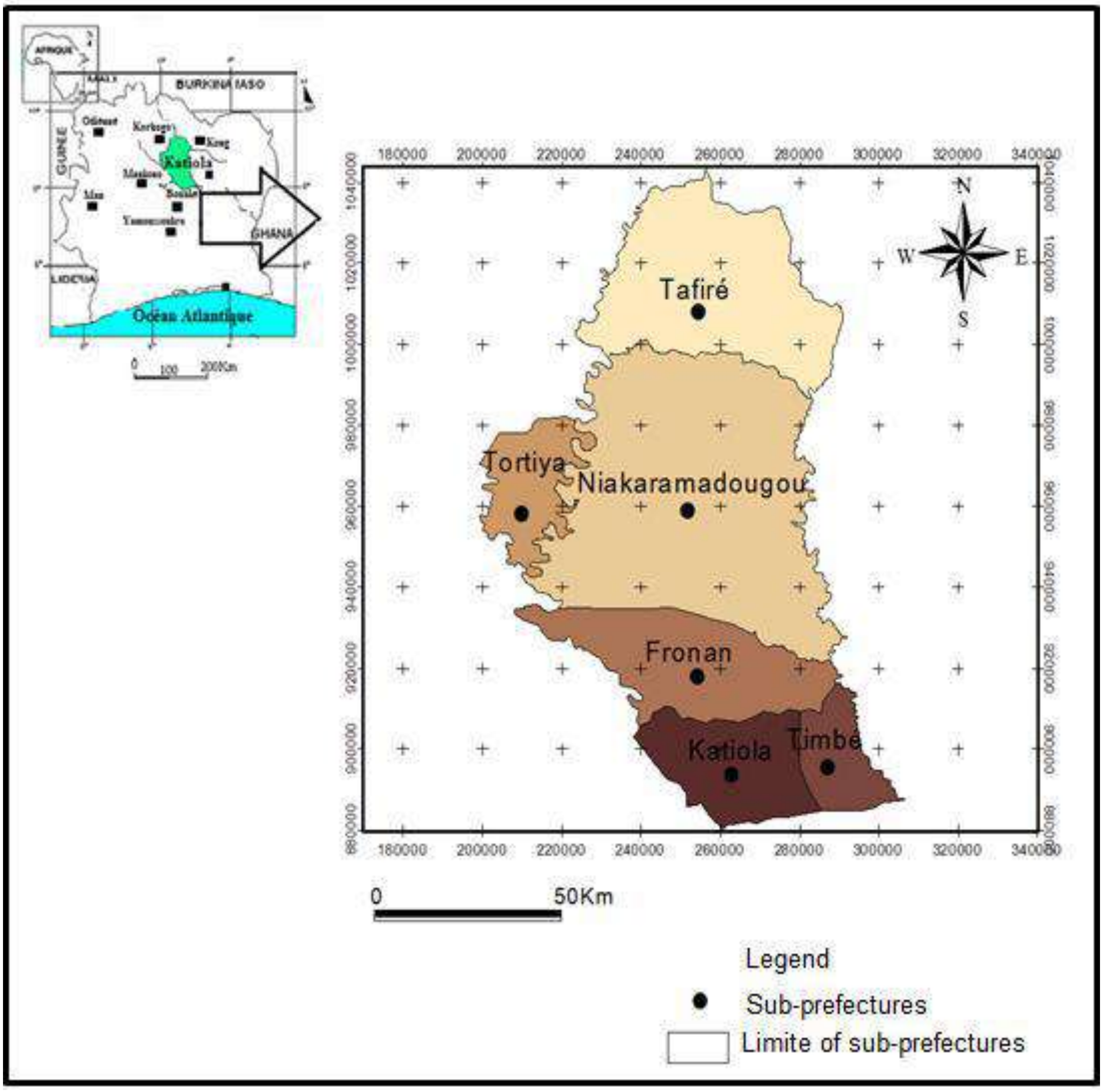

Fig. 2. Location of study area

2.2. Geological and hydrogeological overview of the study area

The region of Katiola belongs to the Eburnéen domain of Côte d'Ivoire. In this area, the geological formations are mainly made up of granitoids. These formations outcrop as slabs, domes and scree. They were affected by the important tectonic phenomena that helped set up the Bandama River. On these granitoids, fractures filled with quartz veins and pegmatite veins of direction N180°. Intrusive microgranites in these fractures are also observed in places. In addition to these crystalline formations, metavolcanites, metasediments and green rock belts are also observed (Figure 3 and 4).

Hydrogeologically, the study area, like most of the country, belongs to the crystalline or crystallophyllian basement. The aquifers found there are composed of three superimposed reservoirs: i) at the top, there are alterations which are composed of lateritic cuirass, clay, arena when the geological formations are of granitoid type. In the region, these alterations are predominantly clay. ii) The middle zone is made up of rock elements and alteration products, with or without the presence of numerous cracks. This zone, when containing water, is the main reservoir of the basement aquifer. iii) The third reservoir is located in fractured basement. It is covered by simple fractures or in some cases by crushed areas due to fairly strong local stresses. This base tank is often the seat of groundwater circulation. The three reservoirs are in fact considered as a bilayer aquifer system consisting of a capacitive semi-permeable reservoir (alterites) overhanging a reservoir formed of cracked rock, captive with essentially conductive function.

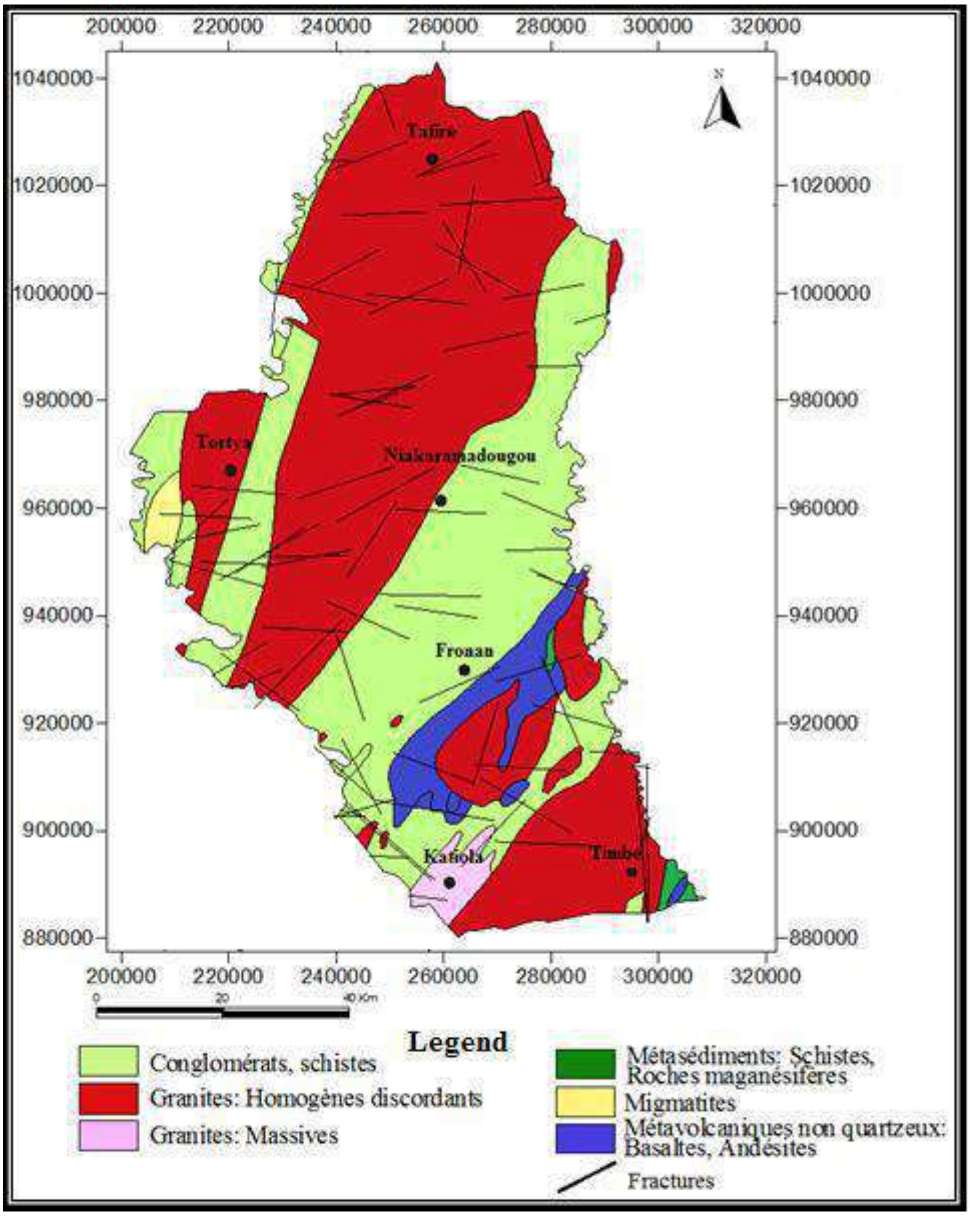

Fig. 3. Geological map of the area study

4a) Outcrops of granite and metavolcanite

4b) Fractures of NE-SW direction

Fig.4.(a, b). Some outcrops and fractures identified during the field campaign

III. MATERIAL AND METHOD

3.1. Data analysis

The data used consists of a RADARSAT-1 scene for the mapping of major lineaments, the characteristics of which have been recorded in Table 1. Unlike optical images, radar or (Radio Detection And Ranging) images allow to visualize large areas, under various atmospheric conditions. Indeed, radar is a measuring system that makes it possible to observe terrestrial areas regardless of the weather conditions due to the penetration of electromagnetic waves through the cloud cover (Bouchemakh et *al*., 2011). In addition, we used end-of-drilling production flow data from nearly 100 boreholes in a village hydraulics programme to study the relationship between productivity and fracturing extracted from satellite images.

Table 1 : Some characteristics of the RADAR scene

Characteristics	
System	RADARSAT-1
Wave length	5,66 cm
Frequency	5,3 GHz
Spectral Band	3,75-7,5cm
Spatiale resolution	50m

3.2. Method

3.2.1. Method of major fractures determination

Lineament processing and extraction was performed using ENVI 4.3 (Environment for visualising image) software. Several spatial filters were used to enhance the radar image and plot the structural lineaments, these were, i) the modified 5x5 Laplacian filter. This is a non-directional filter that enhances the contours of the image components, ii) the 7×7 Sobel filter (assigned with weight 6) in the N-S, E-W, NE-SW and NW-SE directions. This filter shows the lineaments perpendicular to their convolution direction (Figure 5). On the processed images, the discontinuities-images corresponding to supposed structural lineaments were recorded manually, following a visual analysis on the screen. This consisted of representing by a right segment the discontinuities-images and abrupt changes in tone observed on the images. All these extraction operations have resulted in a detailed linear map of the study area.We have extracted the major lineaments map taking into account lineaments larger than 12 km. Lineament validation is carried out on the basis of fracture data measured at outcrops at various sites and fracture data taken from geological maps.

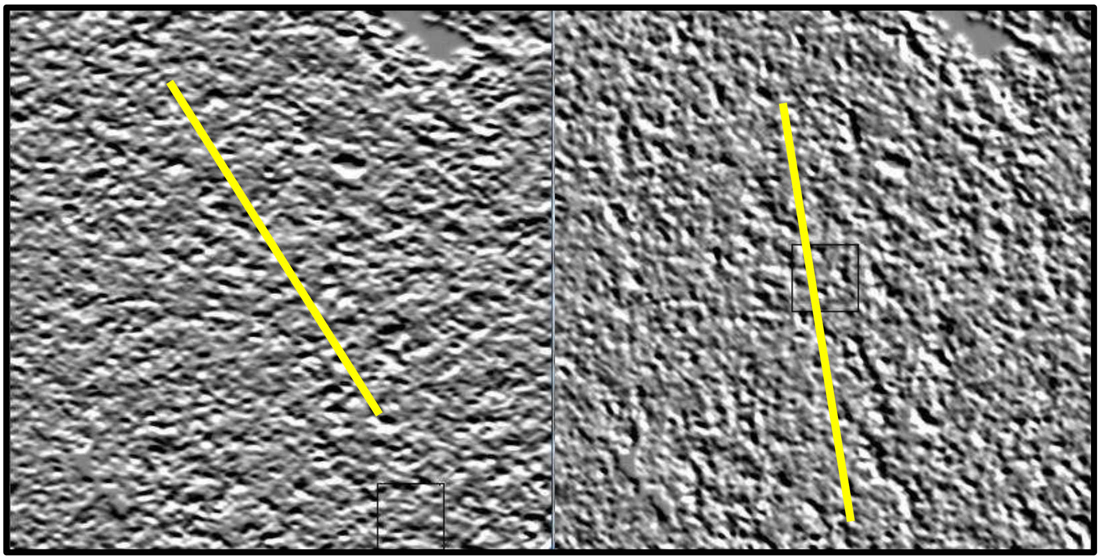

Fig.4. Radar image enhanced with Sobel filter (7 x 7) showing fractures

3.2.2. Calculation method of production flows

The production flows exploited were obtained during short-term test pumping, also known as well testing on isolated boreholes with multiple flow bearings. The minimum number of bearings required to achieve satisfactory results is 3 and the purpose of these tests is to determine the characteristics of the well to deduce the maximum performance of the structure (CIEH, 1978). The tests were carried out by successive short-term pumping with increasing flow rates. Borehole flow rates were measured at capacity for a given filling time, the time being measured using a stopwatch or a handle watch (volumetric method or low-flow measurement method per tank). When the capacity is of sufficient volume and the water is well channelled, the accuracy of the flow measurement is satisfactory (Faillat, 1986). The following formula is then used:

$$Q = \frac{v}{t}$$

3.2.2. Study of relationship between major fractures and productivity of boreholes

The analysis of the relationship between the productivity of the works realized and the lineaments was carried out from different maps. Spatial interpolation flow maps were generated from GIS software, ARCVIEW GIS 3.2 of Environmental Systems Research Institute, Inc, (1992-1999) and SURFER 9.0. These different maps were superimposed (overlayed) in the same plane with the spatial distribution of major fractures to assess the link between these different parameters. Statistical tests generated from Excel made it possible to analysis the influence of major accidents in the positioning of boreholes.

IV. RESULTS AND DISCUSSION

4.1. Results

4.1.1. Analysis of major fractures network

The fracture network map was used to extract large-extension fractures that we considered major fractures (multi-kilometric fractures). There are 323 lineaments identified. These major fractures account for more than 12% of overall fracturing and have spread across the entire study region, but have a relatively smaller distribution in the western sector, at the Tortiya level. Some major lineaments have boundaries that extend beyond the region (Figure 5). The analysis of the map also shows that their large size promotes their interconnection, we see nodes of two or more fractures, which is likely to facilitate the formation of good aquifers.

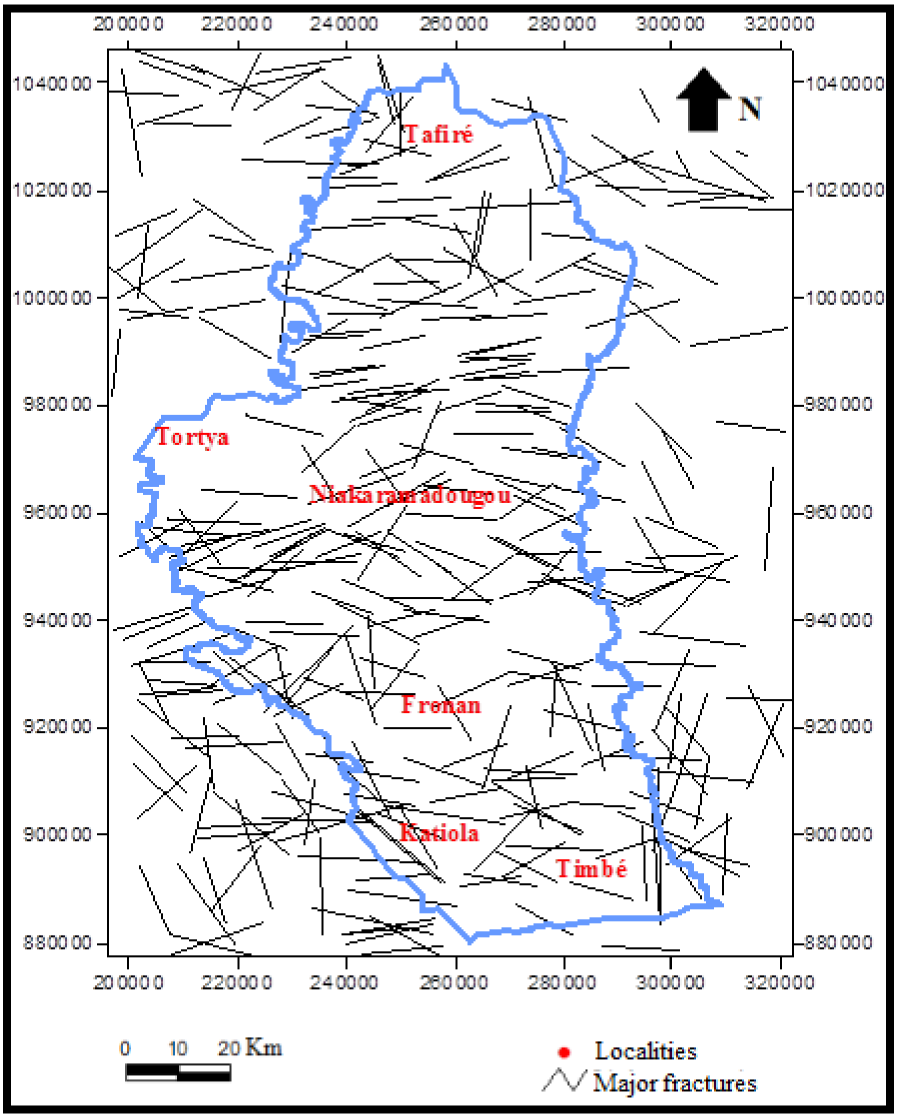

Fig.5. Lineaments major map of Katiola

4.1.2. Statistical analysis of major fractures length

Examination of the table of statistical elements of fracturing shows that the size of the lineaments varies from 12 to about 32 km with an average value of 15.13 km. The class distribution histogram in Figure 6 indicates that most major lineaments (50%) have lengths between 12 and 14 km. We also note that 20% have lengths between 14 and 16 km. The coefficient of variation value Cv (21%) confirms the low heterogeneity in the distribution of major fracture lengths.

Table II : Statistical parameters of major fractures

Parameters	Number	Average	Minimum	Maximum	Standard deviation
Major fractures length (km)	323	15,13	12	31,86	3,23

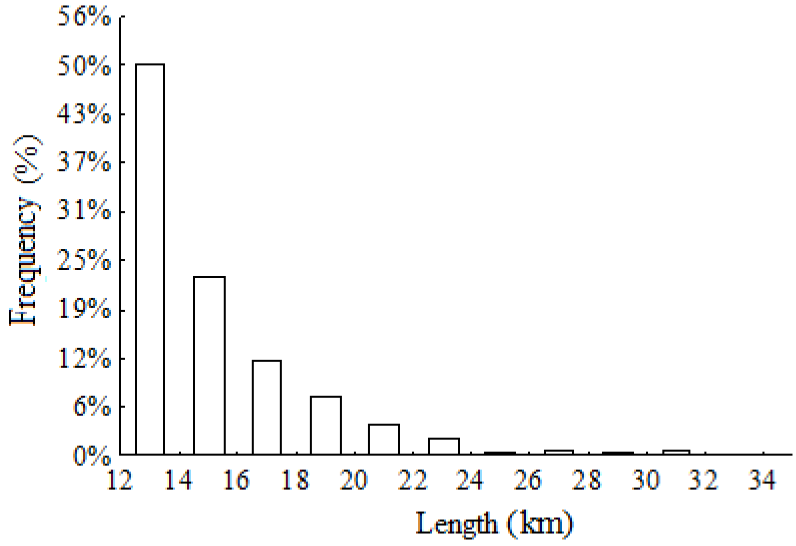

Fig.6. histogram of major lineaments length distribution

3.1.2. Orientation of major fractures distribution

The distribution diagram of major lineaments orientations shows a variability of mega-fracture directional classes (% of frequencies per class of 10%). The N90-100 directional class is the main one with 14%, followed by the N70-80, N100-110 and N80-90 classes that stand out from the others (Figure 7). There are the directions of secondary mega-fractures. The other classes are minority with frequencies sometimes below 5%. Thus, the N30-40 and N40-50 classes are the least represented with respectively 2 and 3% frequency.

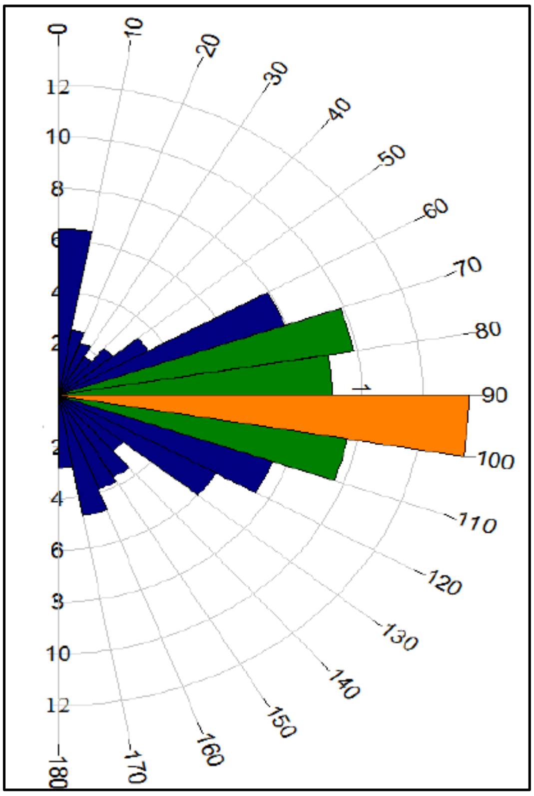

Fig.7. Rose diagram in number of major fractures

3.1.3. Spatial distribution of boreholes flow

Figure 8 represent the spatial distribution map of borehole production flows in Katiola region. Analysis of this map shows any spatialization of the borehole flow classes. The analysis of this map shows any spatialization of the borehole flow classes. We also noted that low flows are spread throughout the region. Medium and high flows are found mainly in the central and southern part of the region. Medium and high flows are found mainly in the central and southern part of the region.

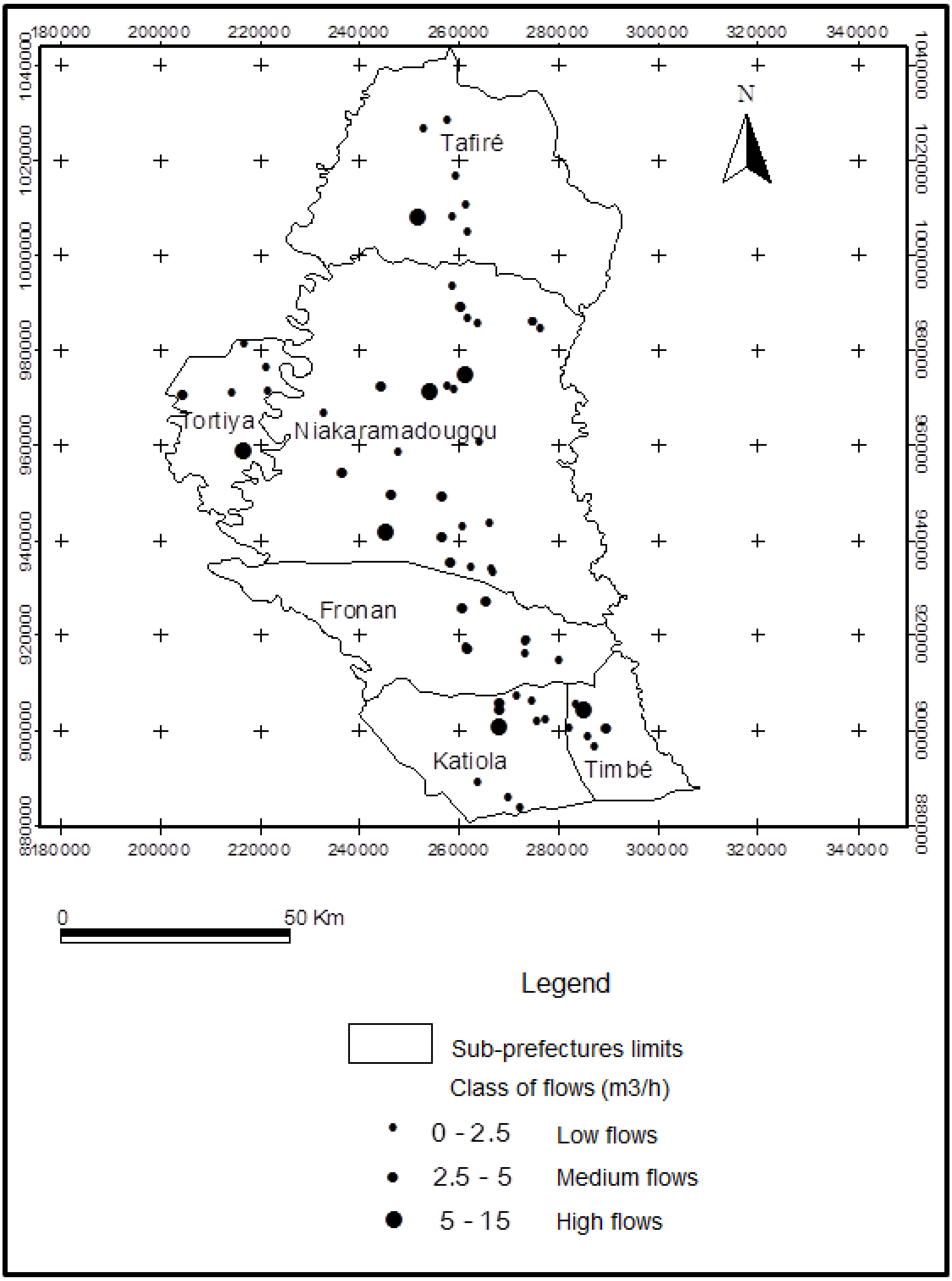

Fig.8. Distribution map of flow classes

3.1.4. Analysis of spatial distribution map of boreholes flow and major fractures

Analysis of the spatial distribution map (Figure 9) indicates that the boreholes with low and medium flow do not overlap with major fractures. Moreover, they are unlikely to have any particular organization in comparison with them. This may account for the high proportion of low-flow boreholes in the study area. However, the map show a few boreholes located either near these major accidents or intersecting major and interconnected fractures (the northern and southern part of the region). It should be noted that these boreholes offer the most interesting flows in this category.

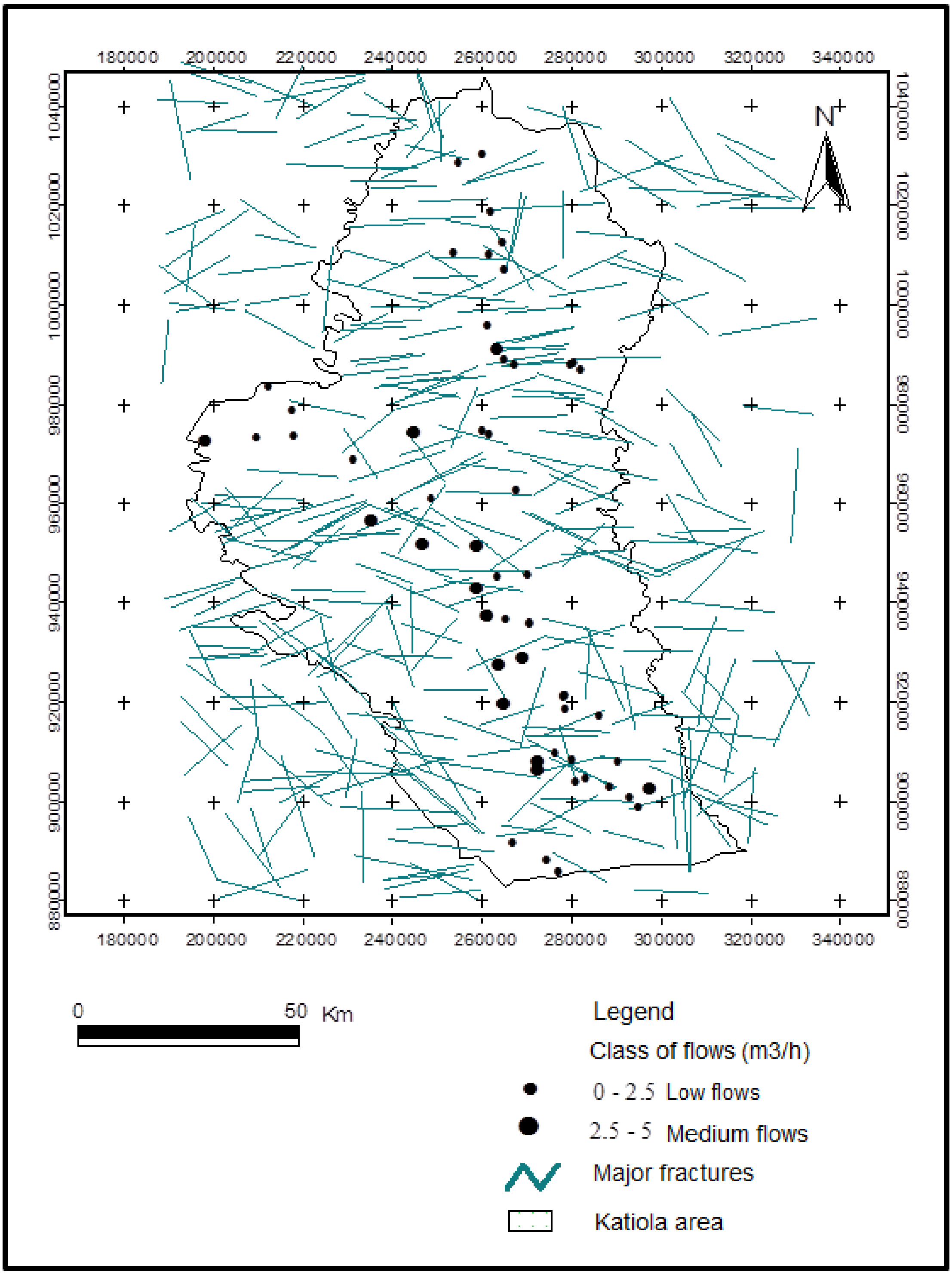

Fig.9. Overlay map of low and medium flows and major fractures

3.1.5. Analysis of spatial distribution map of boreholes high flow and major fractures

Analysis of the spatial overlay map of the high flows with the mega fractures reveals an interesting finding. All high boreholes flow are overlap or very close to major fractures and most often interconnected (figure 10). The Kafiné point (245839.7: 941122) in the figure with the largest flow (14.5 m^3/h) is quite illustrative, as is Kolokaha (252202.19:1006993) with (9.5 m^3/h) and Zanakaha (217239.19: 957737.19) with (9.2 m^3/h). All this confirms the predominant role of open fractures and their connectivity in the formation of good aquifers in the fractured basement of Katiola region.

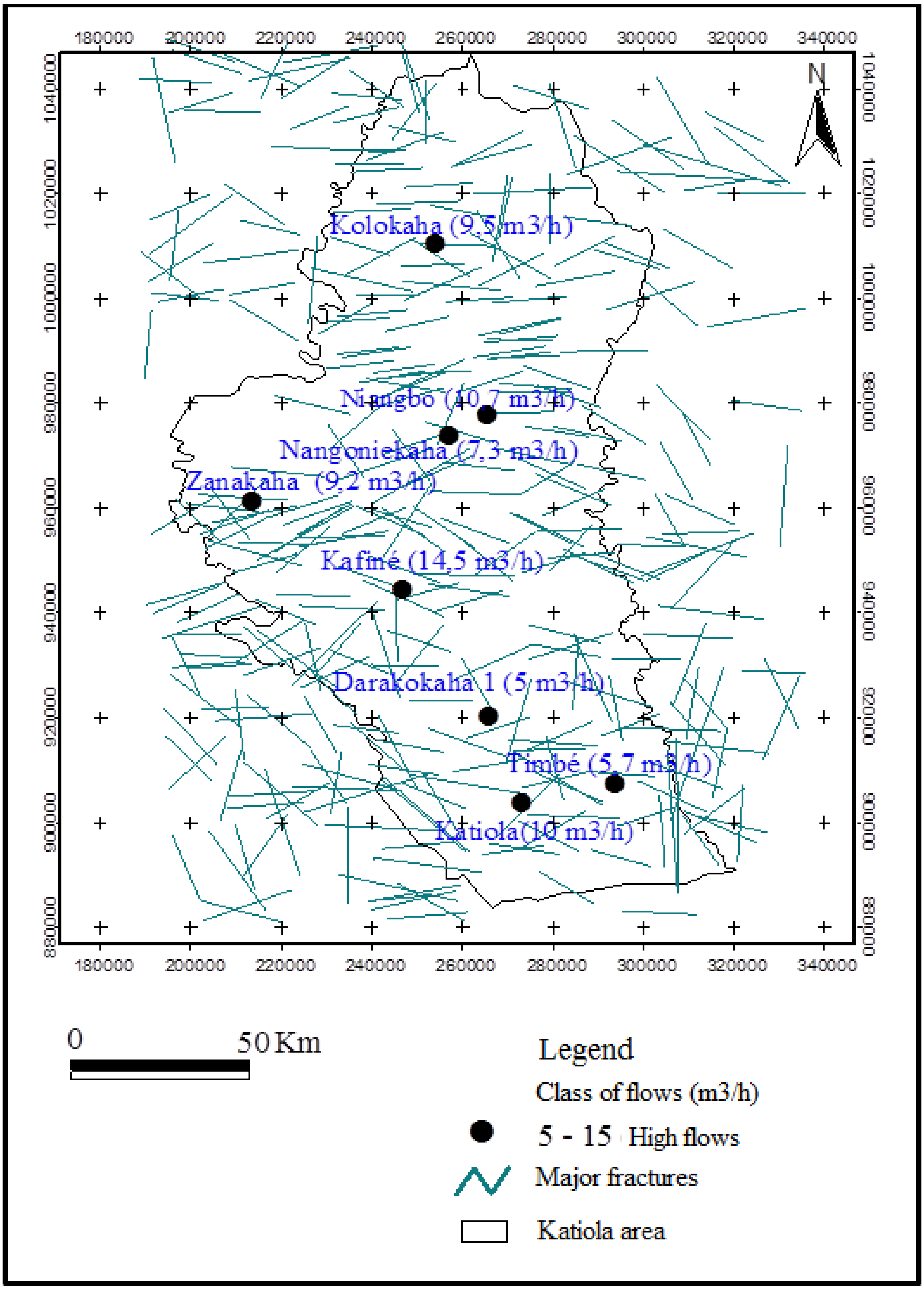

Fig.10. Overlay map of high flows and major fractures

3.1.6. Statistical analysis of the correlation between borehole flow rate and their distance from major lineaments

The statistical analysis of boreholes flows evolution (strong and low) in relation to their distances to nearest lineaments is given by the different graphs in the figure 11. It reveals a clear influence of the distance between boreholes and lineaments on the flow rate evolution. Indeed, we note that the highest proportion (88%) of the flows of structures in the right of a fracture (capturing directly a fracture) is located at the level of the high flows, come next the medium flows (31%) and the low flows (25%). However, at the level of boreholes relatively distant from major fractures, it is paradoxically, the low flow structures that take the lead with more than 75%, followed by medium flows (69%) and less than 10% for high throughputs. The flow therefore evolves in inverse proportion according to the distance between fractures and boreholes.

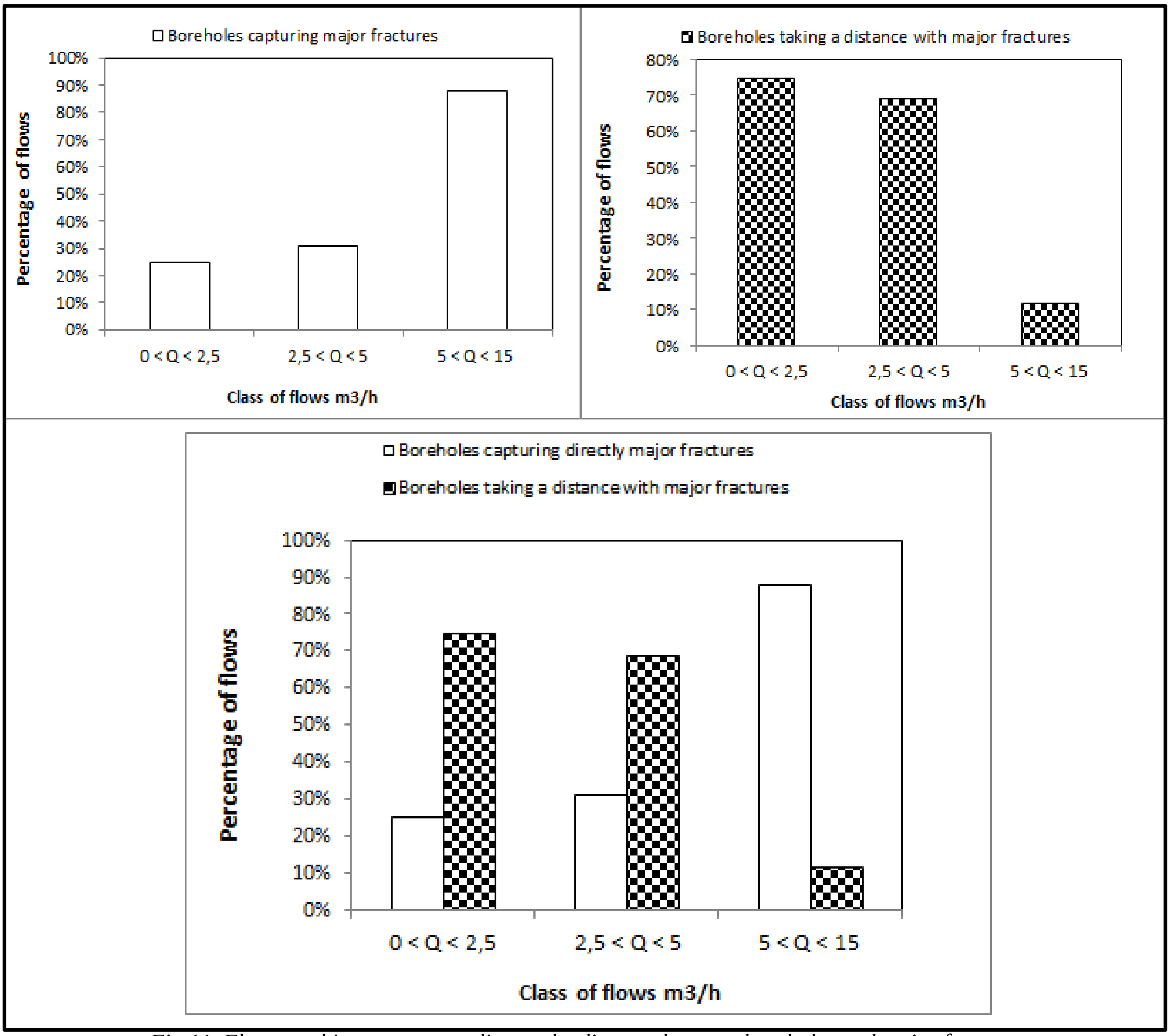

Fig.11. Flow rate histogram according to the distance between boreholes and major fractures

3.1.6. Regression of boreholes percentages according to their relative distance to major accidents

The study of the influence of the positioning of the fractures or their relative distances with the boreholes is completed by the curve of decay of the figure (12). The analysis of the figure corroborates the observations made earlier, namely the influence of the distance between the major lineaments and boreholes on the productivity of the structures. It allows you to make a few comments on the graph, which looks like 3 parts:

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- a straight line of ideal settlements located on the ordinate-axis representing 40% of the holes drilled that directly capture a fracture in the basement of the region. This is the category of drillings offering the best production flows;
- a rapid decay curve between 0 and 2000 m with a peak at 1000 m. It corresponds to the curve of the boreholes closest to the fractures with flow varying between 0.6 m^3/h and 10 m^3/h. This decrease indicates that the further one moves away from the fracture, more the probability of having a good borehole flow decreases. The 1000 m distance between boreholes and lineaments would represent the threshold not to be crossed;
- a relatively moderate decrease curve between 2000 and 8000, it corresponds to the most distant boreholes from fractures with 30% of the number wells. It is the class of boreholes offering the lowest flows in the region varying between 0.4 m^3 / h and 3.8 m^3 / h.

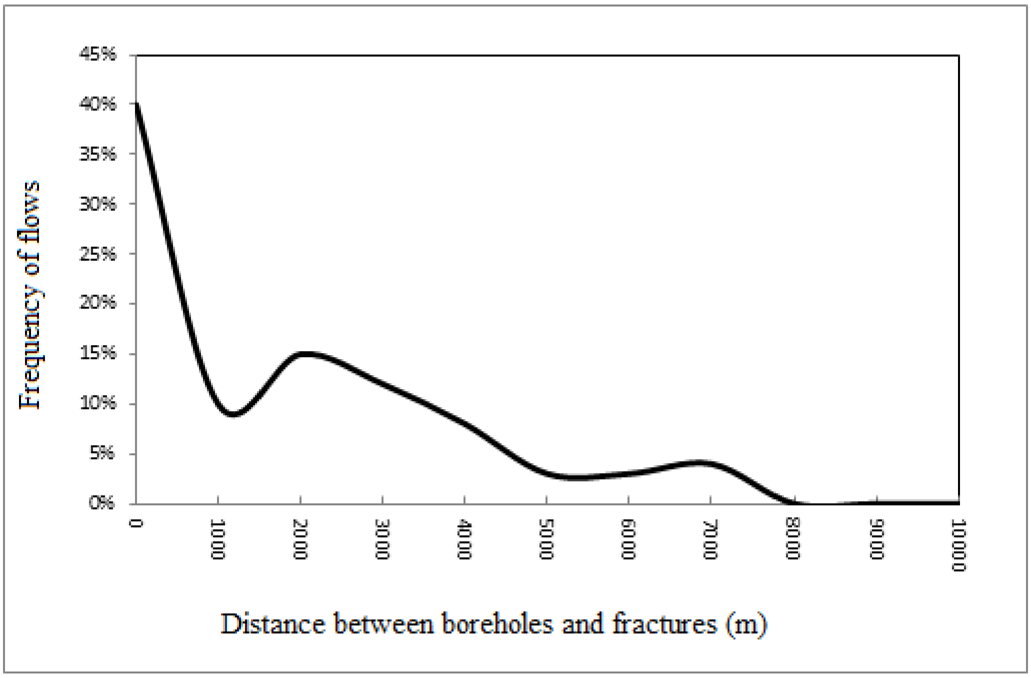

Fig.12. Flow rate histogram according to the distance between boreholes and major fractures

3.2. Discussion

Many works in recent decades have shown that to locate suitable well sites in the environment of the crystalline basement, it is necessary to use satellite images or aerial photography to locate and map fracture traces on the roof of the base (savané, 1997). In Katiola region, the major accidents which also constitute preferential groundwater flow corridors follow the following predominant directions: N90-100, followed by N70-80, N80-90 and N100-110. These main directions are more or less similar to those obtained by Jourda (2005) in Korhogo area and Biémi (1992) in Séguéla region, both close to the study area. The different preferential directions of tectonic accidents identified by their work are (N170-190; N30-40; N90-100; N120-130; N140-160; N60-80). It should be noted that, in general, the major lineaments detected are distributed according to the four main tectonic directions of Africa (N30-60; N80-100; N120-160; N170-190). The N90-100 class being the direction with the most major accidents with 14% of fractures, corresponds according to their work to fairly discrete accidents and not too visible in the field. According to Biémi (1992), these accidents are discontinuous in satellite images, probably due to the frequent influence of other break directions that cut them by shifting them.

Wells dug to the right of these fractures, or better still where two or more fractures intersect, generally provide a large flow of groundwater (Setzer, 1966; Siddiqui and Parizek, 1971). According to Kouadio et *al* (2008), the search for groundwater in basement environments is essentially based on the identification of fractures that are witnesses to tectonic deformations. Thus, a borehole that does not cross any fracture cannot produce water. The results of the analysis of the influence of major accidents on the positioning and productivity of boreholes in the Katiola region corroborate these assertions. It emerges

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from our work that the majority of low flow rates rarely exceeding 1 m^3/h are not located on megafractures, or are for some in the vicinity of such megafractures. For many authors, the boreholes drilled as part of national village hydraulics programmes are largely based on subjective investigations such as geomorphology to the detriment of advanced methods such as geophysics or remote sensing (Faillat, 1986; Biémi, 1992; Gombert, 1997; Saley, 2003; Jourda, 2005; N'Go et *al*., 2010, Mangoua et *al*., 2019). This observation partly justifies the low flows obtained or the very often high failure rate during the implementation of the structures, which can locally exceed 50% in discontinuous basement aquifers according to Gombert (1997). In katiola region, the spatial distribution of the flow classes shows a predominance of modest flows on a regional scale. Assemian et *al* (2014) find similar results in the Bongouanou basement. Moreover, according to Savané (1997), although the number of boreholes drilled during national hydraulic campaigns is significant, borehole flows generally remain low (1.5 to 2 m^3/h) for a drawdown equal to 12 m in the arenas, 5 to 1 m^3/h for a drawdown equal to 20 m in the Birrimian formations.

The overlaying map of major lineaments with the large flows gives quite evocative results. Most borehole points are located at the right of two or even several major fractures. This result corroborates the interest of well-filled and interconnected aquifer discontinuities in the search for large flows in fractured basement environments (Gombert, 1997). According to Engalenc (1979, 1981), one of the main criteria often used in the siting of boreholes in basement environments is: the site must be in a crossing of the longest, cleanest and, if possible, most numerous fractures. In crystalline rocks, the fracture zones and the fracture intersection point are usually the only sites where groundwater is entering. They form an intercalated network of high transmissivity and act as a conduit for groundwater from massive rocks in the interfracture domains (Savané, 1997). However, if the underlying bedrock is resistant to weathering and erosion, it may have several open fractures, but little groundwater can be captured by the well. Therefore, the expected productivity would be small. This could justify the lower air lift flows sometimes observed in some areas even though the well is implanted at the right of a major fracture (south and north of the region).

The results of our work also indicate that the flow rate is inversely proportional to the distance between major accidents and fractures, but not linearly. These results are similar to those of Baka et *al* (2019) in Oumé region, Koita et *al* (2010) region of Dimbokro-Bondoukou, Savané (1997) in Odienné region. All this confirms that these vertical or subvertical accidents of the basement have an influence on the hydrodynamic behaviour of the basement aquifers of the Katiola region.

V. CONCLUSION

This study established the link between the productivity of boreholes at the end of drilling and the discontinuities detected as lineaments in Katiola region. It thus appears from this work that the major preferential directions for groundwater circulation in the area are; N90-100, N70-80, N100-110 and N80-90. The major fractures detected are distributed according to the four main tectonic directions of Africa. Several boreholes with low flows $0 < Q < 2.5$ m^3/h) do not overlap with the major structural lineaments detected and seem to have been drilled using a simple geomorphological criteria, which would justify the low flows observed in most of the works carried out. All high flow boreholes ($Q \geq 5$ m^3/h) match and overlap with open and interconnected fractures. The distance between major accidents and the boreholes influences the productivity of the works carried out. These results indicate that fracture arrangement and degree interconnection play an important role in the storage and conduct properties of aquifer systems in the region. The approach used in this study may be an indirect means of validating the map of the major fractures network that can be used in future drilling campaigns in the Katiola region.

REFERENCES

[1] Assemien A.E., Kouamé K.F., Saley M.B., Affian K., Younta Ta M., Jourda J.P.R. et Biémi J. (2014). Étude de la productivité d'un aquifère de socle et approche statistique pour la détermination des tranches de profondeurs potentiellement productives : cas de la région de Bongouanou, est de la Côte d'Ivoire. Revue des sciences de l'eau / Journal of Water Science, 27 (1), pp. 81–97.

[2] Baka D., Gnamba F.M., Yao K.T. et Youan Ta M. (201). Influence des caractéristiques de l'aquifère sur la productivité des forages dans la région d'oumé (centre-ouest côte d'ivoire). Asian Journal of Science and Technology Vol. 10, Issue, 08, pp. 10005-10013.

[3] Bassolé A., J. Brunner et D. TUNSTALL (2001). SIG et appui à la planification et à la gestion de l'environnement en Afrique de l'Ouest. Rapport de synthèse, World Resources Institute, 48 p.

[4] Biémi J. (1992). Contribution à l'étude géologique, hydrogéologique et par télédétection des bassins versants subsaheliens du socle précambrien d'Afrique de l'Ouest : Hydrostructurale hydrodynamique, hydrochimique et isotopie des aquifères discontinus de sillons et aire granitique de la Haute Marahoué (Côte d'Ivoire). Thèse de

Doctorat ès Sciences Naturelles, Université d'Abidjan, Côte d'Ivoire 479 p.
[5] Biémi, J. (1997) Positionnement, productivité et gestion des forages en milieu fissuré de Côte d'Ivoire par télédétection et système d'information géographique. Atelier de la FAO sur Télédétection et ressources en eau, Montpellier. Séries Water Report 16. Available from http://www.fao.org/docrep/w7320b18.htm [Google Scholar]
[6] Bouchemakh L., Boutarfa S., Smara Y. et Souissi B. (2011). Sur le filtrage du chatoiement dans les images radar SAR polarimétriques : Etude des régions d'Alger et d'Oran. Revue Télédétection, 2011, vol. 10, pp. 113-127.
[7] CIEH (1978). Méthode d'étude et de recherche de l'eau souterraine des roches cristallines de l'Afrique de l'Ouest. Edition Géohydraulique, vol. 1, 190p.
[8] Dewandel B., P. Lachassagne, R. wyns, J.C. Maréchal et N.S. Krishnamurthy (2006). A generalized 3-D geological and hydrogeological conceptual model of granite aquifers controlled by single or multiphase weathering. J. Hydrol., 330, pp. 260– 284.
[9] Engalenc M. (1979). Méthode d'étude et de recherche de l'eau souterraine des roches cristallines de l'Afrique de l'Ouest Géohydraulique CIEH. 194 p.
[10] Engalenc M. (1981). Elément de quantification des ressources hydrauliques dans les roches cristallines. Bulletin B.R.G.M., Série II, Section III, n°. 4, pp. 325-326.
[11] Faillat J. P. (1986) « Aquifères fissurés en zone tropicale humide: structure, hydrodynamique et hydrochimie (Afrique de l'Ouest) », Thèse Université. Languedoc (Montpellier), 534 p.
[12] Faillat J.P. (1986 a). Hétérogénéité et effet d'échelle dans les aquifères fissurés. Approche par pompage d'essai sur station expérimentale (Afrique de l'Ouest). Hydrogéologie, n.° 1, pp. 65-76.
[13] Fernandes, A.J. et Rudolph, D.L., 2001. The influence of Cenozoic tectonics on the groundwater-production capacity of fractured zones: a case study in Sao Paulo, Brazil. Hydrogeol. J., 9: pp. 151-167.
[14] Gnamba F.M. (2014). Hydrochimie et hydrodynamisme des réservoirs de fissures du socle paléoprotérozoïque de la région de Katiola. Thèse de Doctorat, Université Félix Houphouët Boigny, Côte d'Ivoire, 200 p.
[15] Gnamba F.M., Oga Y.M.S., Gnagne T., Lasm.T., Biémi J. et Kouakou Y.K.N (2014). Analyse de la productivité des aquifères de fissures du socle paléoprotérozoîque de la région de Katiola : (Centre-Nord de la Côte d'Ivoire). European Scientific Journal February 2014 edition, vol.10, No.5, pp. 79-98.
[16] Gombert P. (1997). Variabilité spatiale de la productivité aquifère du socle sahélien en hydraulique rurale. Hard Rock Hydrosystems, IAHS Publ., Vol. 241, pp. 113-122.
[17] Jourda J.P.R. (2005). Méthodologie d'application des techniques de télédétection et des systèmes d'information géographique à l'étude des aquifères fissurés d'Afrique de l'Ouest. Concept de l'hydrotechnique spatiale : cas des zones tests de la Côte d'Ivoire. Thèse de Doctorat d'Etat, Université de Cocody-Abidjan, Côte d'Ivoire, 430p.
[18] Koita M., Jourde H., Ruelland D., Koffi K., Pistre S. et Savane I. (2010). Cartographie des accidents régionaux et identification de leur rôle dans l'hydrodynamique souterraine en zone de socle. Cas de la région de Dimbokro-Bongouanou (Côte d'Ivoire), Hydrological Sciences Journal, 55(5), pp. 805-820.
[19] Kouamé K.F. (1999). Hydrogéologie des aquifères discontinus de la région semimontagneuse de Man-Danané (Ouest de la Côte d'Ivoire). Apport des données des images satellitaires. Thèse de Doctorat, Université Cocody-Abidjan, Côte d'Ivoire ,194 p.
[20] Kouame K. F., Akaffou A. G., Lasm T., DE Dreuzy J. R., Davy P. et Bour O. (2005): Simulation des écoulements dans les réservoirs fracturés: Application au socle archéen de Touba (Nord-Ouest de la Côte d'Ivoire). Actes du Colloque Internationale SITIS 05 Yaoundé(Cameroun) 27 Nov.-1er Déc. 2005. pp. 39-46.
[21] Kouamé, K. F., Lasm, T., De Dreuzy, J. R., Akaffou, A. G., Bour, O. et Davy, P. (2010). Contribution d'un modèle hydrogéologique à fractures discrètes à l'étude des aquifères fracturés du socle Archéen de Touba (Nord-Ouest, Côte d'Ivoire). Revue des sciences de l'eau / Journal of Water Science, 23 (1), pp. 41–56.
[22] Larsson I., 1972. Ground water in granite rocks and tectonic models. Nordic Hydrology, 3: pp. 111-129.
[23] Lasm T., Fossou R.M.N, Onetie O.Z., Baka D., Youan TA M., Marie Solange OGA M.S. et SORO N. (2012). Contribution hydrogéologique à la connaissance des aquifères discontinus du département de ferké (nord de la côte d'ivoire) pour une meilleure alimentation en eau potable. Rev. Ivoir. Sci. Technol., 19 (2012) pp.114 – 135.
[24] Mangoua M.J., Yao A.B., Douagui G.A., Kouassi K.A., Goula B.T.A. et BIEMI J. (2019). Evaluation des potentialités en eau souterraines des aquifères fissurés dans le bassin versant du Bandama (Côte d'Ivoire), Larhyss Journal, n°37, pp. 53-74.
[25] Maréchal J.C., B. Dewandel, S. Ahmed, L. Galeazzi et F.K. Zaidi (2006). Combined estimation of specific yield and natural recharge in a semi-arid groundwater basin with irrigated agriculture. *J. Hydrol*., 329, pp. 281-293.
[26] Naik, P.K., Awasthi, A.K., Anand, A.V.S.S., Mohan, P.C., 2001. Hydrogeologic framework of the Deccan terrain of the Koyna River basin, India. Hydrogeol. J., 9(3): pp. 243-264.
[27] Neves, M.A., Morales, N., 2007. Structural control over well productivity in the Jundiaí River catchment, Southeastern Brazil. Anais da Academia Brasileira de Ciências, 79(2): pp. 307-320.
[28] N'Go Y.A., Lasm T., Koita M. et Savane I. (2010). Extraction par télédétection des réseaux de fractures majeures du socle précambrien de la région de Dimbokro (Centre Est de la Côte d'Ivoire). Télédétection, Vol. 9, N°1, pp. 33-42.
[29] RGPH. (2014). Récensement Générale de la population et de l'Habitat 2014, Résultats Globaux, 22 p.

[30] Roques C. (2013). Hydrogéologie des zones de faille du socle cristallin : implications en terme de ressources en eau pour le Massif Armoricain. Thèse de Doctorat ès Sciences Naturelles, Université de Rennes, France, 238 p.
[31] Saley M.B. (2003). Système d'informations à référence spatiale, discontinuités pseudoimages et cartographies thématiques des ressources en eau de la région semi-montagneuse de Man (Ouest de la Côte d'Ivoire). Thèse unique de doctorat, Université de Cocody, 209 p.
[32] Savané I. (1997). Contribution à l'étude géologique et hydrogéologique des aquifères discontinus du socle cristallin d'Odienné (Nord-Ouest de la Côte d'Ivoire). Apports de la télédétection et d'un Système d'Information Hydrogéologique à Référence Spatiale. Thèse d'État, Univ. Abidjan, Côte d'Ivoire, 386 p.
[33] Setzer l, 1966. Hydrologic significance of tectonic fractures detectable on air photo. *Groundwater.* Vol. 4 pp 23-29.
[34] Siddiqui S.H., Parizek R.R., 1971. Hydrologie foctors influencing well yields in folded and faulted carbonate rock in central pennsylvania. *Water Resources Research*, Vol. 7 N° 5, pp 1295-1312.
[35] Wyns R., Baltassat J-M., Lachassagne P., Legchenko A., Vairon J. and Mathieu F. (2004). Application of SNMR sounding for groundwater reserves mapping in weathered basement rocks (Brittany, France). Bulletin Société Géologique France, Vol. 175, N°1, pp. 21-34.

Evaluation and Participatory Selection of Promising Sweetpotato F_1 Genotypes in Uganda

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***Abstract*— *Most of the important sweetpotato (Ipomoea batatas (L.) Lam) traits are sensitive to environmental change. This necessitates evaluating new sweetpotato genotypes in different environments to identify those that are stable. To enhance adoption, the new sweetpotato genotypes should have farmer preferred traits thus the need for farmer involvement during selection. This study was conducted to: evaluate and select promising sweetpotato F_1 genotypes with wide and specific adaptation, in association with performance for farmer preferred traits. Twenty-one promising sweetpotato F_1 genotypes were evaluated at Namulonge, Serere and Kachwekano with Tanzania and NASPOT 1 as checks. The randomised complete block design with three replications was used. Scientists and farmers evaluated the agronomic performance and quality traits of the genotypes before and at harvest. Significantly ($P<0.05$) higher mean total storage root yield (TRY) of 25.5 t ha^{-1} was recorded at Namulonge than at Kachwekano and Serere. Genotypes G67, G13, G14, G24 and G29 were the most stable across the sites for TRY and therefore the most widely adapted for this trait, while G68, G60 and G58 were specifically adapted to Kachwekano and Serere. Very low severity levels of Sweetpotato virus disease (SPVD) were recorded with a mean score of 1.9 across sites with Namulonge having the highest mean score of 2.3. Genotypes G14, G16, G24, G29 and G49 were the most stable across the sites for low Alternaria blight severity and can therefore, be recommended for cultivation in both low and high disease pressure areas. In the participatory selection, before harvest and at harvest, Spearman's rank correlation (r = 0.44) of the scientists and farmers' mean ranking of the genotypes at each site was positive and significant. Thus scientists were capable of selecting for farmer preferred traits. In addition, the study identified and selected five superior genotypes including G13, G14, G24, G49 and G69 for further evaluation.*

Keywords*— *Promising F_1 genotypes, Alternaria blight, farmer preferred traits, agronomic performance, and selection.

I. INTRODUCTION

Plant breeders desire stable genotypes with good performance under all conditions within the target production regions. Stable genotypes with high yield potential can only be identified by testing them in a series of environments (Martin et al., 1988) and it is always important to test them in environments which reveal their maximum genetic potential in terms of the traits under consideration (Frey, 1964). The major objective of any crop improvement programme is the development of cultivars with high yield potential and other desirable traits, and the ability to withstand seasonal fluctuations over a wide range of environments (Kamalam et al., 1978; Lebot, 2009). Most of the important sweetpotato traits, including yield, are strongly affected by environmental conditions associated with sites and years (Ngeve, 1993; Niringiye et al., 2014). In most cases, high yielding genotypes are not yield stable and those that are yield stable are low yielding (Ngeve, 1993; Manrique and Hermann, 2000; Mwanga et al., 2006). However, breeding sweetpotato for high yield and wide adaptation is possible (Grüneberg et al., 2005). In sweetpotato, attention needs to be paid to testing in low-yielding marginal environments if farmers working in such environments are the main beneficiaries of the new cultivars. Hence, yield testing in early stages of a sweetpotato breeding program should use at least one favourable environment and one less favourable environment (Grüneberg et al., 2005).

In Uganda, the National Sweetpotato Program released 22 sweetpotato cultivars between 1995 and 2013 and all these releases were made after conducting on-station, on-farm and standard multi-locational yield trials focusing mainly on high yield, high dry mass and resistance to pests and diseases (Mwanga et al., 2011; Ssemakula et al., 2013; Mwanga et al., 2016). In 2017, five new varieties namely NAROSPOT 1, NAROSPOT 2, NAROSPOT 3, NAROSPOT 4, NAROSPOT 5, were released (MAAIF, 2018) to bring the total of released varieties to 27. Of all these released cultivars, only one, NASPOT 11 had been bred through a participatory plant breeding process (Mwanga et al., 2011) but efforts were made to incorporate farmer preferred attributes in the other cultivars. Despite releasing all these cultivars, farmers still demand new ones to meet their ever changing preferences and some of the cultivars for example NASPOT 2, NASPOT 5 and Sowola 6 have not been well received (Abidin et al., 2002). For this reason, many farmers have continued to cultivate their landraces which underscores the need to involve them in participatory cultivar selection so that their preferences are considered. Participatory cultivar selection, and participatory plant breeding (PPB), are considered the most appropriate strategies to develop cultivars for marginal agricultural systems (Almekinders and Elings 2001; Ceccarelli and Grando, 2006; Dawson et al., 2007). This approach allows incorporation of farmers' knowledge, identification of farmers' selection criteria and priorities. Participation of farmers can allow for exploitation of specific adaptation effects within sites and facilitate seed supply to farmers (Ceccarelli et al., 2000).

Evaluation by farmers helps scientists to design, test and recommend new technologies in light of information about farmers' requirements and needs. It facilitates close interaction among farmers, researchers and other role players in crop genetic improvement, allowing researchers to respond more closely to the needs and preferences of resource-poor farmers and their market clients (Sperling et al., 2001). Farmers can be involved through introduction, evaluation and selection of materials grown on the research station and also collaborate by growing and selecting breeding materials in their own fields (Ceccarelli et al., 2000; Mcharo et al., 2001; Keith et al., 2004). The cultivars obtained from this process are developed more rapidly, are more diverse and have higher adoption rates (Witcombe et al., 2003). Consideration of farmers' concerns and conditions leads to varieties that become widely adapted and more productive hence leading to sustainable agricultural systems (Odendo et al., 2002). Among the preferred attributes that farmers select for during PPB for sweetpotato are good yield, sweet taste and dry texture (Laurie and Magoro, 2008; Marti, 2003; Kwach et al., 2010; Sseruwu et al., 2015).

Twenty one promising F_1 genotypes previously selected from early breeding trials were used in this study which was carried out to identify superior genotypes as possible candidates for advanced yield and on-farm trials. The main objective of this study was therefore to evaluate and identify genotypes with wide and specific stable performance over three sites for Alternaria leaf petiole and stem blight (commonly referred to as Alternaria blight) resistance, total storage root yield (TRY), Sweetpotato virus disease (SPVD) and other farmer preferred traits.

II. MATERIALS AND METHODS

Genotypes and sites

Twenty one F_1 genotypes and two checks Tanzania and NASPOT 1 were planted at three sites during the first rain season of 2015 (2015A). The first site was the National Crops Resources Research Institute (NaCRRI), at Namulonge (0˚32' N, 32˚35' E; 1150 metres above sea level (masl)). The second site was Kachwekano Zonal Agricultural Research and Development Institute (KAZARDI) (01˚16'S, 29˚57'E; 2200 masl) and the third site was at the National Semi-Arid Resources Research Institute at Serere (NaSARRI) (1˚32'N, 33˚27'E; 1140 masl). A randomized complete block design with three replications was used for the trial at the three sites with each plot measuring 5 m long with four ridges spaced 1 m apart. On each ridge, seventeen vine-tip cuttings were planted at a spacing of 0.3 m thus a total of 68 cuttings per plot. No artificial inoculation with

Alternaria pathogens or SPVD virus was done thus all disease infection was by natural spread.

Data Collection

Disease rating

Rating for Alternaria blight and SPVD was conducted at monthly intervals from one month after planting (MAP) until four data sets were obtained. Alternaria blight rating was done using a subjective visual scale of 0 to 5 modified after van Bruggen (1984), where: 0 = no disease; 1 = <1%; 2 = 1 to 10%; 3 = 11 to 25%; 4 = 26 to 50%; and 5 = > 50% foliar infection. The disease severity scores were expressed on a plot mean basis. Disease severity data was used to calculate the Area Under Disease Progress Curve (AUDPC) according to Shaner and Finney (1977).

Rating for SPVD was also done using the subjective 1 to 9 severity rating scale of Grüneberg et al. (2010), where: 1 indicated no virus symptoms; 2 = unclear virus symptoms; 3 = clear virus symptoms at < 5% of plants per plot; 4 = clear virus symptoms at 6 to 15% of plants per plot; 5 = clear virus symptoms at 16 to 33% of plants per plot; 6 = clear virus symptoms at 34 to 66% of plants per plot (more than 1/3, less than 2/3); 7 = clear virus symptoms at 67 to 99% of plants per plot (2/3 to almost all); 8 = clear virus symptoms at all plants per plot (not stunted); 9 = severe virus symptoms in all plants per plot (stunted).

Storage root yield

At harvest the total storage root yield (TRY) was recorded on a per plot basis then the mass per plot was converted to t ha^{-1} for analysis.

Participatory selection data collection

In addition to collecting disease and agronomic data, participatory selection of the F_1 genotypes was also performed at two of the three sites namely; Namulonge (NaCRRI) and Kachwekano (KAZARDI). The genotypes were separately evaluated before harvest and at harvest by a group of five scientists and a group of 10 farmers (five males and five females) at each site. The groups of scientists and farmers at both sites were different. The five scientists at NaCRRI, and five scientists at KAZARDI, had a minimum qualification of a Bachelor's Degree in Agricultural Sciences and were employed by the National Agricultural Research Organisation (NARO). The selected farmers were knowledgeable about sweetpotato production and consumer preferences. At each site, the evaluation before harvest was carried out two days before harvesting the trial and it was preceded by familiarising both groups at each site with the selection procedure and criteria. Both groups used the same evaluation criteria and the traits considered were: Alternaria blight and SPVD severity; growth habit (spreading, erect); leaf morphological traits (broad, small leaves, leaf colour); and general acceptability as a new cultivar (i.e. whether each participant considered the genotype suitable to become a cultivar). A rating scale of 1-5 was used for all the traits. For diseases, a severely infected genotype was scored 1 and a symptomless genotype, 5. For leaf morphology traits, leaf colour and size were used, and for growth habit, a genotype with poor growth habit was scored 1 and excellent growth habit was scored 5.

For selection at harvest, the two groups at each site separately listed the traits that they wanted to use in the evaluation process and ranked them in order of importance. On this basis, each group developed a list of top five traits for scoring the genotypes (Table 1).

Table 1 Attributes used by scientists and farmers at harvest for the participatory selection process at Namulonge and Kachwekano

Namulonge scientists		**Namulonge farmers**		**Kachwekano scientists**		**Kachwekano farmers**	
Attribute	**Rank**	**Attribute**	**Rank**	**Attribute**	**Rank**	**Attribute**	**Rank**
High root yield	1	High root yield	1	High root yield	1	High root yield	1
Root size (big roots)	2	No weevil damage	2	No weevil damage	2	Root size (big roots)	2
Root shape	3	Root size (big roots)	3	Root size (big roots)	3	Root shape	3

Root number	4	Skin colour (red/cream)	4	Shape of roots	4	Straight roots	4
Skin colour (red)	5	Long straight roots	5	Root skin colour	5	No cracking	5
No surface defects	6	Root flesh colour (white)	6	No cracking	6		
Root flesh colour	7	Sap content	7	Root flesh colour	7		
Sap content	8	No cracking	8				

For each trait, the participants individually scored each harvested plot in all three replications on a scale of 1-5 where 1= trait absent and 5 = the genotype expressed the trait at a satisfactory level. Then the mean score for each trait was separately determined for each of the two groups per site. Roots were sampled from each plot of each genotype, boiled, taste tested and then scored for the following attributes: appearance of the flesh after cooking, sweetness, dry mass (hardness), fibre content and acceptability as a new cultivar. The same rating scale of 1-5 was used as mentioned above.

Data analysis

AMMI analysis of the data for the three sites

The genotype x environment interaction (GEI) and associated stability of the genotypes across three sites for area under the disease progress curve (AUDPC) for Alternaria blight severity scores, SPVD severity scores and TRY were analysed using the additive main effects and multiplicative interaction (AMMI) procedure in GENSTAT version 14 (Payne et al., 2011) based on the standard AMMI model (Gauch and Zobel, 1996).

The AMMI analysis partitions the GEI sum of squares (SS) into IPCA axes. Only IPCA1 and IPCA2 were significant and the non-significant IPCA3 was considered as "statistical noise" and accounted for by the residual term. The interaction patterns of the genotypes and the environments were graphically represented in a biplot of the respective IPCA1 scores (y-axis) versus the genotype and environmental means or IPCA2 (x-axis). In the biplot, displacement in the horizontal plane reflects differences in the mean performance, while displacement in the vertical plane reflects differences in interaction effects (Zobel et al., 1988).

Analysis of participatory selection data

The scores for all traits for each genotype at each of the two sites for each group were analysed by ANOVA in GENSTAT version 14 to obtain the mean scores for each trait per genotype, evaluation group and site. Weights were assigned to each scored trait such that the trait ranked first by a group was assigned a weight of 5 and the one ranked fifth was assigned a weight of 1. For each genotype, the mean score for each trait was multiplied by the assigned weight then all five weighted scores were summed up to obtain an aggregate score for each genotype.

Aggregate weighting index used for the both the scientist and farmer groups:

$\sum ATW = (AT_1 * W_5) + (AT_2 * W_4) + (AT_3 * W_3) + (AT_4 * W_2) + (AT_5 * W_1)$

Where: $AT_{1…5}$ = Attributes ranked 1…5; and $W_5…W_1$= assigned weight ranging from 5 to 1.

The aggregate scores of the genotypes at each site for each group were ranked to determine two separate rank orders (one per group) of the genotypes at each site. The ranks for each genotype per group were summed across the two sites (Kang, 1993) and the genotype with the lowest rank sum was the best over the two sites.

III. RESULTS

Genotype x environment interaction and stability of the genotypes

Alternaria blight

The genotypes, environments and genotype x environment interaction (GEI) mean squares (MS) were highly significant ($P<0.001$) for Alternaria blight AUDPC (Table 2). The genotypes, environments and GEI accounted for 16.4, 24.5

and 21.8% of the total SS for AUDPC. Only IPCA1 was significant and accounted for 72.0% of the GEI SS. The genotype G14 had the smallest IPCA1 score of 0.00525 and was therefore the most stable (in terms of the interaction pattern captured by IPCA1) for Alternaria blight (Table 3). Genotype G28 with an IPCA1 value of -3.41636 was the least stable. NASPOT 1 (susceptible check) with the highest mean Alternaria blight AUDPC value of 86.7 across the three sites was more susceptible than all the F_1 genotypes evaluated. Tanzania (resistant check) was more resistant than any of the F_1 genotypes with the lowest mean Alternaria blight AUDPC value of 46.1 across the three sites. Across the sites, G49, G13, G67, G14 and G65 had the lowest Alternaria blight AUDPC values of 46.6, 48.7, 48.7, 49.1 and 51.1, respectively. Genotype G58 had the highest mean Alternaria blight AUDPC value of 79.8 among the genotypes. Of the three sites, genotypes at Kachwekano recorded the highest Alternaria blight severity with an average AUDPC value of 76.6 (Table 3).

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Table 2 AMMI analysis for Alternaria blight severity, sweetpotato virus disease severity score and total storage root yield for 23 sweetpotato genotypes evaluated at Namulonge, Kachwekano and Serere

		Alternaria AUDPC				SPVD				Total storage root yield (t ha^{-1})			
Source of variation	**df**	**SS**	**MS**	**% Total SS**	**% G x E SS**	**SS**	**MS**	**% Total SS**	**% G x E SS**	**SS**	**MS**	**% Total SS**	**% G x E SS**
Total	206	126650	615			262.1	1.27			13574	65.9		
Treatments	68	79424	1168***	62.7		107.4	1.58*	41.0		10535	154.9***	77.6	
Genotypes	22	20809	946***	16.4		27.6	1.26	10.5		1312	59.7***	9.7	
Environments	2	31049	15525***	24.5		20.9	10.45***	8.0		6489	3244.7***	47.8	
Interaction	44	27566	626**	21.8		58.9	1.34	22.5		2734	62.1***	20.1	
IPCA1	23	19857	863***		72.0	39.9	1.73		67.8	1716	74.6***		62.8
IPCA2	21	7709	367		28.0	19.0	0.90		32.2	1018	48.5***		37.2
Error	132	46789	354			148.2	1.12			2701	20.5		

* = significant at 0.05; ** = significant at P<0.01; *** = significant at P<0.001; AUDPC = area under disease progress curve for Alternaria blight severity; SPVD = sweetpotato virus disease severity scores (scores 1-9 used; 1 = no SPVD and 9 = SPVD causing stunted growth); df = degrees of freedom; SS = sum of squares; MS = mean square; % Total SS = percentage of total sum of squares; % G x E SS = percentage of genotype x site sum of squares

Table 3 Mean AMMI performance estimates and ranking of the genotypes for Alternaria blight severity at Namulonge, Kachwekano and Serere

Genotype	Overall mean AUDPC	IPCA1	IPCA2	Namulonge		Kachwekano		Serere	
				Mean	Rank	Mean	Rank	Mean	Rank
G8	67.1	-0.39336	1.83402	48.3	8	90.1	18	63.0	20
G13	48.7	2.46564	-1.92151	56.3	18	45.5	1	44.3	9
G14	49.1	0.00525	-1.71079	51.0	10	63.7	7	32.7	1
G16	58.7	-0.18533	-1.51633	59.1	20	74.9	13	42.0	8
G21	67.8	-2.50861	-0.17353	56.4	19	102.6	19	44.3	10
G24	58.8	0.77041	1.21857	45.6	5	72.6	10	58.3	18
G28	63.3	-3.41636	-0.77916	53.7	16	103.6	20	32.7	1
G29	51.8	0.50558	-1.29120	52.2	13	63.5	6	39.7	5
G30	56.4	-1.43335	0.07224	45.6	6	84.0	16	39.7	5
G38	60.1	2.02679	0.42608	53.7	17	63.7	8	63.0	20
G49	46.6	-0.92678	1.82993	26.8	1	73.3	12	39.7	5
G53	57.7	-1.88434	-0.80294	51.0	11	87.1	17	35.0	3
G58	79.8	-2.32293	-2.78913	83.4	23	109.2	22	46.7	12
G59	57.7	-0.94515	1.41543	40.3	4	83.9	15	49.0	13
G60	63.6	1.79363	-1.85431	69.6	22	65.3	9	56.0	16
G61	54.4	2.31667	-0.01823	51.0	12	55.2	4	57.0	17
G65	51.1	3.15645	0.61656	45.6	7	46.9	2	60.7	19
G67	48.7	1.31573	1.03116	37.6	2	58.3	5	50.3	14
G68	68.8	2.33462	2.15257	53.2	14	72.9	11	80.3	23
G69	55.6	-0.32871	1.71497	37.6	3	77.9	14	51.3	15
G79	66.2	-2.99241	0.82728	48.3	9	106.0	21	44.3	11
NASPOT 1	86.7	-1.16207	1.79274	66.7	21	115.0	23	78.3	22
Tanzania	46.1	1.80864	-2.07441	53.3	15	47.3	3	37.7	4
Mean	**59.3**			**51.6**		**76.6**		**49.8**	

AUDPC = area under disease progress curve for Alternaria blight severity; IPCA = Interaction principal component axes

In the AMMI biplot of IPCA1 versus AUDPC mean values for genotypes and environments (Figure 1), genotypes on the right hand side of the vertical line were the most susceptible to Alternaria blight and those on the left were the most resistant. Genotypes closest to the horizontal line were more stable for the expression of Alternaria blight across the three sites. Genotypes G8 and NASPOT 1 were stable for the disease but they had above average AUDPC values. Genotypes G14, G16, G24, G29, G49, G59 and G69 were stable for the disease with below average AUDPC values. None of the sites was very stable for

Alternaria blight severity but Namulonge was more stable than Serere and had several genotypes specifically adapted to it (Figure1). Kachwekano was a high disease pressure site and the least stable with high interaction with the genotypes.

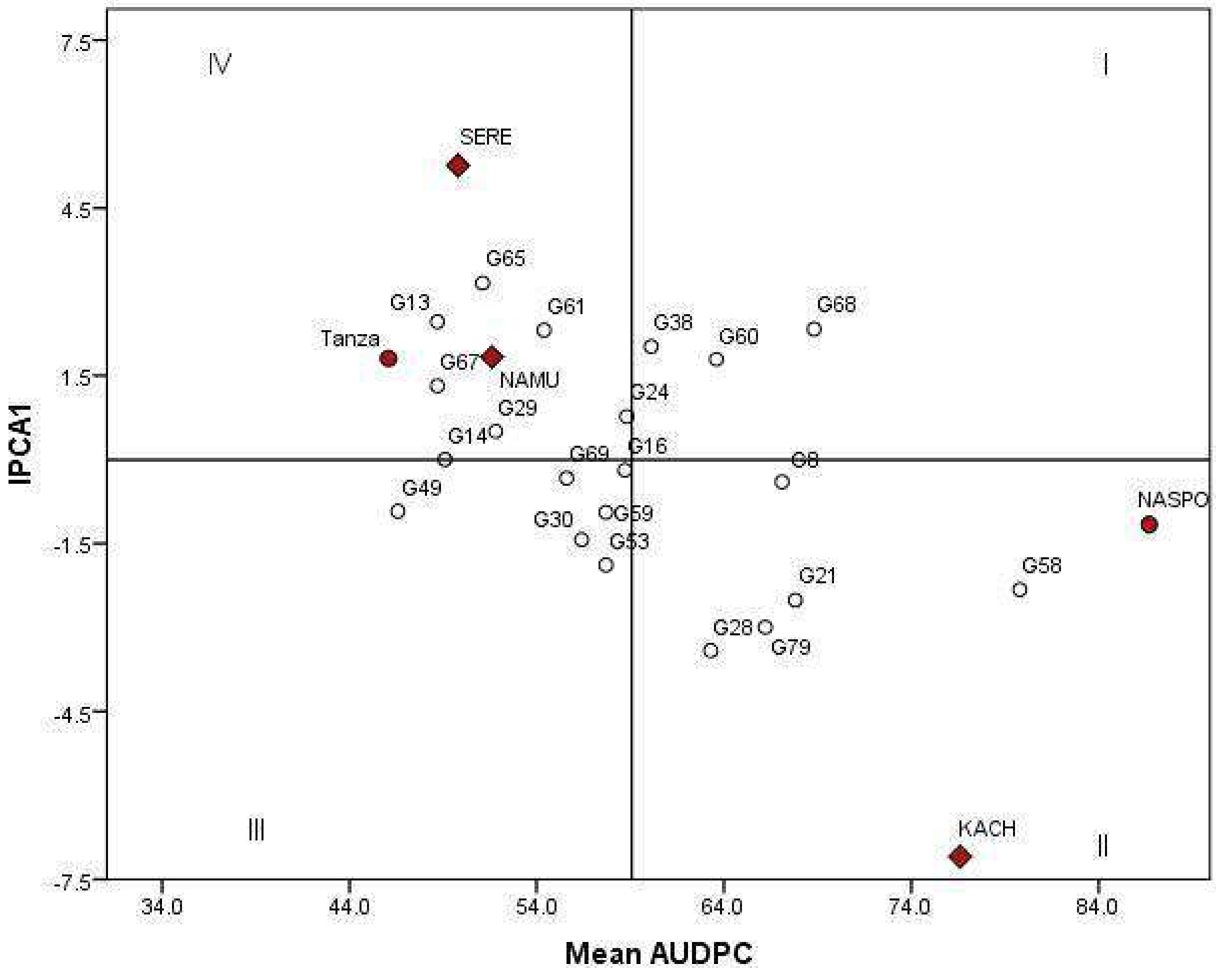

Fig.1: Biplot of IPCA1 scores versus genotype and environment Alternaria blight AUDPC means

Key

Check genotypes: NASPO = NASPOT 1; Tanza = Tanzania

O F$_1$ test genotypes: G8, G13, G14, G16, G21, G24, G28, G29, G30, G38, G49,G53, G58, G59, G60, G61, G65, G67, G68, G69 and G79

Sites: NAMU = Namulonge; KACH = Kachwekano; SERE = Serere

Sweetpotato virus disease

The MS for the environments were highly significant (P<0.001) for SPVD and not significant (P>0.05) for the genotypes and GEI (Table 2). Very low severity levels of SPVD were recorded for these genotypes with a mean score of 1.9 across sites (Table 4). Serere recorded the highest SPVD severity with a mean score of 2.3 while Namulonge had the lowest mean severity score of 1.6.

Table 4 Mean AMMI performance estimates and ranking of the genotypes for sweetpotato virus disease score at Namulonge, Kachwekano and Serere

Genotype	**Mean SPVD**	**IPCA1**	**IPCA2**	**Namulonge**		**Kachwekano**		**Serere**	
				Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
G8	1.3	-0.01957	-0.12729	1.3	4	1.3	2	1.7	6
G13	2.0	-0.63277	-0.39805	2.3	20	2.3	20	1.3	1
G14	1.9	-0.14939	0.29899	2.0	18	1.3	2	2.3	10
G16	2.2	0.47151	0.05500	1.3	4	2.0	15	3.3	20
G21	1.7	-0.38595	-0.39269	1.7	11	2.0	15	1.3	1
G24	1.7	0.35452	-0.37663	0.7	1	2.0	15	2.3	10
G28	2.1	0.10256	-0.03882	1.7	11	2.0	15	2.7	17
G29	1.7	-0.13913	-0.38734	1.3	4	2.0	15	1.7	6
G30	2.4	0.58851	0.48664	1.7	11	1.7	11	4.0	21
G38	2.0	0.35452	-0.37663	1.0	2	2.3	20	2.7	17
G49	1.7	0.22726	-0.12194	1.0	2	1.7	11	2.3	10
G53	1.6	-0.64304	0.28829	2.3	20	1.0	1	1.3	1
G58	1.8	-0.02470	0.21588	1.7	11	1.3	2	2.3	10
G59	2.1	0.11026	-0.55357	1.3	4	2.7	22	2.3	10
G60	2.9	0.84560	-0.19435	1.3	4	3.0	23	4.3	23
G61	2.1	-0.64561	0.45987	3.0	23	1.3	2	2.0	8
G65	1.7	-0.14683	0.12741	1.7	11	1.3	2	2.0	8
G67	1.9	0.22469	0.04965	1.3	4	1.7	11	2.7	17
G68	1.4	-0.39108	-0.04953	1.7	11	1.3	2	1.3	1
G69	1.6	-0.51578	0.03359	2.0	18	1.3	2	1.3	1
G79	1.9	-0.02213	0.04430	1.7	11	1.7	11	2.3	10
NASPOT 1	1.7	0.10000	0.13276	1.3	4	1.3	2	2.3	10
Tanzania	2.6	0.33655	0.82445	2.3	20	1.3	2	4.0	21
Mean	**1.9**			**1.6**		**1.7**		**2.3**	

SPVD = sweetpotato virus disease; IPCA = interaction principal component axes

Total storage root yield

The genotypes, environments and GEI MS were highly significant ($P<0.001$) for TRY (Table 2). The genotypes, environments and GEI SS accounted for 9.7, 47.8 and 20.1% of the total SS for TRY, respectively. Both IPCA1 and IPCA2 were significant and accounted for 62.8 and 37.2% of the GEI SS. Genotypes G14 and G13 were the most stable for TRY across the sites with IPCA1 scores of 0.08633 and 0.18901, respectively (Table 5). Genotypes G58 and G60 were the least stable with IPCA1 values of 2.2542 and -1.74938, respectively. Across sites, G67, G24, G13, G53 and G65 had the highest TRY of 21.6, 21.4, 20.8, 19.9 and 19.4 t ha^{-1}, respectively while genotypes G68, G60, G58, G29 and G21 had the lowest TRY of 12.9, 13.5, 14.0, 14.0 and

15.3 t ha^{-1}, respectively across sites. The mean TRY across genotypes of 25.5 t ha^{-1} recorded at Namulonge was the highest of the three sites while the 12.3 t ha^{-1} recorded at Serere was the lowest. The most outstanding genotypes at Namulonge were G30, G69 and G16 with yields of 34.0, 31.3 and 30.6 t ha^{-1}, respectively. There was no consistency in the ranking of the genotypes in that highly ranked genotypes at one site ranked poorly at the other sites.

Table 5 Mean AMMI performance estimates and ranking of the genotypes for total storage root yield (t ha^{-1}) at Namulonge, Kachwekano and Serere

Genotype	Overall Mean TRY t ha^{-1}	IPCA1	IPCA2	Namulonge		Kachwekano		Serere	
				Mean	**Rank**	**Mean**	**Rank**	**Mean**	**Rank**
G8	20.5	0.49816	0.02743	30.2	4	17.2	8	14.1	7
G13	20.8	0.18901	-0.29646	29.1	7	17.4	7	16.0	4
G14	18.9	0.08633	1.44211	27.7	11	20.5	3	8.6	20
G16	19.0	0.92707	0.52908	30.6	3	16.0	13	10.4	15
G21	15.3	1.03024	-1.23778	26.4	14	7.2	23	12.3	11
G24	21.4	0.13619	-0.90956	29.2	6	16.4	11	18.7	2
G28	17.1	1.05036	-1.81533	28.0	10	7.4	22	15.9	5
G29	14.0	-0.26961	0.32112	20.8	19	13.4	18	7.9	22
G30	19.4	1.69363	0.54536	34.0	1	14.5	14	9.6	18
G38	17.8	-0.65650	0.38515	23.1	18	18.4	5	12.0	12
G49	17.7	0.49669	-0.40570	27.1	13	13.2	19	12.7	10
G53	20.0	-0.13348	-1.50316	26.3	15	14.0	17	19.5	1
G58	14.0	-2.25420	-1.11901	12.2	23	14.4	15	15.5	6
G59	17.7	0.88763	-0.14469	28.8	9	13.0	20	11.3	13
G60	13.5	-1.74938	0.12783	14.4	22	16.1	12	10.2	16
G61	18.6	-0.95907	1.59782	23.3	16	23.3	1	9.3	19
G65	19.4	0.64465	0.87561	30.1	5	18.1	6	10.1	17
G67	21.6	-0.22108	1.00442	28.9	8	22.7	2	13.1	9
G68	12.9	-1.08135	0.27452	16.4	21	14.2	16	8.0	21
G69	18.4	1.17342	1.16814	31.3	2	16.5	10	7.3	23
G79	16.0	0.99172	-0.44067	27.3	12	10.2	21	10.4	14
NASPOT 1	20.2	-1.13006	-0.19483	23.3	17	20.3	4	17.0	3
Tanzania	16.4	-1.35035	-0.23140	18.7	20	17.0	9	13.7	8
Mean	**17.9**			**25.5**		**15.7**		**12.3**	

IPCA = Interaction principal component axes

In the AMMI biplot of the two significant axes IPCA1 vs IPCA2 for TRY (Figure 2), the genotypes and the three environments generally dispersed around the origin (centre) of the biplot (the sites more so than the genotypes) indicating strong interactions between the genotypes and environments in response to the abiotic or biotic factors underlying or driving the IPCA1 & 2 scores. Genotypes G13, G8, G49 and G29 were positioned close to the origin indicating minimal interaction of these genotypes with the environments. The remaining 17 genotypes and checks (Tanzania and NASPOT 1) were positioned further away from the origin and therefore had strong interactions with some of the environments.

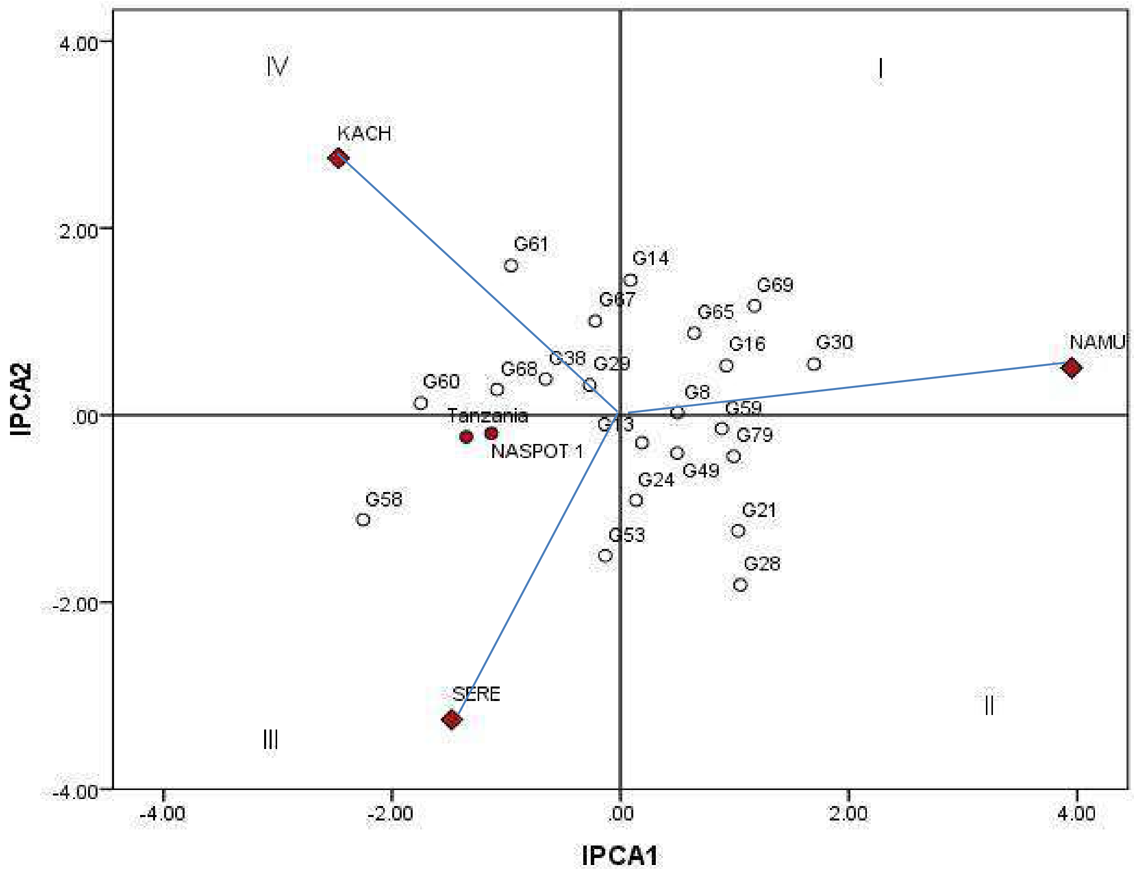

Fig. 2: Biplot of IPCA1 scores versus IPCA2 scores for genotype and environment mean total storage root yield (t ha^{-1})

Key

Check genotypes: NASPOT 1; Tanzania

O F$_1$ test genotypes: G8, G13, G14, G16, G21, G24, G28, G29, G30, G38, G49,G53, G58, G59, G60, G61, G65, G67, G68, G69 and G79

Site: NAMU = Namulonge; KACH = Kachwekano; SERE = Serere

Participatory genotype selection

Genotype evaluation before harvest

For the evaluation done before harvest, the scientists and farmers at both sites selected different genotypes (Table 6). Based on the selection index, the scientists at Namulonge ranked NASPOT 1, G58, G79, G69 and G2 and those at Kachwekano ranked G60, G67, NASPOT 1, G49 and G16 as their most preferred genotypes. Similarly, the farmers at Namulonge ranked G58, G59, NASPOT 1, G21 and G29 and at Kachwekano G14, G29, NASPOT 1, G60 and G16 as their most preferred genotypes. NASPOT 1, G21, G53, G58 and 65 were ranked as the best across the groups and sites. The Spearman's correlation between scientists and farmers' rankings before harvest at Namulonge was significant ($P<0.05$) and positive ($r = 0.324$). Similarly, the Spearman's correlation between scientists and farmers' rankings at Kachwekano was also significant ($P<0.05$) and positive ($r = 0.282$) (Table 7).

Table 6 Scientists and farmers' selection and ranking of genotypes before harvest at Namulonge and Kachwekano

Genotype	Namulonge scientists		Namulonge farmers		Kachwekano scientists		Kachwekano farmers		Rank sum	Overall rank
	Aggregate	Rank	Aggregate	Rank	Aggregate	Rank	Aggregate	Rank		
G8	36	10	27	8	23	22	16	9	49	14
G13	37	5	28	4	23	22	15	11	42	9
G14	20	23	24	12	29	10	30	1	46	11
G16	24	21	20	18	34	5	21	5	49	15
G21	38	5	28	4	29	10	21	5	24	2
G24	32	13	17	21	29	10	13	17	61	20
G28	23	22	25	11	30	9	12	21	63	21
G29	27	19	28	4	29	10	29	2	35	6
G30	37	5	21	17	29	10	13	17	49	16
G38	32	13	27	8	25	20	14	13	54	18
G49	31	16	22	15	35	4	18	8	43	10
G53	37	5	28	4	29	10	16	9	28	3
G58	47	2	32	1	28	17	14	13	33	4
G59	31	16	32	1	26	18	12	21	56	19
G60	33	12	16	22	42	1	24	3	38	8
G61	34	11	18	19	24	21	13	17	68	22
G65	38	4	27	8	34	5	13	17	34	5
G67	27	19	22	15	39	2	14	13	49	17
G68	31	16	16	22	29	10	12	21	69	23
G69	45	3	24	12	31	7	14	13	35	7
G79	45	3	23	14	26	18	15	11	46	12
NASPOT 1	50	1	30	3	39	2	24	3	9	1
Tanzania	32	15	18	19	31	7	21	5	46	13

Aggregate = sum of the weighted attributes for each genotype per group; Rank sum = sum of the genotype rank across the four groups

Table 7 Spearman's rank correlations between the scientists and farmers' genotype rankings before harvest at Namulonge and Kachwekano

NS	-			
NF	**0.342***	-		
KS	-0.153	**-0.305***	-	
KF	-0.04	0.103	**0.283***	-
	NS	NF	KS	KF

NS = Namulonge scientists; NF = Namulonge farmers; KS = Kachwekano scientists; KF = Kachwekano farmers;
* = Significant at P<0.05

Genotype evaluation at harvest

For the evaluation at harvest, each group listed their own set of traits that they considered important for desirable sweetpotato genotypes and they ranked these attributes (7). Storage root yield was the most important trait ranked first by all four groups followed by root size, weevil resistance, root shape and skin colour.

At harvest, on the basis of the selection index, the ranked order of the scientists' selected genotypes at Namulonge was: G30, G28, G49, G67 and G24; and at Kachwekano was: G29, G49, G30, NASPOT 1 and G14 (Table 8). The ranked order of the farmers' selections at Namulonge was: G8, G30, G53, G29 and G49; and at Kachwekano was: G21, G24, G30, G29 and G14.

At harvest, the Spearman's correlation between scientists and farmers' rankings at Namulonge was highly significant (P<0.01) and positive (r = 0.412) and that between scientists and farmers at Kachwekano was also highly significant (P<0.01) and positive (r = 0.440) (Table 9). The other rank correlations were non-significant.

Table 8 Scientists and farmers' selection and ranking of genotypes at harvest at Namulonge and Kachwekano

Genotype	Namulonge scientists		Namulonge farmers		Kachwekano scientists		Kachwekano farmers		Across four groups	
	Aggregate	Rank	Aggregate	Rank	Aggregate	Rank	Aggregate	Rank	Rank Sum	Final rank
G8	52.0	16	71.3	1	36.7	18	23.6	22	57	16
G13	41.6	23	60.3	22	38.3	14	34.0	13	72	23
G14	52.7	14	64.7	16	49.6	5	42.7	5	40	7
G16	47.0	22	64.3	18	44.3	9	37.7	10	59	18
G21	48.7	19	64.7	15	34.3	23	50.0	1	58	17
G24	61.3	5	65.3	12	47.7	6	49.0	2	25	4
G28	67.4	2	66.3	8	40.3	11	40.0	7	28	5
G29	56.4	12	67.7	4	56.0	1	44.7	4	21	2
G30	70.0	1	68.7	2	52.7	3	47.0	3	9	1
G38	47.7	20	64.3	19	39.0	12	30.0	19	70	22
G49	66.7	3	67.3	5	55.4	2	36.7	11	21	3
G53	57.4	10	67.7	3	35.0	20	21.0	23	56	13
G58	58.6	6	56.3	23	35.0	20	31.0	16	65	20
G59	57.7	8	65.3	10	35.7	19	40.6	6	43	8
G60	47.3	21	65.3	11	45.4	7	40.0	7	46	9
G61	57.0	11	67.0	6	34.6	22	32.3	15	54	11
G65	52.3	15	62.6	20	37.3	15	35.0	12	62	19
G67	62.7	4	67.0	7	37.0	16	30.0	19	46	10
G68	50.0	18	65.7	9	38.7	13	31.0	16	56	14
G69	58.6	7	64.7	17	41.7	10	27.0	21	55	12
G79	51.0	17	65.0	14	37.0	16	30.7	18	65	21
NASPOT1	57.7	8	65.2	13	52.1	4	39.6	9	34	6
Tanzania	53.4	13	60.4	21	44.5	8	32.9	14	56	15

Aggregate score based on weighted selection index

Table 9 Spearman's rank correlation between the scientists and farmers' genotype rankings at Namulonge and Kachwekano at harvest

NS	-			
NF	**0.412****	-		
KS	0.206	0.093	-	
KF	0.115	0.028	**0.440****	-
	NS	NF	KS	KF

NS = Namulonge scientists; NF = Namulonge farmers; KS = Kachwekano scientists; KF = Kachwekano farmers;

** = significant at P<0.01

The quality traits (mostly organoleptic) of the genotypes that were evaluated at harvest included sweetness (taste), root firmness (hardness), root fibre content, appearance and general acceptability based on taste and appearance. At Namulonge, scientists ranked G24, NASPOT 1, Tanzania, G38 and G28 as the best and at Kachwekano G68, NASPOT1, G14, G60 and G29 were ranked as the best genotypes. Farmers at Namulonge ranked NASPOT 1, G28, G38, G68 and Tanzania as the best genotypes, and at Kachwekano G14, G29, G68, G60 and NASPOT 1 were ranked as the best. Genotypes NASPOT 1, G68, G24, G60 and G53 were the best ranked across the groups (Table 10). The positive Spearman's correlation (r = 0.605) between scientists and farmers' rankings at Namulonge and rank correlation (r = 0.552) between scientists and farmers' ranking at Kachwekano were highly significant (P<0.01) (Table 11).

Table 10 Scientists and farmers' selection and ranking of quality traits of genotypes at harvest at Namulonge and Kachwekano

Genotype	Namulonge scientists		Namulonge farmers		Kachwekano scientists		Kachwekano farmers		Rank sum	Overall rank
	Aggregate	Rank	Aggregate	Rank	Aggregate	Rank	Aggregate	Rank		
G8	47.0	13	39.5	18	41.0	18	39.5	18	67	19
G13	42.0	20	35.0	21	40.5	19	45.5	11	71	21
G14	39.5	21	43.0	12	55.5	3	56.5	1	37	6
G16	37.5	23	42.5	13	45.0	16	43.0	15	67	20
G21	45.0	16	32.0	23	35.5	23	33.0	21	83	23
G24	61.5	1	50.0	6	48.5	10	44.5	12	29	3
G28	54.5	5	55.5	2	38.0	21	42.0	16	44	9
G29	43.5	17	42.5	14	55.0	5	55.0	2	38	7
G30	51.0	7	48.5	8	50.5	8	31.5	22	45	12
G38	55.0	4	53.5	3	46.0	14	30.5	23	44	10
G49	43.5	18	39.0	19	47.0	12	41.5	17	66	18
G53	47.5	11	49.5	7	50.5	9	49.5	7	34	5
G58	46.5	15	47.5	10	38.5	20	51.5	6	51	14
G59	43.5	19	48.5	9	45.5	15	47.5	9	52	15
G60	48.5	9	40.0	16	55.5	4	53.5	4	33	4
G61	48.5	10	46.0	11	51.0	7	44.0	14	42	8
G65	49.0	8	41.5	15	48.5	11	37.0	19	53	17
G67	53.5	6	40.0	17	46.5	13	48.5	8	44	11
G68	47.5	12	52.5	4	62.0	1	54.0	3	20	2
G69	38.0	22	36.5	20	36.0	22	44.0	13	77	22

G79	47.0	14	34.0	22	51.5	6	46.0	10	52	16
NASPOT 1	58.5	2	57.0	1	56.0	2	53.0	5	10	1
Tanzania	58.0	3	51.0	5	43.5	17	36.5	20	45	13

Aggregate score based on weighted selection index (sweetness (taste), root firmness (hardness), root fibre content, appearance and general acceptability).

Table 11 Spearman's rank correlation between scientist and farmers' genotype rankings for quality traits at harvest at Namulonge and Kachwekano

NS	-			
NF	0.605**	-		
KS	0.217	0.275*	-	
KF	-0.229	0.058	0.552**	-
	NS	NF	KS	KF

KF = Kachwekano farmers; KS = Kachwekano scientists; NF = Namulonge farmers; NS = Namulonge scientists;

** = significant at P<0.01; * = significant at P <0.05

Discussion

The objectives of this study were to evaluate and identify F_1genotypes with wide and specific stable performance over three sites for Alternaria blight resistance, SPVD, TRY and other farmer preferred traits. Additionally, the ranking of the genotypes by two different groups of scientists and farmers at two of the sites for selected traits were compared using Spearman's rank correlations.

Performance and stability of the genotypes

The severity of Alternaria blight was higher at Kachwekano than at the other two sites (Table 3). The AMMI analysis revealed that the Alternaria blight was influenced more by environmental effects, than by the GEI effects and least by genotypes effects. During the season, Kachwekano did not receive as much rainfall as Namulonge but the disease was more severe at this site. This is consistent with what was reported by Sseruwu et al. (2015) where some farmers in Luwero district reported the disease to be more severe during the dry season than during the wet season. It is possible that the disease infected the crop during the first month after planting when there was sufficient moisture and the symptoms became visible later on when the crop was stressed due to insufficient moisture. Mwanga et al. (2007b) described Serere as a low pressure area for Alternaria blight. However, this study has provided an indication that the effect of Alternaria blight under natural infestation in Serere is increasing since the severity was not significantly less than that of Namulonge. However, this can only be confirmed after obtaining data for several seasons. Should the trend be confirmed, farmers at Serere will require Alternaria blight resistant genotypes and this would necessitate evaluation of all the popular sweetpotato genotypes in the area for resistance to the disease in order to identify those with good levels of resistance.

Resistance of the genotypes across sites to Alternaria blight was not consistent, with some genotypes having lower Alternaria blight AUDPC values at one site and higher values at another site. However, some genotypes maintained lower Alternaria blight AUDPC values across sites and if these genotypes can maintain this consistency in subsequent evaluations (particularly over more seasons) and also meet the required performance levels for other important traits then they will be recommended to the farmers for cultivation in all the tested and similar sites/environments. Those that have consistent, good performance at particular sites will be recommended for those sites. Genotypes G49, G67, G69, 59 and G24 were the best genotypes at Namulonge. Genotypes G13 and G65 performed better than the check, Tanzania at Kachwekano. Similarly, G14, G28 and G53 were also better than the Tanzania at Serere. Thus these genotypes are well adapted to those sites. Genotypes G49, G13, G67 and G14 recorded lower mean AUDPC values across sites and should be further evaluated for even wider adaptation.

The AMMI biplot provided an indication of the stability of the different genotypes for Alternaria blight. In this context, stability means a genotype that maintains the same level of disease severity, either high or low across sites. Genotypes that are stable for low Alternaria blight severity and good yields are desired for this programme. Stability of genotypes G14, G16, G24, G29, G49, G59 and G69 for low Alternaria blight severity implies that these genotypes can be grown in all of the test sites and maintain low disease severities. They can also be used as sources of resistance in breeding for Alternaria blight resistance. Genotypes NASPOT 1 and G8 expressed stable but above average AUDPC values. This implies that these genotypes can only be grown in areas of low Alternaria blight pressure or may need fungicide protection when grown in high disease pressure areas. Kachwekano is a high Alternaria blight pressure site; therefore, it is ideal for evaluating the resistance of germplasm to the disease while Namulonge and Serere are ideal for germplasm multiplication.

The high significance (P<0.001) of the effects of genotypes, environments and GEI for TRY implied that all these factors are important in determining the expression of this trait. However, environmental effects were more important than genotypes and GEI effects. Namulonge was the highest yielding site with a mean TRY of 25.5 t ha^{-1} and Serere was the lowest yielding site with a mean of 12.3 t ha^{-1}. The cause of such high variation in yield was in all likelihood the amount of rainfall received during the season. At Kachwekano and Serere, the crops received reasonable amounts of rainfall only during the first month after planting but very little in the subsequent months unlike Namulonge which had good rainfall for the first three months after planting (Appendix 1).The yield recorded at Namulonge which ranged between 12.2 (G58) and 34.0 t ha^{-1} (G30) is an indication of the high yield potential of this set of genotypes. However, the full genotype yield potential was not realised at the other two sites possibly due to moisture stress. However, the best genotypes for TRY across the three sites were G67 (21.6 t ha^{-1}) and G24 (21.4 t ha^{-1}).

The AMMI biplot provided an indication of the stability of the genotypes for TRY. Genotypes G53, G67, G14, G13, G29 and G24 were very close to the horizontal line and therefore the most stable. These genotypes are widely adapted and can be grown at any of the three test sites and should give good yields. Provided the necessary agronomic requirements are available, they can be recommended to farmers at all three sites. Genotypes G68, G60 and G58 were low yielding and specifically adapted to the low yield potential sites of Kachwekano and Serere hence may not perform well outside these sites.

Participatory Clonal selection

At the two selection stages, before harvest and at harvest, the scientists and farmers at the two sites ranked some of the genotypes similarly and in other instances differently. The significant ($P<0.05$), positive Spearman's rank correlation between scientists and farmers at each site ($r = 0.342$ for Namulonge, $r = 0.283$ for Kachwekano) before harvest indicated that the two groups ranked many genotypes in the same way before harvest. Therefore, at each site the scientists in this study were capable of selecting genotypes that had farmer preferred traits. The groups of scientists at the two sites selected different genotypes and so did the farmers. Since they based their selection on crop vigour, the cause of the difference in genotype selection was likely to be the differences in the performances of the genotypes across the sites due to the poor weather conditions at Kachwekano, which did not receive enough rainfall during the trial (Appendix 1). Ranking of genotypes before harvest may be influenced by the amount of aboveground foliage produced particularly the leaves which at that stage are the economic yield component of the crop. On the other hand, farmers may prefer genotypes with more upright growth habit than prostrate growth habit with spreading vines. However, the aboveground characteristics of any genotype may not always be a good indicator of belowground performance.

At harvest, most of the attributes identified by the scientists and farmers were similar but the ranking of the genotypes differed. Just as in any formal selection system where yield is considered as a major criterion (Joshi et al., 1997), yield was ranked the number one trait by the groups. Scientists and farmers at both sites preferred high-yielding genotypes with big storage roots which implied the converse that high yielding genotypes that produce small storage roots are not preferred. This is certainly the case where the farmers are market oriented. The buyers select and pay only for the large storage roots and leave the small ones or take them at no cost. Abidin et al. (2002) in north-eastern Uganda, also reported that farmers prefer genotypes that produce numerous, large storage roots, which tend to also have large overall yields. Similarly, Ndirigwe et al. (2005) in Rwanda reported that farmers rejected one cultivar which was high yielding because it had small size storage roots. In addition to storage root size, shape of the storage root was identified as an important trait by all groups except farmers at Namulonge. Grooved roots are not preferred because they are difficult to peel and will not be bought in the market unless they are the only ones available. Skin colour was important to all groups except the Kachwekano farmers. Red skin colour was mostly preferred by the groups and this is also the market preference. That skin colour was not identified as an important trait by the Kachwekano farmers, probably because most of them produce for home consumption. In previous studies by Abidin et al. (2002) in north-eastern Uganda, the preferred skin colour was white/tan and flesh colour was yellow. Therefore, the importance of skin colour depends on the region where the evaluation is carried out. According to Ndirigwe et al. (2005), in Rwanda the reddish skin was also preferred by both the farm household and the market.

At harvest, the significant ($P<0.01$), positive Spearman's rank correlation coefficient between scientists and farmers at Namulonge ($r = 0.412$) and between scientists and farmers at Kachwekano ($r = 0.440$), indicated that it is possible for the evaluation to be carried out by scientists only and successfully identify farmer preferred traits. This would obviously enable considerable savings for research budgets and will facilitate quicker selection processes.

For the cooking qualities of the genotypes, the farmers also represented the consumer since they also consume sweetpotato and they interact frequently with other consumers. The highly significant ($P<0.01$), positive Spearman's rank correlations between the rankings of scientists and farmers at Namulonge ($r = 0.605$) and scientists and farmers at Kachwekano ($r = 0.552$) for cooking quality traits indicates that the scientists at each site are capable of selecting for the same cooking qualities preferred by farmers. Therefore, it is not necessary to use site specific groups in the selection process. NASPOT 1, which is a popular cultivar, emerged as the best genotype across the groups for cooking quality traits with G68 and G24 ranking second and third respectively. Since NASPOT 1, which is already a very popular cultivar in Uganda, was ranked as the best by the groups this provides some validation of the outcome of the current study.

IV. CONCLUSION

Some of the F_1 genotypes selected from the crosses conducted in this breeding programme are highly adaptable and have farmer preferred attributes. Genotypes that exhibited stability for resistance to Alternaria blight as well as stability for high storage root yield were G14, G16, G24, G49 and G59. These genotypes can be recommended to farmers on a trial basis at the three test sites and other associated sites. However, a full investigation of the stability of these genotypes across a representative range of environments will have to be performed. Stability for the scientist and farmer evaluated traits will be the basis upon which any genotype will be advanced.

The good correlations between scientist and farmer rankings of genotypes at each of the two sites in this study demonstrated that the identification of selection criteria and application thereof by scientists and farmers was not that different. The practical implication of this study is that selection within sites can be generally carried out by experienced scientists who have a good understanding of the production requirements of sweetpotato and consumer preferences. Importantly, the selection has to be conducted by site specific sets of scientists.

Overall, genotype G49 was ranked well both for stability by GEI analysis and for scientist and farmer preferred traits by the participatory selection process. In the participatory process it was ranked tenth before harvest and third at harvest. It is an above average yielder with good yield stability, and is stable for Alternaria blight with below average Alternaria blight AUDPC value. This genotype will be recommended for cultivation by selected farmers on a trial basis, as further evaluations are done at more sites.

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REFERENCES

[1] Abidin, P.E., F.A. van Eeuwijik, P. Stam, Struik.P.C, D.P. Zhang, M. Hermann and E.E. Carey. 2002. Evaluation of sweepotato (*Ipomoea batatas* (L.) Lam.) germplasm from North-eastern Uganda through a Farmer Participatory Approach. In: Ames, T., editor, Proceedings of the First InternationalSymposium on Sweetpotato. Acta Horticulturae 583, ISHS. p. 61-68.

[2] Almekinders CJM, Elings A. 2001. Collaboration of farmers and breeders: participatory crop improvement in perspective. Euphytica 122: 423–424.

[3] Ceccarelli S, Grando S. 2006. Decentralized-participatory breeding: an example of demand driven research. Euphytica 155:349–360.

[4] Ceccarelli, S., S. Grando, R. Tutwiler, J. Baha, A.M. Martini, H. Salahieh and A. Goodchild. 2000. A methodological study on participatory barley breeding.I. Selection phase. Euphytica 111: 91-104.

[5] Dawson J.C, Murphy K.M, Jones S.S. 2007. Decentralized selection and participatory approaches in plant breeding for low-input systems. Euphytica 160: 143–154.

[6] Frey, K.J. 1964. Adaptation reaction of oats strains selected under stress and non-stress environmental conditions. Crop Science 4: 55-58.

[7] Gauch, H.G. and R.W. Zobel. 1996. AMMI analysis of yield trials. In: Kanga, M. S. and H. G. Gauch, editors, Genotype by Environment Interaction. CRS, Boca Raton, Florida, USA. p. 85-122.

[8] Gibson, R.W., E. Byamukama, I. Mpembe, J. Kayongo and R.O.M. Mwanga. 2008. Working with farmer groups in Uganda to develop new sweetpotato cultivars: Decentralisation and building on traditional approaches. Euphytica 159: 217-228.

[9] Grüneberg, W.J., K. Manrique, D. Zhang and M. Hermann. 2005. Genotype x environment interactions for a diverse set of sweetpotato clones evaluated across varying ecogeographic conditions in Peru. Crop Science 451: 2160-2171.

[10] Joshi, K., M. Subedi, R. Rana, K. Kadayat and B. Sthapit. 1997. Enhancing on farm varietal diversity through participatory varietal selection: a case for Chaite rice in Nepal. Experimental Agriculture 33: 335-344

[11] Kamalam, p., R.S. Biradar and N. Hrishi. 1978. Stability parameters in sweetpotato (*Ipomoea batatas* (L.) Lam.). Journal of Root Crops 4: 35-39.

[12] Kang, M.S. 1993. Simultaneous selection for yield and stability in crop performance trials. Consequences for growers. Agronomy Journal 85: 754-757.

[13] Keith, T., Rwiza, E., Nyango, A., Amuor, R., Ngendello, K.R., Jolliffe, D.F. (2004). The use of sensory evolution and consumer preference for the selection of sweetpotato cultivars in East Africa. Journal of the Science of Food and Agriculture 84(8): 791-799.

[14] Kwach, J. K., Odhiambo, G. O., Dida, M. M. and Gichuki, S. T. 2010. Participatory consumer evaluation of twelve sweetpotato varieties in Kenya. African Journal of Biotechnology. 9(11): 1600-1609

[15] Laurie S.M and Magoro M.D. 2008. Evaluation and release of new sweetpotato varieties through farmer participatory selection. African Journal of Agricultural Research 3 (10): 672-676

[16] Lebot V. 2009. Tropical root and tuber crops: cassava, sweetpotato, yams and aroids. Wallingford: CABI Publishing.

[17] MAAIF. 2018. Ministry of Agriculture Anima Industry and Fisheries. 2018. Performance Report. Kampala, Uganda. p.15

[18] Manrique, K. and M. Hermann. 2000. Effect of Genotype x Environment interactions on root yield and beta-carotene concentration of selected sweetpotato (*Ipomoea batatas* (L.) Lam.) varieties and breeding clones. International Potato Centre. Program report 1999-2000, Lima, Peru. p. 281-287.

[19] Marti, R.H. (2003). Estimation of sample size in skin and flesh colour measurements of dry flesh sweet potato (Ipomoea batata (L) Lam). Scientia Horticulturae 98:331-336.

[20] Martin, W.F., A.N. Flores and G.S. Carmer. 1988. Identification of a key environment for determination of yield stability in sweetpotato. Tropical Agriculture (Trinidad) 65: 313-316.

[21] Mcharo, M., Carey, E.E., Gichuki, S.T. (2001). Performance of selected sweetpotato varieties in Kenya. African Crop Science Journal 9: 49-59.

[22] Mwanga, R., Lemaga, B., Nsumba, J., Ndolo, P., Agili, S., Marandu, E.2006. Evaluation of stability performance of orange-fleshed sweetpotato clones in East African regional trials. In: Book of Abstracts of the 14th Symposium of the International Society for Tropical Root Crops, 20–26 November 2006, Thiruvananthapuram, India. pp 62–63.

[23] Mwanga R.O.M., G. Kyalo., G. N. Ssemakula., C. Niringiye., B. Yada., M. A. Otema., J. Namakula., A. Alajo., B. Kigozi., R.N.M. Makumbi., A. Ball., W.J. Greuneberg., J.W. Low and G.C. Yencho. 2016. ‘NASPOT 12 O’and ‘NASPOT 13 O’Sweetpotato. HortScience.51(3): 291–295.

[24] Mwanga, R.O.M., B. Odongo, A.Alajo, B. Kigozi, N. Niringiye, R. Kapinga, S. Tumwegamire, R.M. , E. Lugwana, J. Namakula, B. Lemaga, J. Nsumba and C. Yencho. 2007. Submission to the Variety Release Committee for the release of sweetpotato varieties. National Agricultural Research Organisation (NARO)/National Crops Resources Research Institute (NaCRRI), Kampala, Uganda.

[25] Mwanga, R.O.M., B. Odongo, C. Niringiye, R. Kapinga, S. Tumwegamire, P.E. Abidin, E.E. Carey, B. Lemaga, J. Nsumba and D. Zhang. 2007. Sweetpotato selection releases: lessons learnt from Uganda. African Crop Science Journal 15: 11- 23.

[26] Mwanga, R.O.M., C. Niringiye, A. Alajo, J. Namakula, I. Mpembe, S. Tumwgamire, R.W. Gibson and G.C. Yencho. 2011. 'NASPOT 11', a sweetpotato cultivar bred by a participatory plant breeding approach in Uganda. HortScience 46: 317-321.

[27] Ndirigwe, J., S. Muyango, R. Kapinga and S.Tumwegamire. 2005. Participatory on-farm selection of sweetpotato varieties in some provinces of Rwanda. African Crop Science Conference Proceedings. 7: 1205-1209.

[28] Ngeve, J.M. 1993. Regression analysis of genotype x environment interaction in sweetpotato. Euphytica 71: 231-238.

[29] Niringiye, C.S., Ssemakula, G.N., Namakula, J., Kigozi, C.B., Alajo, A., Mpembe, I and Mwanga R.O.M. 2014. Evaluation of promising sweetpotato clones in selected agro-ecological zones of Uganda. Time Journals of Agriculture and Veterinary Sciences 2(3):81-88.

[30] Odendo, M., H.D. Groote, O. Odongo and P. Oucho. 2002. Participatory Rural Appraisal of Farmers’ Criteria for Selection of Maize Varieties and Constraints to Maize Production in Moist-Midaltitude Zone of Western Kenya. A

case study of Butere-Mumias, Busia and Homa Bay Districts. Final Technical Report. CIMMYT, Nairobi, Kenya. pp. 17.
[31] Payne, R.W., S.A. Harding, D.A. Murray, D.M. Soutar, D.B. Baird, A.I. Glaser, S.J. Whelham, A.R. Gilmour, R. Thompson and R. Webstar. 2011. The guide to Genstat release 14, Part 2: Statistics. VSN International, Hemel Hempstead, UK.
[32] Sperling, L., J.A. Ashby, M.E. Smith, E. Weltzien and S. Mcguire. 2001. A framework for analysing participatory plant breeding approaches and results. Euphytica 122: 439-450.
[33] Ssemakula, G., C. Niringiye, B. Yada, M. Otema, G. Kyalo, J. Namakula, A. Alajo, B. Kigozi, R. Makumbi, G.C. Yencho, and R.O.M. Mwanga. 2013. Submission to the variety release Committee for the release of sweetpotato varieties. National Agricultural Research Organization (NARO)/National Crops Resources Research Institute (NaCRRI), Kampala, Uganda.
[34] Sseruwu G., P. Shanahan, R. Melis, G. Ssemakula. 2015. Farmers' awareness and perceptions of Alternaria leaf petiole and stem blight and their preferred sweetpotato traits in Uganda. Journal of Plant breeding and Genetics. 3:1-13
[35] Witcombe, J.R., A. Joshi and S.N. Goyal. 2003. Participatory plant breeding in maize for low-input systems. A case from Gujarat, India. Euphytica 130: 413-422.
[36] Zobel, R.W., M.J. Wright and H.G.Gauch. 1988. Statistical analysis of yield trials. Agronomy Journal 80:388-393.

Appendix 1.Rainfall (mm) received at each site from planting to harvesting

Location	**2015A**				
	March	**April**	**May**	**June**	**July**
Namulonge	150.4	226.0	104.1	87.73.4	33.0
Kachwekano	161.3	69.9	54.7	22.8	00
Serere	152.2	56.6	37.1	1.5	0.0

Entomopathogenic Nematodes against Insect Pests of Rice

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Abstract*— *Rice is one of the important staple crop in the world. Rice pests cause yield reduction as well as value of the crop. A number of insect pests that attack rice plants account for yield losses. In the rice agro ecosystems, many types of entomopathogens such as nematodes, fungi, bacteria, and viruses can reduce pest population. More emphasis should be placed on using an IPM approach where biological control with entomopathogens is also one of the main components. Entomopathogenic nematodes (EPN) as a safe alternative to the use of insecticides against insect pests in IPM of rice.

***Keywords*— Rice, Insect pests, Biological control, Entomopathogenic Nematodes.**

I. INTRODUCTION

Rice being a tropical plant is also adaptable to a broad range of climatic, edaphic and cultural conditions. It provides 20% of the per capita energy and 15% of the per capita protein for humans worldwide (Mikkelsen and Datta, 1991). Rice is grown in more than a hundred countries, with a total harvested area of approximately 158 million hectares, producing more than 700 million tons annually. Nearly 640 million tons of rice is grown in Asia representing 90% of global production (Way and Bowling, 1991). Rice production should be increased to supply a rapidly expanding population; however, it has been hindered by a number of diseases and insect pests. Moreover, rapid changes in rice production technologies have created greater frequencies of pest epidemics (Reissig *et al*., 1986).

II. INSECT PESTS STATUS

A number of insect pests that attack rice plants account for yield losses of 24% worldwide. Insect pest cause at least 20 per cent field losses in rice in India (Pathak *et al.*, 1982). More than 70 species of insect pests are known to feed on rice, and at least 20 of them can seriously affect rice production. A variety of factors can contribute to pest outbreaks, including climatic factors, improper irrigation, high rates of nitrogen fertilizer application, overuse of insecticide. Insect pests attack all parts of the rice plant at all growth stages and some serve as vectors of viruses that adversely affect the plant.

Brown planthopper (*Nilaparvata lugens*) and white-backed planthopper (*Sogatella furcifera*) are serious pests of rice. They occur in tropical to temperate areas with high reproductive potential and can cause extensive damage through their feeding activity and transmission of viral diseases like rice grassy stunt and ragged stunt. Nymphs and adults suck sap from the base of plants, just above the waterline. In heavy infestation, these planthoppers can cause hopperburn resulting in browning and wilting of some or all tillers in a hill (Kuno, 1968). Most of the rice varieties are susceptible to this pest.

The rice stem borer, *Chilo suppressalis* has been one of the most important pests. It can cause significant damage by reducing tiller number. Females are capable of laying eggs in masses near the base of rice leaves or leaf sheaths. The larvae penetrate tillers and feed on the inner surface of the stem walls, interrupting the movement of water and nutrients. The central leaves of damaged tillers of young plants turn brown (called dead hearts).If the damage occurs after formation of spikelets, panicles turn white (called whiteheads) and no grain filling occurs. The plant often dies and the larvae move to another stem.

Rice water weevil (*Lissorhoptrus oryzophilus*) the larvae cause much more damage, as they feed on roots and prune them, than the adult weevils feeding on leaves. The yield loss could reach up to70-80% with heavy infestation.

III. MANAGEMENT OF INSECT PESTS

Control of insect pests has primarily depended on the application of chemical insecticides. Chemical insecticides are expensive, besides other disadvantages, including secondary pest resurgence, insecticide resistance, environmental pollution, and impact on nontarget organisms. The utilization of pathogenic microorganisms holds a high possibility for the suppression of rice insect pests (Otake, 1979; Chatterjee *et al.* 1983; Pathak *et al*., 1982; Heong, 1983). In recent years more emphasis has been placed on using an IPM approach where biological control is also one of the main components. In the rice agro ecosystems, many types of entomopathogens such as nematodes, fungi, bacteria, and viruses can reduce pest population.

Nematodes have been found associated with most of the insect orders. There are more than 3100 natural associations between insects and nematodes involving 11 orders of nematodes and 23 orders of insects. The association may range from a phoretic relationship to obligate entomoparasitism leading to host death, sterility, reduced fecundity or delayed development. Some that are associated with one host and its special ecology are highly specialized and difficult to propagate on an artificial medium while other less specialized forms have wide host range, can be mass produced on artificial media, and are currently used for control of agricultural pests. Entomopathogenic nematodes (EPN) as a safe alternative to the use of insecticides in IPM of different crops, including rice, have gained worldwide attention (Table 1).

IV. MODE OF ACTION

Mermithids are a large group of obligate entomopathogenic nematodes that are considered important regulators for some insect populations (Kaiser,1991). Mermithid parasitism results in nutritional depletion, retarded growth, organ disruption, reduced fecundity or sterility and death. The newly hatched second stage mermithid is the infective stage (pre-parasite).Once the mermithid contacts the plant hopper nymph, it uses its stylet to penetrate through the cuticle into the host hemocoel to initiate the parasitic phase. The 3rd and 4th stage juveniles occur in the hemocoel.Two to three weeks after parasitization, the 4th stage juvenile (post parasite) exits its adult host by boring through the thin intersegmental area of the abdominal segment, causing death of the host. After emergence, the postparasite burrows into the soil, molts, and overwinters as an adult (Sutanov *et al*., 1990; Vandergast and Roderick,2003; Kamminga *et al*.2012).

Steinernematidae and Heterorhabditidae have attracted most attention as they contain the EPNs *Steinernema* and *Heterorhabditis*. The nematodes musualistically associated with insect-pathogenic bacteria. The bacteria *Xenorhabdus* and *Photorhabdus* (Family: Enterobacteriaceae) are symbiotically associated with *Steinernema* spp. and *Heterorhabditis* spp. respectively. These bacteria are ecologically obligate to EPNs, with specific mechanisms of pathogenicity and their existence in free form in nature is believed to last a very short while due to photo and thermo sensitivity. These nematode bacterium associations meet many criteria for augmentative control of insect pests through inundative releases including: broad host range; ability to kill hosts rapidly : a durable infective stage capable of storage; distribution; and persistence; available mass inexpensive mss production technologies; no evidence of insect immunity; safety to plants and vertebrates; and application with existing spray equipment. The third stage dauer juvenile (DJ) occurs free in the soil and its role is to seek out and infect an insect larva. These free-living, non-feeding juveniles and developmentally arrested third stage juvenile ranging in length from 0.4mm to 1.1mm. *Steinernema* gains entry to the insect larva through natural openings (mouth, anus and spiracles). In addition to these modes of entry, *Heterorhabditis* also gains entry by abrading the intersegmental membranes of the insect. Once in the haemocoel of the insect the DJ releases cells of a symbiont bacterium that it carries in its intestine. The insect haemolymph provides rich medium for the bacterial cells and these begin to grow, release toxins and exoenzymes and kill the insect. The insect dies rapidly, usually within 24-48 h. Generally life-cycle of entomopathogenic nematodes is completed within 12-15 days at room temperature. Depending on the availability of food resource, both heterorhabditis and steinernematids generally complete 2-3 generations within insect cadaver and emerge as infective juveniles to seek new hosts.

PARASITISM / BIOEFFICACY

Imamura (1932) reported that Mermithidae were parasitic on *Chilo simplex.* Grewal *et al*., (2006) recorded it in Asia. Pena & Shepared (1985) recorded 50% parasitisation of BHP by *Hexamermis* sp. in Phillippines. Heong (1983) reported that an entomopathogenic nematode *Amphimermis unka* caused high mortality of hoppers pest in China. Natural incidence of parasitism by *Hexamermis* sp. on BPH was first reported in south India by Manjunath (1978) and in eastern india by Satpathi (1999). Ramani, (2003) also reported that *Hexamermi*s sp. was most important nymphal /adult parasitoid from India. Satpathi *et al*., (2008) studied in detail about the factor affecting abundance of parasitic nematode *Hexamermis* sp. in eastern India. Jayanthi *et al*., (1987) recorded parasitism (12%) by mermithids in rice planthopper. *Rhabditis* sp. (*Oscheius* sp.) was found to be effective against egg mass and neonate larvae of *Scirpophaga incertulas* (Padmakumari *et al*.,2007;2008).

Agamermis species infecting insects have been reported in North America (Cobb *et al*.,1923; Christie,1936), Asia (Kaburaki and Imamura 1932; Choo *et al*.1995), Australia (Baker and Poinar,1995), Africa (Igbinosa,1988), China (Bao et al.,1992) and Europe (Rubtsov,1969;1977). *Agamermis unka* is the most important and common natural enemy in temperate regions. *Agamermis* species live in the soil and infect hosts from the soil directly or after short migration up to plant stem (Nickle, 1981; Choo *et al*., 1995). *Agamermis unka* was first collected by Esaki and Hasimoto,1931 from BPH and WBPH at Oita, Japan and described as a new species (Kaburaki and Imamura,1932; Fuse and Sato,1968). Esaki and Hasimoto (1931) found that >40% BPH and >70% of WBPH populations were parasitized. Mermithid nematodes have received attention as possible biocontrol agents of brown planthopper (BPH). A number of studies were initiated to determine its role as a mortality factor in plant hopper population. *Agamermis* parasitism castrates the reproductive organs of BPH and WBPH. In Philippines, 50% parasitism of BPH by an unidentified mermithid was recorded during the wet season (Otake, 1979), but parasitism was low throughout the year and its impact as a natural control agent of BPH was negligible. Parasitism of the host usually occur at the lower part of the rice stem where most planthoppers are found (Cho *et al*.,2002; Choo and Kaya, 1990;1991;1993). In Korea, *Agamermis unka* is a major natural enemy of the brown planthopper, *Nilaparvata lugens*(Stal) (Hemiptera: Delphacidae), and the whitebacked leafhopper, *Sogatella furcifera* (Horvath) (Hemiptera: Delphacidae), and has been widely studied regarding future inoculative releases and conservation approaches to mange populations in rice (Choo and Kaya,1994).When the pre parasites of *A. unka* were released at a mermithid to BPH of 10:1, parasitism of BPHs ranged from 33 to 63% (Choo *et al*.,1995). *Agamermis* can be redistributed by artificial releases. *Agamermis unka* is an important mortality factor in planthopper populations in Korea. It kills the adult and reduces the fecundity of the females. Males of BPH and WBPH are susceptible to parasitism by *A.unka* (Kuno,1968). As egg production and hatchability of *Agamermis* are high, inoculative releases into areas where the mermithid population is low or nonexistent appear feasible. To affect plant hopper populations; the mermithid must parasitize a high number of progeny of the migrating population. Both the short-winged (brachypterous form) and the long-winged (macropterous form) adults are susceptible to mermithid parasitism, but the brachypterous form (57%) had higher parasitism than the macropterous form(8%)(Choo *et al*.,1989).The brachypterous form is usually found lower on the rice plant where the mermithid is more likely to encounter it. *A. unka* would be most effective when the migrating adult insects produce few progeny and the parasitic stage of the mermithid occurs in high numbers. About 30% of the natural controls of brown plant hopper in eastern India are due to parasitic nematode (Satpathi *et al*., 2008). A control strategy would be to reduce the number of progeny produced by migrating adults. This can be accomplished through an integrated manner with chemical or biological insecticides, resistant cultivars, cultural methods, or a combination of these control tactics. Before an integrated pest management system can be incorporated in the field, further studies on the biology of the nematode and its compatibility with current control tactics are needed. Rice water weevil adults can be parasitized by mermithid, as has been reported in native regions (Bunyarat *et al*., 1977).These parasites may reduce fecundity and cause high mortality in infected adults. Rice blue beetle (*Leptispa pygmaea*) was reported to be parasitized by *Hexamermis* (Patel and Shah,1988). This mermithid is already established in the rice fields in Korea, appears to be compatible with some chemical pesticides, and reduces the fecundity of its host (Choo *et al*., 1998). Cultural practices such as tilling and irrigation can increase the performance of *A.unka*. We have to enhance the effectiveness of the naturally occurring mermithid into an IPM program to reduce BPH population. Genus identification through molecular technique can help predict and infer mermithid biology, which can ultimately assist in rearing protocols, if mermithids are to be used for future research and incorporation into current management protocols.

In India, efforts were made during 1970s to study the effectiveness of exotic EPNs, *S.carpocapsae* (DD-136) against insect pests of rice (Rao *et al*., 1968). However, the nematode was not able to become established in field trials. *S. carpocapsae* (DD-136) causing up to 98% mortality and fast multiplication on 5th instar larvae of *Cnaphalocrocis medinalis* (Srinivas and Prasad,1991). Choo *et al.* (1989) reported that *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* are very effective against the rice yellow stem borer, *Scirpophaga incertulas* causing mortality and proved EPN as a potential biocontrol agent in rice eco system. *S. carpocapsae* or *H. bacteriophora* were sprayed at the rates of 250, 500, 1000 or 2000 IJs for *S. carpocapsae* and 100, 200, 400 or 800IJs for *H. bacteriophora*. Seven days after treatment, showed that both nematodes were effective causing more than 91% mortality. On the other hand, mortality was 42.6% and 63.1% when stems were dipped into a nematode suspension at the rate of 100 or 200 IJs of *H. bacteriophora.* Because the moist habitat of rice stems were favorable to nematode survival and searching abilities, entomopathogenic nematode, were confirmed to be a potential biological control agents against rice stem borers (Choo *et al*., 1991). Prasad *et al*., (2006) reported that *H.indica* caused 100% mortality of rice leaf folder, *Cnaphalocrocos medinalis* within 18 to 20 hours of exposure. Padmakumari *et al.* (2008) reported that lethal time of 19.8 h was recorded by *H. indica* and 37.8 h by *S. asiaticum* on *C. medinalis* in a bioassay study. Combined inoculation of *H. indica* and *S. asiaticum* each at 75 IJs/larva resulted in faster larval mortality on *C. medinalis* (24.6 h). The percent larval mortality caused by *H. indica* alone was resulted significantly more (60%) than *S. asiaticum* (40%) on *C. medinalis*. Progeny produced by *H indica* and *S. asiaticum* 4843 and 4330 IJs/larva respectively on *C. medinalis* larva (Sankar *et al*., 2009). *Heterorhabditis indica* caused 66.67 to 91.67 per cent mortality at concentrations of 5IJs to 9IJs in the grubs of *L. pygmaea* (Karthikeyan, and Jacob, 2009). Spraying of *H.indica* @ 3000 IJs /ml was effective in reducing the white ear incidence,@ 2500 IJs /ml was also equally effective in reducing the gall midge incidence at 25 DAT, *H. indica* applied @ 3000 IJs /ml was the superior treatment at 25 days after transplanting while at the later stage (55 days after transplanting), a lower dose @ 2500 IJs /ml was found to be sufficient to bring about significant control of leaffolder (Karthikeyan and Jacob,2010). Among the three entomopathogenic nematodes evaluated for their biological control, maximum reduction of BPH was observed with *Steinernema glaseri* followed by *Metarhabditis amsactae* isolate Drr-Ma2 (Annon.2015).Rice stem borer (*Chilo suppressalis*) was reported to managed by *S.carpocapsae* Pocheon strain, *S.carpocapsae* Iksan strain, *S.monticolum* Hwasun strain, *H.megidis* Hwasun strain in Korea (Jung *et al*.,2018). Efficacy of EPNs was evaluated was evaluated against the African white rice stem borer, *Maliarpha separatella*. Significant virulence was obtained with all the nematodes species at 200 IJs after 48 hours of exposure in the following order *H.indica* > Ex Nakuru (local isolate) > *S.carpocapsae* > Ex Mombosa (local isolate) > *S.karii* (Kega *et al*.,2013;2020). *Steinernema carpocapsae* was found to cause mortality against rice water weevil, *Lissorhoptrus oryzophilus* under laboratory setting but failed to work in the field in Japan (Nagata,1987).In Cuba, there was success using *Steinernema* spp. against the rice water weevil with up to 80% control in field trials(Carbonell, 1983; Meneses, 1983).In California, research with both *S.carpocapsae* and *Heterorhabditis* spp. found that nematodes provided control of rice water weevil larvae when applied to drained soil that was reflooded 8 d later (Grigarick and Oraze,1990).In China, the Otio strain of *S. feltiae* was found to cause high mortality for larvae (Sun *et al*.,2006) and mortality rate was affected by time and dose. Efficacy of *S.feltiae*, *S. carpocapsae* A24 strain, *S.glaseri* NC 34 strain, *H.bacteriophora* and *H.zealandica* have also been detected in adult weevils (Kisimoto *et al*.,1987). Mortality of 82.5% and 97.5% was observed in adults of *L. oryzophilus* treated with *S.feltiae* and *H.bacteriophora*, respectively, at 10d after incubation with nematodes (Li *et al*.,2007). However, the widespread application or adoption of nematodes against rice water weevil in Asia or North America has not been possible for economic reasons (Choo and Rice, 2007).

V. CONCLUSION

Understanding the ecological and behavioral relationships between the nematode and insects could result in proper use of compatible insecticides or other biological control agents in providing an integrated approach to insect management. *In vivo* production of mermithid has been accomplished with the mermithids from the banded cucumber beetle (Creighton and Fassuliotis, 1982) and from mosquitoes (Peterson, 1984). Similarly, if BPH can be mass produced easily, *in vivo* production of the mermithid may be used to augment natural population. In addition to production, methods to store the eggs and adults and timing of introduction into BPH populations need to developed. Using the conservation of naturally-occurring population of mermithid, there is a need to implement an effective IPM programme. By understanding the biology and ecology of these entomopathogens, we may be able to use them effectively in the integrated pest management of rice through augmentation or inundative release. The microclimate of rice culture with high humidity and moderate temperature is also conductive for the survival, movement, tracking and invasion of the host by EPN and their establishment as a bio-control agent. However, there is a need for development of suitable delivery mechanisms including formulation technology for field application of this EPN. Studies are required to evaluate their bioefficacy against other rice pests as well.

REFERENCES

[1] Acharjee P, Satpathi CR,Modak MK.2020.Long term studies on the role of parasitic nematode *Hexamermis* sp. in controlling brown planthopper in farmer's rice field of West Bengal, India. International Journal of Current Research and Review.12 (3). DOI: http: //dx. doi. Org /10. 31782 / IJCRR. 2020.12032.

[2] Anonymous. 2015. ICAR-IIRR Annual Report, Hyderabad, India.

[3] Baker GL and Poinar,GO.Jr. 1995. *Agamermis catadecaudata* n.sp.(Nematoda: Mermithidae), a parasitoid of Orthoptera in South eastern Australia. *Fundamental and Applied Nematology*.18: 139-148.

[4] Bao XC, Lou QG, Luo DM.(1992). A new species of *Agamermis* from China (Nematoda: Mermithidae). *Acta Zootaxon Sinica* 17: 20-25.

[5] Bunyarat M. Tugwell P. Riggs R.D.1977.Seasonal incidence and effect of a mermithid nematode parasite on the mortality and egg production of the rice water weevil *Lissorhoptrus oryzophilus*. *Journal of Economic Entomol*ogy 6: 712-714.

[6] Carbonell R.M. 1983. Pathogens and nematodes for control of rice water weevil in Cuba. *International Rice Research Newsletter*. 8: 16-17.

[7] Chatterjee, P.B., Sen, S.C., Sarkar, D. and Hikimindran.1983. On the implementation of IPM in rice. *Pest Management in Rice*, TNAU, Coimbatore. pp. 270-279.

[8] Cho SR, Lee DW, Choo HY, Kim HH. 2002. Distribution of *Agamermis unka* (Nematoda: Mermithidae), a mermithid parasite of brown planthopper (*Nilaparvata lugens*) in Korean rice paddies. *Korean Journal of Applied Entomology*.41:129-136.

[9] Choo HY, Kaya HK, Kim JB, Park YD. 1991. Evaluation of entomopathogenic nematodes, *Steinernema carpocapsae* (Steinernematidae) and *Heterorhabditis bacteriophora* (Heterorhabditidae) against rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae). *Korean Journal of Applied Entomology*. 30:50-53.

[10] Choo HY, Kaya HK, Lee SM, Lee YI, Choo DJ.1987. Detection and identification of biological control agents from planthoppers and leafhoppers. II. Distribution of nematode parasite of brown plant hopper in the soil, with notes on bionomics. Journal of the Institute of Agricultural Research Utilization, Gyeongsang National University 21:81-85.

[11] Choo HY, Kaya HK.,Kim JB.1989. *Agamermis unka* (Mermithidae) parasitism of *Nilaparvata lugens* in rice fields in Korea. *Journal of Nematology*. 21:254-259.

[12] Choo HY, Kaya HK.1990. Parasitism of brown plant hopper and white backed plant hopper by *Agamermis unka* in Korea. *Journal of Nematology*. 22 (4): 513-517.

[13] Choo HY, Kaya HK.1994. Biological control of the brown planthopper by a mermithid nematode. Korean J Appl. Entomol. 33:207-215.

[14] Choo HY, Kim HH, Kaya HK.1998.Effects of selected chemical pesticides on *Agamermis unka* (Nematoda: Mermithidae), a parasite of the brown plant hopper, *Nilaparvata lugens*. *Biocontrol Science and Technology*. 8(3):413-427.

[15] Choo HY,Kaya HK,Kim HH.1995. Biological studies on *Agamermis unka* (Nematoda: Mermithidae), a parasite of the brown planthopper *Nilaparvarta lugens*. *Biocontrol Science and Technology*. 5: 209-223.

[16] Choo HY,Kaya HK.1993.Parasitism of the brown planthopper by the mermithid nematode *Agamermis* in rice. In: Nematodes and the Biological control of insect pests. R.Bedding, R Akhurst, H.Kaya (Eds.). pp.21-26. CSIRO, East Melbourne, Australia.

[17] Choo HY.1991.Ecological study of *Agamermis unka*, a nematode parasite of brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). Res. Rept. Rural Development Agency 33:185-191.

[18] Choo H.Y. Rice W.C. 2007. Evaluation of microbial agents against rice pests *In*. Lacey L.A. Kaya H.K. Field manual of techniques in invertebrate pathology: application and evaluation of pathogens for control of insects and other pests. 2nd ed. Springer, -loc Dordrecht, The Netherlands.

[19] Choo,HY,Lee SM,Chung BK,Park YD,Kim HH.1995.Pathogenicity of Korean entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) against local agricultural and forest insect pests. *Korean Journal of Applied Entomology*. 34:314-320.

[20] Christie,JR.1936. Life history of *Agamermis decaudata*, a nematode parasite of grasshoppers and other insects. *Journal of Agricultural Research*.52: 161-198.

[21] Cobb NA,Steiner G,Christie JR.1923. *Agamermis decaudata* Cobb, Steiner, and Christie: A nema parasite of grasshoppers and other insects. *Journal of Agricultural Research*.23:921-926.

[22] Creighton CS and Fassuliotis, G.1982. Mass rearing of a mermithid nematode, *Filipjevimermis leipsandra* (Mermithida: Mermithidae) on the banded cucumber beetle (Coleoptera:Chrysomelidae). *Journal of Economic Entomology*.75:701-703.

[23] Esaki T and Hashimoto S.1931.Report on the leafhoppers injurious to the rice plant and their natural enemies. Entomological Laboratory, Department of Agriculture, Kyushu Imperial University Publication 2, 54-59.

[24] Fuse H, and Sato M.1968.The parasitism of the *Agamermis unka* to the rice planthoppers in Yamagata Prefecture. *Japanese Journal of Applied Entomology and Zoology*.12 (2):97-99.

[25] Greathead DJ.1979. Critical review of natural enemies of insect pests on rice in South and South East Asia and their potential for biological control: proposals for biological studies to assist in development of integrated pests control in rice in South and South East Asia. Commonwealth Institute of Biological Control. Slough, England. pp.126.

[26] Grewal P.S., Ehlers,R. and Shapiro, D.I. (2006) Nematodes as bio control agents Wollingford, UK. CBA international pp 480.

[27] Grigarick A.A. Oraze M.J. 1990. Impact of water management on the effectiveness of nematode applications for control of the rice water weevil p. 72. In: Proceedings, 23rd Rice Technical Working Group 26-28 February 1990 Biloxi, MS Texas A&M University, College Station, TX.

[28] Heong K.L.(1983) Studies on natural enemies of rice insect Pest. *Acta Phytophlacica Sinica,* 10:224.

[29] Igbinosa,IB.1988.Parasites of the immature stages of Latoia viridissima Holland (Lep. Limacodidae), a pest of palms in Nigeria. *Journal of Applied Entomology*.106:527-530.

[30] Imamura S.1932. Mermithidae parasitic on *Chilo simplex* butler and leafhopper. *Oyo-Dobutsugaku Zasshi* 4: 176-180.

[31] Israel P, Rao YRVJ, Rao PSP, Varma A.1969. Control of paddy cut worms by DD-136, a parasitic nematode. *Current Science* 38(16):390-391.

[32] Jayanthi M, Shankar G, Bhaskaran P. 1987.Parasitic nematode in white striated plant hopper (WSPH) of rice. *International Rice Research Newsletter*.12:23.

[33] Jung YH, Choo HY, Choi JY, Choo YM.2018.Occurence and environmentally friendly control of rice stem borer, *Chilo suppressalis* in Amur Silver grass (*Mischanthus sacchariflorus*) plantation. *Korean Journal of Pesticide Science*.22(1):36-44.

[34] Kaburaki T , Imamura S.1932.Mermithid worm parasitic in leafhoppers, with notes on its life history and habits. *Proceedings of Imperial Acadamy*.8:139-141.

[35] Kaiser H.1991.Terrestrial and semiterrestrial Mermithidae.pp.899-965.In: WR Nickle.ed. Manual of Agricultural Nematology.New York,Marcel Dekker.

[36] Kamminga KL,Davis JA,Stock SP.,Richter AR. 2012.First report of a mermithid nematode infecting *Piezodorus guildinii* and *Acrosternum hilare* (Hemiptera: Pentatomidae) in the United States. *Florida Entomologist*. 95:214-217.

[37] Karthikeyan K and Jacob.S. 2010. *Pseudomonas flourescens* and *Heterorhabditis indica* Poinar for the management of major insect pests of rice. *Journal of Biopesticides* 3(1): 96-99.

[38] Karthikeyan, K. and Jacob, S. 2009. Bioefficacy of white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill. and entomopathogenic nematode, *Heterorhabditis indica* (Poinar) against rice blue beetle, *Leptispa pygmaea* Baly. *Journal of Biological Control,* 23 (1): 79-81.

[39] Katti, G,Prasad JS, Padmakumari, A. P.Sankar M.2006. Effect of storage periods on survival and infectivity of indigenous entomopathogenic nematodes of insect pests of rice. *Nematol.Medit*.34: 37-41.

[40] Katti, G., Padmakumari, A. P. and Jonnalagadda, P. S. 2003. An entomopthogenic nematode infecting rice stem borer, *Scirpophaga incertulas. Indian Journal of Plant Protection,* 32(2): 80-83.

[41] Kega VM, Kasina M, Olubayo F, Nderitu JH. 2020. Effectiveness of entomopathogenic nematodes against the African white rice stem borer *Maliarpha separatella* Rag. *Universal Journal of Agricultural Research* 8(2): 41-45.

[42] Kega VM, Kasina M, Olubayo F, Nderitu JH.2013.Management of *Maliarpha separatella* Rag using effective entomopathogenic nematodes and resistant rice cultivars. *Journal of Entomology*.10(2): 103-109.

[43] Kisimoto, R., Yamada, Y. and Suzuki. K. 1987. The entomogenous nematode *Steinernema feltiae* as a biological control agent of the rice water weevil, the brown rice planthopper, and the diamondback moth. In: *Recent Advances in Biological Control of Insect Pests by Entomogenous Nematodes in Japan*, N. Ishibashi (ed.). Ministry of Education, Culture and Science, Japan, Saga University, Japan, pp. 111-115.

[44] Kuno E.1968.Studies on the population dynamics of rice leafhopper in a paddy field.The Bulletin of the Kyushu Agricultural Experiment Station.14(2):131-246.

[45] Li JJ, Qian HT, Dong H, Cong B. 2007. Laboratory test on the infectivity of entomopathogenic nematode to *Lissorhoptrus oryzophilus*. *Chinese Journal of Biological Control* 32: 188-190.

[46] Manjunath TM.1978.Two nematode parasites of rice brown plant hopper in India. Intern. Rice Res. Newsl. 3:11-12. *Hexamermis* sp. and *Mermis* sp. isolated from *Nilaparvata lugens*.

[47] Meneses CR. 1983. Pathogens and nematodes for control of rice water weevil in Cuba. *International Rice Research Newsletter*. 8(6):16-17.DD-136

[48] Mikkelsen DS and Datta, SKD.1991.Rice culture .In: Volume Rice Production(BS Luh (ed).pp.103-186.Van Nostrand Reinhold,New York.

[49] Nagata, T. 1987. Infectivity of *Steinernema feltiae* on the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel. In: *Recent Advances in Biological Control of Insect Pests by Entomogenous Nematodes in Japan*, N. Ishibashi (ed.).

Ministry of Education, Culture and Science, Japan, Saga University, Japan, pp. 65-70.

[50] Nayak P., Rao YRVJ, Yadava CP., Rao YS.1977.Occurrence of a new entomophilic nematode on rice stem borer, *Sesamia inferens*. *Oryza* 14:51-54.

[51] Nayak P.,Rao YS.1978. Occurrence of a new entomopathogenic nematode *Parasitorhabditis* on rice stemborer *Sesamia inferens*. Oryza J. Asso. Rice Res. Work.14: 51-54.

[52] Nickle,WR.1981.Mermithid parasites of agricultural pest insects. *Journal of Nematology*.13: 262-266.

[53] Otake A.1979. Natural enemies of the brown planthopper. pp. 42-57. In: Brown Planthopper: Threat to rice production in Asia. International Rice Research Institute, Los Banos, Laguna, Philippines.

[54] Padmakumari AP, Prasad JS, Katti G, Sankar M. 2007. *Rhabditis* sp (*Oscheius* sp), a biocontrol agent against rice yellow stem borer, *Scirpophaga incertulas*. *Indian Journal of Plant Protection*. 35(2):255-258

[55] Padmakumari, A.P., Katti, G., Sankar, M. and Prasad, J.S.2008. *Entomopathogenic nematodes in Rice Pest Management.* Technical Bulletin No. 32. Directorate of Rice Research (DRR), Rajendranagar, Hyderabad, A.P., India. 21 P.

[56] Patel CB, Shah AH.1988.Nematodes (Mermithids) as a parasite of larvae of *Leptispa pygmaea* Baly. *Indian Journal of Entomology* 14:354.

[57] Pathak, M. D., Khelsa, M. S. and Varma, S. K. 1982. Controlling major pests of rice plant. *Indian Farmers Digest*, 15 (10): 6-9.

[58] Pena NP, Shephard SM. 1985. Parasitism of nematodes on three species of hopper pests of rice in Laguna, Philippines. *International Rice Research Newsletter*. 10:19-20.

[59] Peterson,JJ.1984. Nematode parasites of mosquitoes. In: Plant and Insect Nematodes.WR Nickle(Ed.). Marcel Dekker, New York, USA. Pp.797-820.

[60] Poinar GO Jr.,Chang PM.1985.*Hexamermis cathetospiculae* n.sp. (Mermithidae: Nematoda), a parasite of the rice stemborer, *Tryporyza incertulas* (Wik.) (Pyralidae: Lepidoptera) in Malaysia. J.Nematol.17:360-363.Scirpophaga incertulas

[61] Prasad JS, Katti G, Padmakumari AP, Sankar M. 2006. Bioefficacy of indigenous entomopathogenic nematodes against leaf folder, *Cnaphalocrocos medinalis* Guenee in rice.pp.418.In: Proceedings of the 2nd International Congress held on October, 9-13,at National Academy of Agricultural Sciences,New Delhi.

[62] Prasad JS, Katti G, Padmakumari AP,Pasalu IC.2003.Exploitation of indigenous entomopathogenic nematodes against insect pests of rice.pp.121-126. In: Current status of research on entomopathogenic nematodes in India (Hussaini SS, Rabindra RJ, Nagesh M, Eds.). Project Directorate of Biological Control, Bangalore, India.

[63] Prasad JS, Padmakumari AP, Somasekhar N, Katti G. 2016. Entomopathogenic nematodes as a component in rice pest management: A journey from lab to land. In: Plant Health Management for Food Security: Issues and Approaches (Varaprasad KS, Katti G, Kodaru A). Daya Publishing House. New Delhi, 199-212.

[64] Ramakrishnan N.and Kumar, S.1981.Recent advances in insect pathology in India.In: Recent Advances in Entomology in India(ed. Ananthakrishnan TN).Pp.79-97. Madras University Press,Madras.India.

[65] Ramani S. (2003) Biological control of rice insect pests, Biological control of crop pests in India, Kalyani Publisher. pp 349.

[66] Rao, V.P, Basy AN, Phalak VR, Checko MJ, Rao HD.1968.Some new records of parasites of rice stem borer in India .Indian Academy of Sciences, Section B,68:91-110.

[67] Rao, V.P., Manjunath, T.M., 1966. DD-136 nematode that can kill many insect pests. *Indian Farming* 16, 43-44.

[68] Rao, YRVJ, Prakasa Rao, P. S., Varma, A. and Israel, P. 1971.Tests with an insect parasitic nematode DD-136 (Nematoda: Steinemematidae) against rice stem borer, *Tryporyza incertulas* Walk. *Indian Journal of Entomology*, 33: 215 - 217.

[69] Reissig WH, Heinrichs EA,Litsinger JA,Moody K, Fiedler L,Mew TW and Barrion AT.1986.Illustrated guide to integrated pest management in rice in tropical Asia. Intern. Rice Res. Inst. Los Banos, Laguna, Phillippines.

[70] Ring DR, Browning HW. 1990. Evaluation of entomopathogenic nematodes against the Mexican rice borer (Lepidoptera: Pyralidae). *Journal of Nematology*. 22:420-422.

[71] Rubtsov,IA.1969. On a new species of *Agamermis* (Mermithidae) of a bug in Poland. *Acta Parasitologica Polonica*.16:97-99.

[72] Rubtsov,IA.1977. New genus and two new species of mermithids from bugs.*Parazitologiia.*11:541-544.

[73] Sankar,M, Prasad, J.S., Padmakumari, A.P, Katti G. and Divya K.2009. Combined application of two entomopathogenic nematodes, *Heterorhabditis indica* and *Steinernema asiaticum* to control the rice leaf folder, *Cnaphalocrocis medinalis* (Goen.). *Journal of Biopesticides* 2(2): 135-140.

[74] Satpathi CR,Sarkar A, Acharjee P.2008. Factors affecting abundance of *Hexamermis* sp. in rice plant in Eastern India. *International Journal of Nematology* 18(1): 75-78.

[75] Satpathi CR.1999. Record of nematode (*Hexamermis* sp.) on brown planthopper in West Bengal. Insect Environment 5:89.

[76] Satpathi CR.2009. Relationship between parasitic nematode, *Hexamermis* sp. and brown planthopper, *Nilaparvata lugens* Stal in rice crop in Eastern India. International Journal of Nematology 19(1):120.

[77] Srinivas, P.R. and Prasad, J.S. 1991. Record of DD-136 nematode infection in rice leaf folder (*Cnaphalocrocis*

medinalis). *Indian Journal of Agricultural Science* 16(5): 348-349

[78] Sun W,Cong B,Dong H,Qian H.2006. Study on the efficacy of *S.feltiae* Otio on rice water weevil in laboratory. Journal of Anhui Agricultural Science 34:1620-1621.

[79] Sutanov MA,Artyukhovskii AK,Lysikova EA.1990.Hosts of mermithids-The most important pests of farm and forest plants in Uzbekistan.pp.186-191.In: MD. Sonin and VS Kothekar.eds. Helminths of insects. New Delhi, India, Oxonian Press.

[80] Torii T. 1975. Feasibility of using the entomophilic nematode, DD-136 as a biotic insecticide against rice stem borer,as a biotic insecticide against rice stem borers. In: Approaches in Biological Control.Yasumatsu K.and Mori H.(Eds).pp.87-88.University of Tokyo Press,Tokyo.

[81] Vandergast AG,Roderick GK.2003.Mermithid parasitism of Hawaiian *Tetragnatha* spiders in a fragmented landscape. Journal of Invertebrate Pathology.84:128-136.

[82] Way MO, Bowling CC.1991.Insect pests of rice. In: Volume Rice Production (BS Luh Ed).pp.237-268.Van Nostrand Reinhold, New York.

[83] Yadav CP, Rao YS. 1970. On the effectiveness of entomopathogenic nematode, DD-136 in the biological control of insect pests of rice. *Oryza.* 7:131-136.

[84] Yan S, Qian H, Xia K, Ling L.1986.Life cycle of *Amphimermis* sp., a nematode parasite of the brown plant hopper in China. *Acta Phytophylacica Sinica* 13:79-84.

[85] Zhao HT., Hu Z.L, Zhang S.L1987.Field occurrence of *Agamermis* sp., a nematode parasite of rice hoppers. *Chinese Journal of biological control*.3:73-77.

Effect of Probiotic Supplementation on Milk Yield in Lactating Holstein Fresien Cross Bred Cows

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***Abstract*— *On farm trial was conducted on 20 lactating crossbred cows were randomly divided into two groups on the basis of milk yield (10 to 13 kg/day) and day of calving less than 60 days to see the effect of supplement probiotic on milk yield for continuous three year (2014 to 2017). Cross bred cows were fed concentrate, green and dry fodder and wheat straw in control groups and addition of 20 grams of Probiotic was given in treatment group. Experimental feeding was continued up to 90 days. The average milk production and fat percentage was significantly higher in treatment group. Milk production efficiency was also significantly higher in probiotic supplemented in comparison to control group. It was concluded that probiotic supplementation @ 20 gram per day per animal in cows significantly increase the milk production over the control group.*

***Keywords*— probiotic, cows, milk production.**

I. INTRODUCTION

Probiotics are defined as "live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal micro flora" (Fuller, 1989). The concept of microbial manipulation in the gastro-intestinal tract was first appreciated by Metchnikoff who viewed the consumption of yoghurt by Bulgarian peasants as conferring a long span of life. In dairy farming, feed is the most important factor which accounts for about 60-70% of the total cost of rearing. In order to get maximum profitability, the feed must be balanced nutritionally and also be economical. The economy of feed is not only determined by the apparent cost per kg of feed but also by the cost of feed required to produce a kg of milk.

Therefore, in order to achieve maximum profitability in dairy farming there is need to adopt the scientific feeding strategies for dairy animals.Probiotics like yeast culture to improve livestock productivity, and the underlying mechanisms for such improvement, have attracted increasing attention during recent years. Yeast cells are known to be a rich source of vitamins, enzymes and yeast is also observed to stimulate cellulolytic bacteria in the rumen, increase fiber digestion and flow of microbial protein from the rumen. Hence, yeast culture supplementation has been shown to improve the growth rate and feed conversion efficiency. However, the effect of dietary yeast supplementation on milk yield and milk composition is varied. In some studies, yeast culture supplementation was shown to increase milk production and milk fat percentage (Ayad*et al.*, 2013).

Probiotics have been observed to improve the performance of crossbred cows by increasing the count of favorable micro-flora present in the rumen and by improving feed utilization by animal, respectively. Keeping this view in mind, the present investigation was proposed to explore the possible feed additive probiotic-yeast culture, which will be more beneficial and economic in order to produce profitable performance of crossbred cows. Therefore, present experiment was planned to study the effect of supplementation of probiotic on the performance of lactating crossbred cows.

II. MATERIAL AND METHODS

Twenty lactating crossbred cows were selected from villages of Mehsana District with average milk yield of 10 to 12 kg and lactation stage below sixty days. The study was conducted during winter month of November to January for 90 days after the adoption period of two weeks for consecutive three years (2014-2017). The control animal fad concentrate, green and dry fodder and wheat straw whereas experimental group fed additional 20 grams probiotic (Gausac- product from Indian Immunological Limited). The animals were kept in well-ventilated byres with access to fresh water and having separate mangers for fodder and concentrate. The animals were milked twice a day morning and evening and the concentrate (Total quantity divided into two parts) was given as each milking time. Probiotic was added and mixed in concentrate uniformly in morning and fed individually to each animal. Milk sample from each animal in both groups were collected daily and analyzed for milk production at their village cooperative dairy regularly for 90 days. All the periodicals data with regards to milk yield were statistically analyzed by paired ‘t’ test with Systat 7.0 (1997).

III. RESULT AND DISCUSSION

Feeding of probiotic resulted in significant (P<0.05) increase in milk yield a (Table 1). Milk yield increased by 7.6 % in probiotic fed group over the control group.

Table 1.Milk production performance in cows.

Year	Particulars	Control	Treatment group	Significance
2014-15				
	Average milk yield per animal	8.90 ± 0.92	9.50 ± 1.06	< 0.05
2015-16				
	Average milk yield per animal	9.10 ± 0.49	9.80 ± 0.69	< 0.05
2016-17				
	Average milk yield per animal	9.30 ± 0.42	10.10 ± 0.48	< 0.05
Pooled of three years	Average milk yield per animal	9.10 ± 0.61	9.80 ±0.74	< 0.05

Values in rows are significance at P < 0.05

Hossain*et al.* (2014) reported significant (P<0.05) improvement in milk yield after supplementing probiotics (0.3 litre/ day/ cow). Vibhute*et al.* (2011) noticed improvement in milk yield of cows after supplementation of yeast culture. Wohlt*et al.* (1998) also noticed significant improvement in milk yield in Holstein Friesian cows supplemented with 10 and 20 g yeast culture per day.Similar results were also observed by Williams et al. (1991), Wohlt et al. (1991), Piva et al. (1993), Dutta et al. (2008), Yalcin et al. (2011), Bruno et al. (2009) reported that Cows fed yeast culture produced 1.2 kg/d more milk. Jacquette et al. (1988) and Ware et al.(1988) reported increased milk yield (1.8 kg/day) when feeding cows *Lactobacillus acidophilus* (2 x 109 cells/day) compared with the control group. Gomez-Basauri et al. (2001) observed an increase in milk production (0.73 kg/day) when feeding cows a mixture of *L. acidophilus*, *L. casei* and *Enterococcus faecium*. More recently, Stein et al. (2006) reported an 8.5% increase in 4% fatcorrected milk in cows receiving 6 x 1010 *Propionibacterium*/day from 2 weeks pre-partum to 30 weeks post-partum. More recent studies have looked at the combination of yeasts and bacteria. The results of the present trial are in accordance with the above findings.

IV. CONCLUSION

On Farm Test result showed that supplementation of probiotic to high yielding crossbred cows is beneficial in terms of increasing milk yield. Further research is necessary to find out the supplemental effect of the probiotic on dairy animals fed various types of basal rations at different productive levels and stage of lactation.

REFERENCES

[1] Ayad, M.A., B. Benallou, M. S. Saim, M. A Smadi and T. Meziane (2013) Impact of feeding yeast culture on milk yield, milk components and blood components in algerian dairy herds. J. Vet. Sci. Tech **4**: 135.

[2] Bruno RGS, Rutigliano HM, CerriRL, Robinson PH and Santos JEP (2009).Effect of feeding *Saccharomycescerevisiae*on performance of dairy cows during summer heat stress.Animal and Feed ScienceTechnology, 150:175-186.

[3] Dutta TK and Kundu SS (2008). Response of mixed viable probiotics culture on milk production and nutrient availability in crossbred mid lactating cows. Indian Journal of Animal Sciences. 78 (5): 531-535.

[4] Fuller R (1989).Probiotics in man and animals. Journal of Applied Microbiology, 66:365-378.

[5] Gomez-Basauri J, de Ordanza MB and SicilianoJJ (2001). Intake and milk production of dairy cows fed lactic acid bacteria and manna oligosaccharide. Journal of Dairy Science, 84(Suppl.1): 283.

[6] Hossain, F. M. A., M. M. Islam, A. Ara, N. Iliyas (2014) Supplementing probiotics (*saccharomyces cerevisiae*) in multiparous crossbred cows ration provoke milk yield and composition. J. of Anim. and feed Res. **4(2)**: 18-24.

[7] Jacquette RD, Dennis RJ, Coalson JA, Ware DR, Manfredi ET and Read PL (1988). Effect of feeding viable *Lactobacillus acidophilus* (BT1386) on performance of lactating dairy cows. Journal of Dairy Science,71 (Suppl. 1): 219.

[8] Piva G, Belladonna S, Fusconi G and Sicbaldi F (1993). Effects of yeast on dairy cow performance, ruminal fermentation, blood components and milk manufacturing properties. Journal of Dairy Science, 76: 2717-2722.

[9] Stein DR, Allen DT, Perry EB, Bruner JC, Gates KW, Rehberger TG, Mertz K, Jones D and Spicer LJ (2006).Effects of feeding propionibacteria to dairy cows on milk yield, milk components and reproduction.Journal of Dairy Science, 89(1): 111-125.

[10] Systat (1997).Users manual and reference manual for systat V 7.0 for windows. SPSInc, Chicago, IL.

[11] Vibhute, V. M., R. R. Shelke, S .D. Chavan and S. P. Nage (2011) Effect of probiotics supplementation on the performance of lactating crossbred cows. Vet. World Vol. 4(12): 557-561.

[12] Ware DR, Read PL and Manfredi ET (1988). Lactation performance of two large dairy herds fed *Lactobacillusacidophilus* strain BT138 in a switchback experiment. Journal of Dairy Science, 71 (Suppl.1): 219.

[13] WilliamsPE, Tait CA, Innes GM and Newbold CJ (1991). Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. Journal of Animal Science, 69(7): 3016-3026.

[14] Wohlt JE, Finkelstein AD and Chung CH (1991). Yeast culture to improve intake, nutrient digestibility, and performance by dairy cattle during early lactation. Journal of Dairy Science, 74 (4):1395-1400.

[15] Wohlt, J. E., T. T. Corcione and P. K. Zajac (1998) Effect of yeast on feed intake and performance of cows fed diets based on corn silage during early lactation. J. Dairy sci. **81 (5)**: 1345-1352.

[16] Yalcin S, Can P, Gurdal AO, Bagc B and Eltan O (2011). The nutritive value of live yeast culture (*saccharomyces cerevisiae*) and its effect on milk yield, milk composition and some blood parameters of dairy cows. Asian-Aust. J. Anim. Sci. Vol. 24(10): 1377–1385.

A Study on Growth and Performance of Dairy Sector in Nepal

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Abstract— *Dairy sector has been a vector in providing dairy products to the urban population and pull the urban capital into the rural areas. Dairy industries have been successful in creating a strong network between the dairy farmers and the consumers and have been established as a bridge between the urban and rural trade across the country. The entire dairy chain is dependent upon milk production. At present, Nepal contributes approximately 0.247% of the world's total milk production. Nepal's milk output is estimated to be 2.05 million metric tons. The per capita availability of milk in Nepal is around 158.9 grams per day, which is far below than the value recommended by World Health Organization (WHO). The current milk production growth rate should be raised to 4 percent per annum so as to meet the WHO recommended minimum value of 250 gm per day per-capita milk consumption by the year 2025.The dairy sector in Nepal is the most important sub sector in Nepalese livestock production. Almost 28% of the national GDP comes from the agriculture and livestock sector. However, the share of dairy sector in agricultural GDP of Nepal is 8% of the national GDP. Beside contributing to the GDP, the agriculture sector also provides employment to the two-thirds of the country's population. The cooperative sector in Nepal has been in the emerging state and is playing an important role in socioeconomic development of millions of rural families.*

Keywords— ***Dairy; GDP; Per Capita availability; Milk production; Livestock; Sustainability.***

I. INTRODUCTION

The organized dairy development activities in Nepal began only after 1952. The establishment of a Yak cheese factory in Langtang of Rasuwa district under the assistance of Food and Agriculture Organization (FAO) in 1953 is considered to be the pioneer activities in the dairy development of Nepal (FAO, 2010).

A large share in agricultural Gross Domestic Product comes from the Nepalese dairy sector. The co-operatives play an important role in agriculture and livestock sector, whose share in agricultural GDP of country has been 28 percent (NRB, 2018; Tiwari and Shingh, 2020). Dairy co-operatives help in establishing strong network and linkages in millions of rural households scattered across the country. Dairy co-operatives have helped immensely to establish a strong network and linkages among the rural population all over the country. The co-operative sector in Nepal has been in the emerging state and is playing an important role in socio-economic development of the country. At present the dairy sector of our country is contributing 0.247% percent of the world's total milk production. Nepal's milk output is estimated to be 2.05 million metric tons (FAOSTAT, 2019). The per capita availability of milk has also increased to a level of about 158.9 gram per day (RAN, 2015). This sector has its importance in reducing poverty through creating employment and income generating opportunities that is ensured through regular cash flow from urban to the rural areas (Neupane *et al.*, 2018). The population growth rate of Nepal is found to be 1.35 percent per annum. Under this assumption, the current milk production should be raised by 4 percent so as to meet the WHO recommended minimum value of 250 gm per day per-capita milk consumption by the year 2025. In contrast, current milk production growth stands only at 3.09 percent per annum (Upadhyay, 2017).

Present paper focuses on growth and performance of dairy sector in Nepal and provides recommendations to meet future challenges. A major fraction of milk is found to be handled by the unorganized sector in Nepal. Sweet shops, hotels, restaurants and tea shops which manufactures short to medium shelf-life milk products and are not recognized by Nepalese Dairy Act comes under the unorganized sector. There is no doubt regarding the dairy co-operatives playing a vital role in alleviating rural poverty. The formal sector or organized sector shares 20% of the total annual milk produced in the country (NEPC, 2017). The result of the study indicates that product development, milk quality, infrastructure support, and global competition could be the future challenges of Nepalese dairy sector.

II. OBJECTIVES OF THE STUDY

The objectives of this research are as followings:

1. To analyze the progress of dairy sector in Nepal.
2. To find out the limitations and opportunities of dairy sector in Nepal.
3. To examine the role of cooperatives in development of dairy sector and their challenges.
4. To propose the sustainable remedial measures for improving the overall performance of Nepalese dairy sector.

III. MATERIAL AND METHOD

The present research paper is descriptive and is based on secondary data. The secondary data has been obtained from various sources such as, Ministry of Agriculture and Livestock Development (MoALD, Nepal), Ministry of Finance (MoF, Nepal) and various reports of Dairy development Cooperation (DDC), Food and Agricultural Organization (FAO), National Agricultural Research council (NARC) and National Dairy Development Board (NDDB). The basic statistical tools such as percentage, growth rate, variation etc. is used for the economic analysis. The similar methodology was adopted by (Deshmukh, 2014) for analyzing the growth and performance of dairy sector in India.

IV. RESULT & DISCUSSION

1.1. Status of Dairy Sector in the World

The dairy sector of Nepal is emerging, and its share to the global milk production is very low i.e0.247 percent of the global milk share. India has been established as the largest milk producer in the world, producing 176.27 MT of milk per year sharing 21.32% of global milk production, followed by USA (97.76 MT/year,11.82% of global milk production) and Pakistan (44.29 MT,5.35% of global milk production). The annual milk production of Nepal as per the report published by FAOSTAT (2019) is 2.05 MT. Nepal's share in global milk production is 0.247% in the year 2017.

From table 1 it is clear that the global milk production is rising tremendously to meet the requirements of the growing population. The world milk production in the year 1975 was only 424.73 MT, which is almost half of the present global milk production (826.75MT). Table 1 also reveals a slight drop in the annual milk production of several countries like China, New Zealand, UK and France. The top ten countries of the world are contributing around 62 % of the world milk output till 2017.

The setup of modern dairy processing units with the application of scientifically advanced processing techniques and with several investments, innovations in all possible scale would make Nepal a major player in the world dairy market.

Table 1: Largest Milk producing countries in the world (FAOSTAT, 2019)

Rank in 2017	Country	Production (Million tons)					Share in percentage				
		1985	1995	2005	2015	2017	1985	1995	2005	2015	2017
1	India	44.02	65.37	95.62	155.69	176.27	8.58	12.10	14.75	19.43	21.32
2	USA	64.93	70.44	80.25	94.634	97.76	12.65	13.04	12.38	11.81	11.82
3	Pakistan	10.86	19.01	29.44	41.59	44.29	2.11	3.51	4.54	5.19	5.35
4	China	4.76	9.46	32.02	36.28	34.87	0.92	1.75	4.94	4.52	4.21
5	Brazil	12.57	17.13	25.53	34.86	33.74	2.45	3.17	3.94	4.35	4.08
6	Germany	33.63	28.63	28.48	32.71	32.69	6.55	5.30	4.39	4.08	3.95
7	Russian Federation	0.00	39.31	31.15	30.79	31.18	_	7.27	4.80	3.84	3.77
8	France	28.40	26.069	25.71	25.93	25.26	5.53	4.82	3.96	3.23	3.05
9	New Zealand	7.88	9.29	14.64	21.94	21.37	1.53	1.72	2.25	2.73	2.58
10	UK	16.02	14.84	14.47	15.32	15.26	3.12	2.74	2.23	1.91	1.84
Nepal's share to global milk production											
	Nepal	0.81	1.01	1.35	1.86	2.05	0.15	0.18	0.20	0.23	0.24
	World	512.98	540.07	648.22	801.13	826.75	100	100	100	100	100

1.2. Dairy Sector Scenario in Nepal

As the dairying sector in Nepal is in the developing stage, its position in terms of per capita availability is one of the lowest. The per capita availability of milk was about 132.88 gm per day in 1985 which has declined to 129.30 gm per day in 1995. However, the present level of per capita availability is 158.9 gm which is much lower than the recommended value of WHO (250gms) and even less than 220 gm recommended by the Nutritional Advisory Committee of the Indian Council of Medical Research (ICMR). The current milk production of Nepal as per the report of Food and Agricultural Organization in the year 2017 is 2.05 MT.

As per the statistical information collected from Nepalese Agriculture- 2014/15, MoAD, total milk production in Nepal was 1724823 MT out of which 1153838MT is from buffalo and 468913 MT from cow. The total buffalo population was estimated to be 5133139 and that of cattle population was 557669. Likewise, the total milking cattle were 1025947 and milking buffaloes were 135164 and they produced 1167154 milk. Out of the total livestock population, only 13% of the cattle and 26% of the buffaloes are of improved breeds (NARC, 2016). Jersey, Holstein, Brown-Swiss, Ayrshire and Sahiwal and their cross-bred cows were the breeds of cows, whereas the buffaloes included local, improved such as Murrah and their crosses. Buffalo milk shares about 65% of the total milk production in Nepal (MoAD, 2016).

The decentralized system of governance in Nepal shifted from the development region to the province system in the year 2015.The data tabulated below in the table shows various milch animal population in different provinces of Nepal. Province no.1 holds the maximum cattle population whereas the maximum buffalo population is found in province no.5. Province no.2 doesn't hold any yak whereas the maximum yak and sheep population is hold by province no.6. Likewise, province no.1 holds the first position on the goat and pig population. The total cattle population as per the report of livestock statistics of Nepal,2017 is 6430397.

Table 2: Population of animals in various provinces of Nepal (Livestock statistics ofNepal,2017)

Province	Cattle	Buffalo	Yak/Chauri	Sheep	Goat	Pig
Province 1	1601707	455638	13007	48365	2277659	458723
Province 2	697881	424711	-	12118	1306800	27839
Province 3	832320	588984	11354	39836	2484855	84763
Province 4	476367	526689	10664	67954	1283467	81939
Province 5	1040251	675601	11	134320	1835436	134424
Province 6	717636	170314	13083	223272	994927	27471
Province 7	1064235	332452	746	87019	1041986	55038
Total	6430397	3174389	48865	612884	11225130	870197

Table 3: Animal Population Trend over different years in Nepal

(Livestock Statistics of Nepal, 2016)

Year	Total Animal	Total Milking Animal	% of Milking Animal
2005/06	11207802	1988140	17.74
2006/07	11411092	2033166	17.82
2007/08	11587221	2073711	17.90
2008/09	11855684	2144371	18.09
2009/10	12036244	2207450	18.34
2010/11	12219700	2265766	18.54
2011/12	12378083	2330000	18.82
2012/13	11515895	2395387	19.14
2013/14	12422528	2370350	19.08
2014/15	12409480	2371111	19.11
2015/16	12471617	2381519	19.10
2016/17	12525485	2539041	20.27

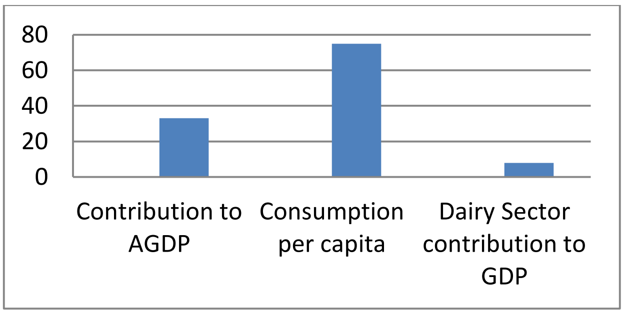
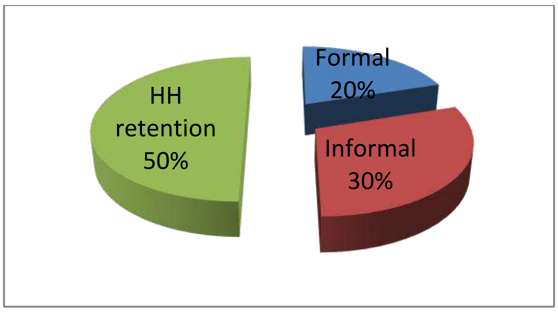


Fig 1: Nepal Dairy sector at a glance.



HH Retention: Household Retention

Fig 2: Distribution of Milk (COMP-NDDB,2017)

From the bar graph mentioned above it is clear that, Nepal is an importer of milk and milk products to fulfill the present needs of its population. Milk, cream powder, butter, butteroil, cream liquid and cheese are the major products that are imported to Nepal. Dairy sector contributed nearly 33 percent of the AGDP (Agricultural Gross Domestic Product) and 8 percent of total GDP. The buffalo population in Nepal bears a considerably shorter lactation period of 242days, whereas the cows of Nepal has an average lactation period of 286 days which is sound.

1.3. Ration and its effect on milk production in Nepal

Balanced Ration is the quantity of feed that provides the necessary nutrients required for proper growth, development, gestation and lactation of animals. In Nepal, crop residues, straw of wheat and rice, stovers of maize, leaves of trees and other green fodders cultivated by farmers are the prime sources of feed for animals. Beside these, maize is used as concentrate followed by brans of rice and wheat, oilseed cakes of soybean, mustard, sunflower and other byproducts of legumes. These concentrates used are not sufficiently produced in Nepal. Thus, they are imported from India and other countries. Grains of legumes are substantial source of protein. Osti et. al, (2013) found that milk production was less (8 kg/d/head) prior to bypass protein (BP) feeding, while higher during BP feeding (10.0 kg/animal/day) was provided. The feed supply of Nepal is not sufficient to meet the demand of dairy animals. There is shortfall of 38% in crude protein, 42% in metabolizable energy and 33% in dry matter (Osti,2020).

Table 4: Crop residues and by-products available (%) in Nepal (MoAD,2013)

Crops	Main Product	Residues	Oil Meals	Oil Cakes	Bran/Husk
Rice	14.99	9.27	-	-	-
Maize	66.56	82.30	-	97.71	76.28
Millet	1.018	1.68	-	-	-
Wheat	5.75	3.55	-	-	6.57
Barley	0.113	0.07	-	-	17.15
Buckwheat	0.033	0.021	-	-	-
Oilseeds	0.596	0.143	99.95	0.76	-
Sugarcane	9.75	1.01	-	-	-
Cotton	0.005	0.000069	0.05	0.000732	-
Pulses	1.19	1.96	-	1.53	-
Total	100	100	100	100	100

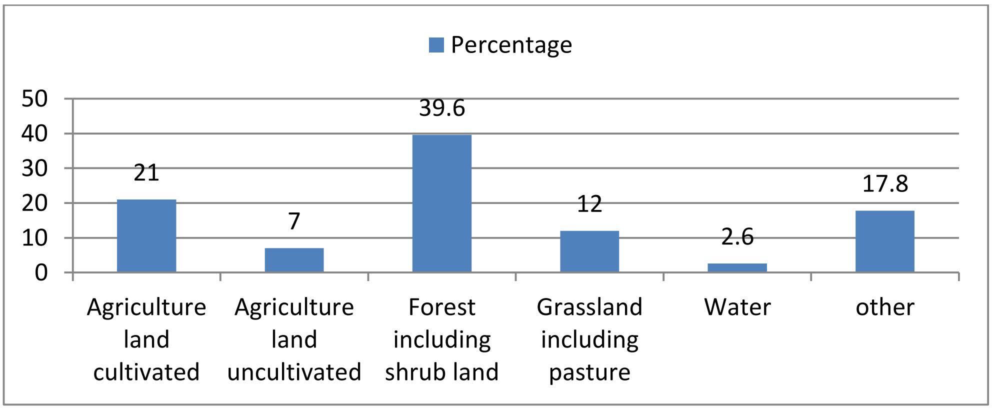


Fig 3: Land Use Statistics in Nepal

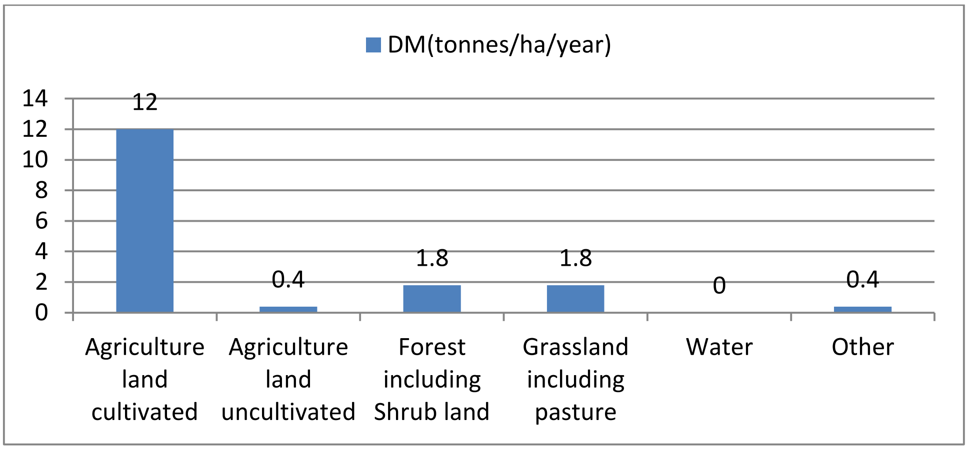


Fig.4: DM (Dry Matter) in tonnes/ha/year in different land use pattern

When we see prior to 100years then it is found that people used to feed only roughages to their animals and later use of concentrates increased the yield of milk and then the concept of total mixed ration (TMR) was popular and it is found that animals supplemented with TMR were found to be free from nutritionally related off feed, milk fat depression and indigestion problems (Schingoethe, 2017) .Total mixed rations are commonly being fed to dairy animals in other countries but still people in Nepal are compelled to feed roughages to their animals due to poor quality, lesser availability and higher prices of the concentrates (De Vries and Kaylegian, 2018).

1.4. Pattern of Flow of Raw milk

The latest milk production of Nepal as per the data obtained in the year 2017 was 2.05 MMT (FAOSTAT, 2019). Out of the total milk production, only 20% of the total milk was utilized by the formal sector (NEPC, 2017).

The milk is produced by the dairy farmers and the surplus milk is distributed either via formal and informal trading methods. The formal sector in Nepal comprises of the MPCS (Milk Producers Cooperative Society), MCC (Milk Chilling Centers), Milk processing plants and dairy cooperatives. The flow of milk in the formal sector passes from the MPCS to the MCC and finally to the milk processing plants. The milk producer's cooperative society is the organization comprising of the dairy farmers and functions to collect the raw milk at village and grassroot level. The milk collected from the MPCS is transported to the milk chilling centers where they are chilled and temporarily stored. From the MCC the milk is then transferred to the milk processing plants in the large tankers which are facilitated with adequate refrigeration requirements. Similarly, a small volume of milk directly passes from MPCs to Cow milk cheese factories and from farmers to the Yak cheese factories (FAO, 2010).

Tracking the milk passing through various channels either formal or informal is not an easy task in Nepal due to the lack of advances in the traceability system. The milk in Nepal is mainly supplied to the informal sectors which accounts for approximately 80% of the total annual milk production. In the informal sector, the milk is mostly handled by the individual households, tea shops and the sweet meat shops. The vector for the informal milk trading in Nepal is mostly individual farmers and the milk contractors. This pattern of flow of raw milk in Nepal can be illustrated via a chart shown below.

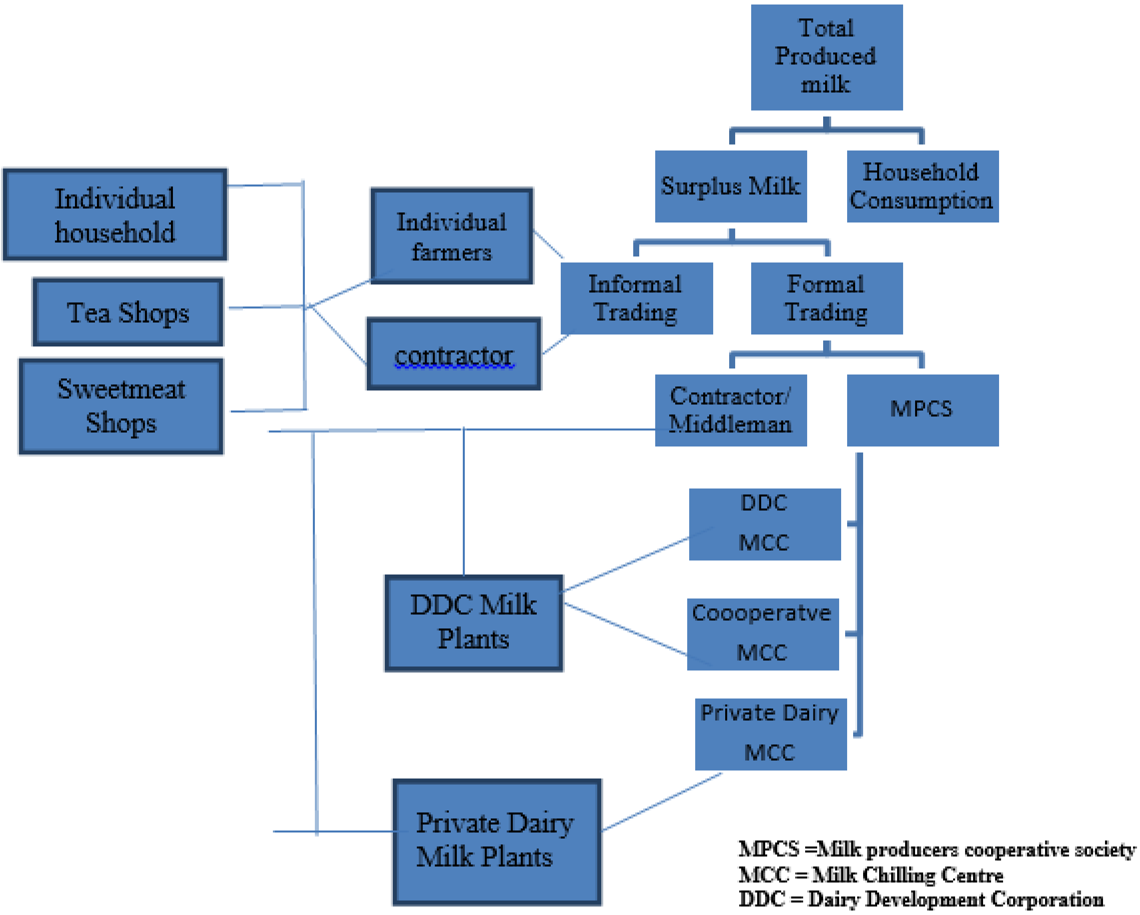


Fig 5: Flow patterns of milk in Nepal (FAO,2010)

1.5. Milk Production and Per Capita Availability Projection

According to the data taken from FAOSTAT, (2019) and (Nepal Population, 2020), the annual milk production and the human population in the year 2017 were 2.05 MMT and 27632681 respectively. The per capita availability was calculated by dividing the total milk production with the human population and was expressed in grams per day. The per capita availability was found to be 203.49 gm per day in the year 2017 which was far less than the recommended value of WHO (250 gm). It has been estimated that the annual milk production growth rate of Nepal is 3.09% (Upadhyay, 2017) and the population growth rate is 1.35% per annum (Nepal Population, 2020).

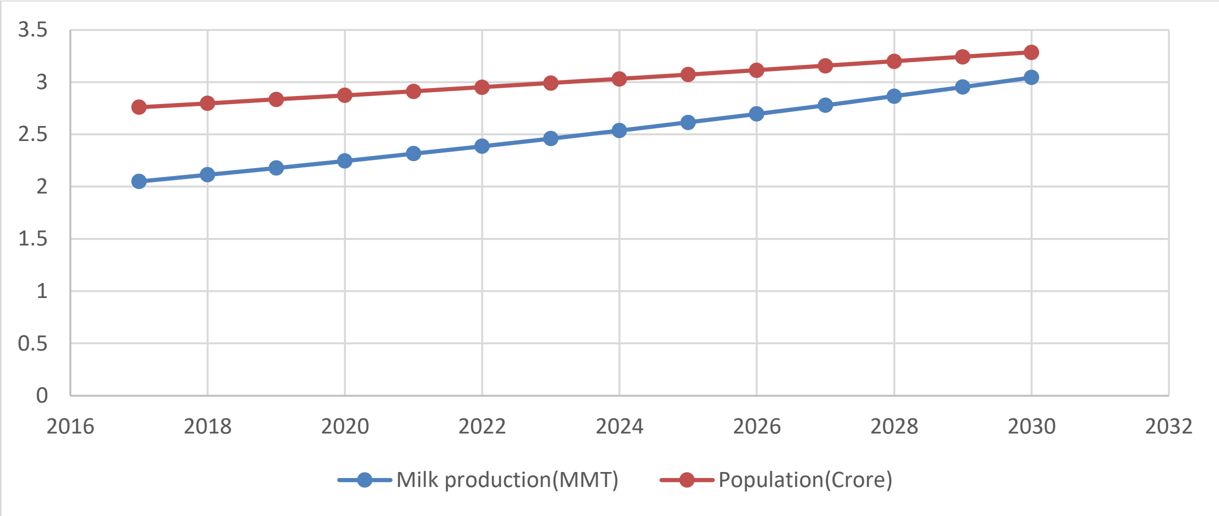


Fig 6: Projection on Annual Growth Rate Required to meet Milk Consumption Recommended By FAO/WHO By 2030.

The projected value of milk production was calculated by considering 3.09% annual growth rate i.e. current rate and the total milk production for the year 2017-2030 were extrapolated. Likewise, the human population for the various years were estimated by considering 1.35% annual growth rate and the population for the year 2017-2030 were extrapolated. The population and the milk production for the year 2030 was estimated to be 3.28 crores and 3.044 MMT respectively. The value of the per capita availability determined on the year 2030 is 253.89 gm per person per day which is just above the recommended value provided by WHO (250gms). Thus, with the same growth rate in milk production (3.09% per annum) and population (1.35% per annum), the recommended value of milk availability would be achieved by 2030.

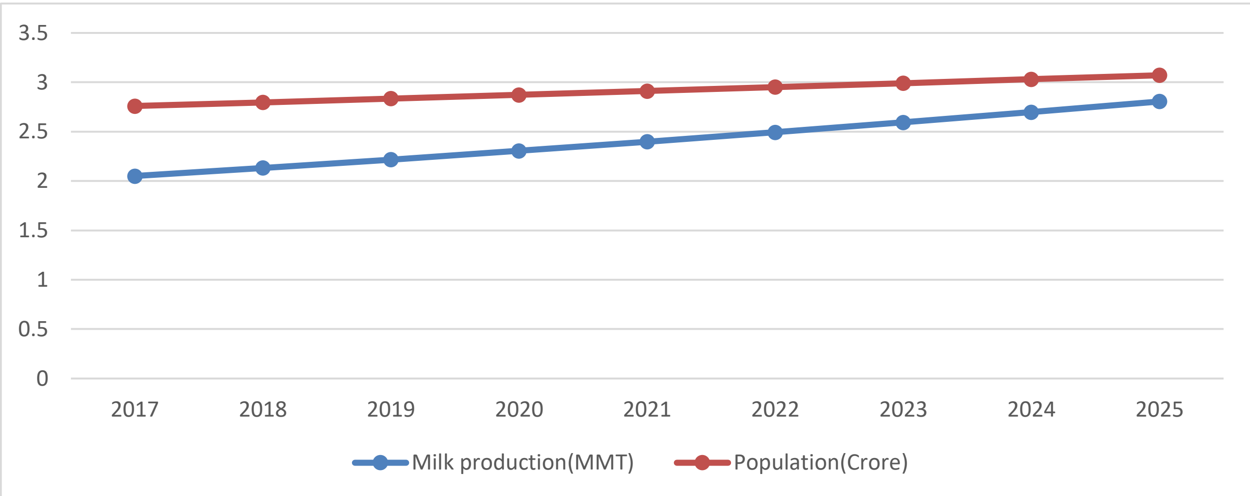


Fig.7: Projection on Annual Growth Rate Required to meet Milk Consumption Recommended By FAO/WHO By 2025

Figure 7 represents the projection on annual growth rate required to meet the milk consumption recommended by FAO/WHO by 2025. In order to attain the recommended value of per capita availability by 2025, the current milk production growth rate has to be increased to 4% per annum assuming the static population growth rate i.e. 1.35% per annum. The total milk production in the year 2025 at 4% annual growth rate would be 2.80 MMT while the population at that period of time would be 3.07 crores.

Hence, the value of per capita availability estimated by dividing the total milk production by total population is 250.16 gm per person per day which is just above the recommended value of WHO (250gms).

1.6. Limitation and Opportunities of Dairy sector in Nepal

According to the World Bank collection of development indicators, the rural to urban population ratio of Nepal is approximately 8:2 i.e approximately 80% of the Nepalese reside in the rural sector. A major fraction of rural population of Nepal seem to be engaged in agriculture and livestock rearing which makes the rural sector a major milk producer in the nation. But because of the lesser availability of the assured market, the producers do not have an incentive to invest in good breeding stock, feeds, or veterinary medicine and services. Lack of these inputs affect the productivity which eventually reduces profit (Sharma and Banskota, 2002). Moreover, the dense population, lower milk production and availability causes the demand escalation of milk and milk products in the urban region. Similarly, because of the low purchasing power of the consumers, the effective demand for milk at local levels is also low. Hence, it is necessary to transport milk to major urban areas for marketing. At the same time the price the cooperatives pay to farmers does not match growing feed prices (Shrestha, 2000). Nepal has tremendous potential for increasing dairy livestock production and productivity. Buffalos in Nepal are also slaughtered for the production of meat and meat-based food products. Buffalo meat accounts for 54% of the total meat produced in the country (MoAD, 2016). Milk market in formal sector or organised sector contributes about 20% of the total annual milk produced in the country (NEPC,2017).The bovine population of 9604786 produces 2.05 million tonnes of milk annually (per capita availability of milk is 158.9 gms per day), whose contribution is yet below the recommended value of WHO (i.e. 250gms per day). The production and productivity of cattle in Nepal is very low with 519.56 litre per lactation as compared to world average 2038kg per lactation, which requires a lot of improvement (Thompkinson and Sabikhi, 2012).

Hence, there is need of great improvement in the dairying and animal husbandry systems in Nepal. There is huge variation in productive and reproductive performance of cattle in Nepal. To make dairy sectors more commercial there is need and opportunities to increase productive and reproductive performance of cattle which can be achieved by cross breeding and hybridization (Paneru *et al.*, 2015).

All over the country, the cattle and buffalo population are evenly distributed. It creates opportunity for the farmers to generate benefits from longer duration of lactation in cattle and high fat content of buffalo milk, both the factors leading to sound income. Huge population density in urban areas has created a significant demand for milk and dairy products which is impacting the milk market to grow and flourish (FAO, 2010). The increasing number of dairy plant schemes under DDC and strong channel of dairy cooperatives from the grassroot level to the central level has strengthened the dairy industry and provided a supportive environment to the dairy farmers. The influencing activities of NGOs like providing technical support, veterinary care service and involvement of private dairy sector has become sensational support and hope to the small-scale milk producer (Sharma and Banskota, 2002).

Despite the opportunities mentioned above, there are several other constraints relating to dairy sector development in Nepal. The depletion of animal feeding base resulted due to the deterioration of the forest areas for the various purposes like timber and fuel wood has affected the dairy sector adversely (Pande,1997). The unavailability of green fodder and quality feed adversely affects animal productivity. Moreover, poverty and illiteracy among the livestock raisers severely hits their ability to respond to the new opportunities and cope with the dynamic situation. Illiteracy complicates the extension learning process as they require more face to face communication. Above all, the uncommonness of the heifer rearing practice and lack of cost-effective heifer rearing technology has resulted in the critical shortage of the productive dairy animals (Sharma and Banskota, 2002). Upadhyaya et.al, (2000) studied the scenario of the dairy cooperatives in Nepal and concluded that the dairy cooperatives do not buy milk from their regular suppliers during the flush seasons. Those days of the week, when the private and public dairies do not buy milk from the dairy farmers are termed as “milk holidays.” The main reason behind the milk holidays could be the lesser demand of milk and milk products among the consumers and lack of milk storages capacities in the processing plants. With an aim to compensate a possible milk holiday, Nepal is going to export 30000 litres of milk to India. Due to low domestic demand during the flush period exporting milk is an effective measure to avoid the problem. Production cost of milk in Nepal in comparison to India is naturally higher as Nepal is dominated by non-commercial farmers. Milk production drops by almost 30% during lean season (April-August) which becomes insufficient to fulfill the market demand as milk consumption is going high. The deficit in milk production can be well combated by promoting powder milk as an alternative source of milk production as well as good support from government in building farmers capacity and introducing better dairying technologies. Also, low milk price is one of the major limitations in Nepal followed by lack of proper government milk policy and inadequate milk processing industries (Timsina and Regmi, 2009).

1.7. Dairy Co-operatives and their challenges

The establishment of dairy cooperatives in Nepal was the result of the implementation of the first five-year plan in the year 1956-61. The first dairy cooperative was formed at Tusal village of Kavre district. Though the dairy cooperative activities got initiated in the early 60s' their effective activities were observed only after December 1981, when DDC initiated the milk producers-oriented program by participating the farmers to form their own Milk Producer Association (MPAs). The MPAs thus formed were not provided with the legal status and they functioned for milk trade and support to milk production. Later on, MPAs were transformed into Milk Producers cooperative society (MCPS) in February 1989 to make them function autonomously. The MPCS are governed by cooperative Act 1992. The functions of these MPCSs is to gather milk from the dairy farmers, test its quality, transport it for selling to the nearest milk processing plants, receive payment for the milk and distribute the payment to the individual milk supplier farmer. The dairy cooperatives in Nepal functions in 3-tier system (FAO,2010).

The first tier is MPCSs primary level cooperatives, second level is District Milk Producers Cooperative Unions (DMPCUs) of different MPCSs as District bodies. Their main theme is to deliver programs designed to support the increased production and processing of milk and milk products and also to contribute to the financial and social upliftment of the rural milk producers. The third tier is Central Dairy Cooperative Association Limited Nepal (CDCAN). CDCAN is registered as their central-level cooperative organization established in 1993, mainly focuses on increasing economic benefits to the milk producers and making the country self-reliant in clean and high-quality milk and milk related products. Moreover, it also implements policy advocacy activities at the central level to represent the interest of member organizations (Upadhyay et.al., 2001).

The milk processing plants functions for the processing of raw milk and manufacturing the value-added products from the milk. They are the key elements of the formal sector of milk distribution. They help in maintaining a regular standard and balance of milk distribution throughout the region. They are the bridge via which the rural milk and resources and urban capital can be interlinked. The dairy sector in Nepal is characterized by scattered, small scale, unorganized milk animal holders; inadequate and inappropriate animal feeding and health care; low productivity; an inadequate basic infrastructure for provision of production in puts and services. Moreover, lack of an assured year-round remunerative producer price for milk, inadequate basic infrastructure for collection, transportation, processing and marketing of milk is another aspect of the Nepalese dairy sector. Low productivity of the milch animals is a serious constraint to the dairy development in Nepal (GOEC Nepal, 2012).

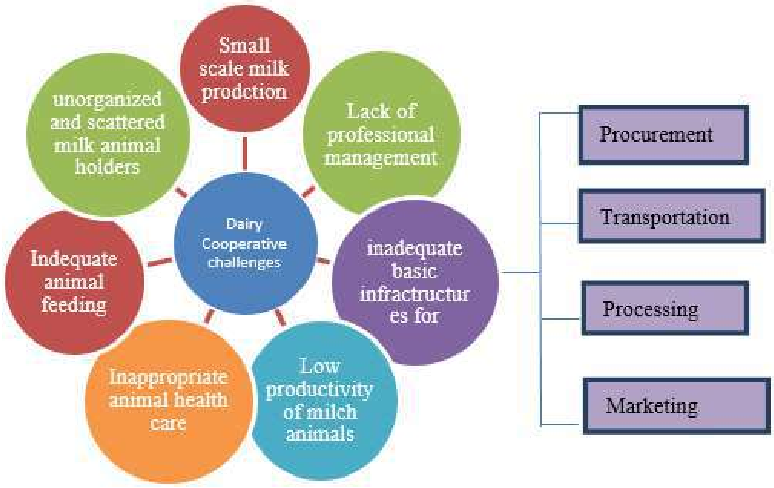


Fig. 8: Challenges faced by the Dairy Co-operatives

1.8. Sustainable remedial measures and suggestions

To overcome the challenges faced by dairy cooperatives and to strengthen the entire dairy sector, the principles of sustainable development is to be taken in account. For upgrading dairying to a larger scale, firstly the farmer's dairy capacity and needs should be improved. Farmers need to improve the quality standards of milk, which would require a strategy to motivate them that dairy is a profitable business other than an optional business. (KUBK-ISFP, 2015).

The combination of proper provision of animal breed, nutrition, health care, processing and marketing is key for bringing success in dairy sectors. Awareness program should be initiated to provide knowledge about animal science and sanitation (Santra, 2018). Moreover, the government should direct, coordinate and regulate the activities of institutions and organizations involved in dairy sector to create and provide favorable environment for small scale dairy farmers (Pant,2017).

There is an utmost need for the disease control mechanisms to be developed as epidemics of FMD, black quarter, HS and other adversely animal health, which eventually reduces milk production and effects the entire dairy sector. Special and aseptic transportation utensils should be assessed by the producers for the safer handling and delivery for the products thereby minimizing the risks of food contamination and spoilage. In this way, consumers can get milk from their choice. Incentives for better quality milk could be suggested around the collection centres on competitive basis. Further, transparent pricing system for respective cattle and buffalo milk could be encouraged and adequate pricing should be offered based on the fat and SNF content. This might eventually reduce the mal adulteration practices.

Application of block chain technology should be introduced and promoted for ensuring efficient traceability and food security (Shingh et. al, 2020). Adequate trainings should be offered to the farmers regarding the advanced technologies and systematic cattle rearing for generating efficient and active man powers in the dairy sector. The income and price elasticity of consumers should also be considered in the long run for a better pricing system (KUBK-ISFP, 2015)

The total mixed ration can be used to feed the animals in order to get higher milk production. This is in line with the findings of (Schingoethe, 2017). The proper provision of animal breeds, nutrition, health care, processing, marketing along with awareness program is key for bringing success in dairy sectors. This suggestion is in line with (Santra, 2018).

V. CONCLUSION

The present study results revealed that the daily milk production in Nepal had been found in small scale and suffering from many obstacles. The per capita availability of milk in Nepal (158.9 grams per day) is far below than the value recommended by WHO (250 grams per day). The intermittent supply of milk via the formal channels causes the dairy cooperatives to function inefficiently which adversely affect the country's GDP. Dairy sector contributed nearly 33 percent of the AGDP and 9 percent of total GDP. The dairy sector of Nepal is emerging, and its share to the global milk production is very low (0.247 percent of the global milk share). By implementing the solutions and remedies discussed in the paper and via active people's participation, Nepal can be a major player of the dairy sectors in the days to come.

REFERENCES

[1] CBS, (2012). National population and housing census, Government of Nepal, National planning commission secretariat, national report, Volume 1

[2] COMP-NDDB, (2017). Study on cost of Milk production, Final report, NEPC Research Pvt. Ltd.

[3] Deshmukh, M.S, (2014), Growth and performance of dairy sector in India, *Voice of Research*,3(2),39-44.

[4] De Vries, A. and Kaylegian, K. (2018). Rapid assessment of the gaps in dairy cattle feeding, management and milk processing that constrain milk quality and quantity in Nepal. Gainesville, FL, USA: Feed the Future Innovation Lab for Livestock Systems

[5] FAO (2010). Food and Agriculture Organization of the United Nations. Dairy Sector Study of Nepal. http://nepalagritech.com.np/

[6] FAOSTAT (2019). Milk production across Countries. Retrieved from https://www.nddb.coop/information/stats/across

[7] GOEC Nepal (2012). Milk marketing strategies study, Final report, National dairy development Board, Harihar bhawan, Lalitpur, Nepal. Retrived from : http://nddb.gov.np/download/publications/Milk%20Marketing%20Strategy%20Study-Report,2012.pdf

[8] KUBK-ISF, (2015). Study and analysis of Dairy value chain in Nepal, Retrieved from http://kubk.gov.np/authore_gov/images/Dairy%20Value%20Chain%20Report.pdf

[9] Landes, M., Cessna, J, Kuberka, L. and Jones, k. (2017). India's dairy sector: structure, performance and prospectus, a Report from economic research service, USDA

[10] Livestock statistics of Nepal, (2016). Ministry of livestock development, Planning, monitoring and evaluation division.

[11] Livestock statistics of Nepal (2017). Ministry of livestock development, Panning, monitoring, and evaluation division.

[12] MOAD (2013). Statistical Information on Nepalese Agriculture. Ministry of Agricultural Development. Agri-business Promotion and Statistics Division. Agri-Statistics Section. Singha durbar, Kathmandu, Nepal.

[13] MoAD (2014/15). Statistical information on Nepalese Agriculture, Ministry of Agricultural Development, Agribusiness Promotion and Statistics Division, Agri Statistics Section, Singha durbar, Kathmandu Nepal.

[14] MOAD (2016). Statistical Information on Nepalese Agriculture. Ministry of Agriculture Development, Singh Durbar, Kathmandu, Nepal.

[15] MOALD (2019). Statistical Information on Nepalese Agriculture. Ministry of Agriculture Development, Singh Durbar, Kathmandu, Nepal.

[16] Ministry of Finance (2015). Economic Survey, Fiscal Year 2014/2015. Government of Nepal.

[17] NARC (2016). National Agricultural Research Council. Animal Breeding Division. Retrieved from: http://narc.gov.np/animal-breeding-division/

[18] Nepal Population (2020). Retrieved July 07, 2020, from https://www.worldometers.info/world-population/nepal-population

[19] NEPC (2017). Study on efficiency of dairy industries in Nepal, final report, Nepal environment protection center. RFP no.: NDDB/C/RFP/2074/75/02

[20] Neupane N, Neupane H and Dhital, B (2018). A socioeconomic view of status and prospects of goat farming in rural areas of Nepal. *J. Inst. Agric. Anim. Sci* **35**:1-8

[21] NRB. (2018). Nepal Rastriya Bank Monetary Policy 2018/19. Kathmandu: Nepal Rastriya Bank.

[22] Osti, N.P, (2020). Animal Feed Resources and their Management in Nepal. *Acta Scientific Agriculture* 4.1 :02-14.

[23] Osti N.P., Mandal P, Bholas and Shrestha (2013). Milk Yield Response of Bypass Protein feeding (soybean meals) in Dairy animals. *Buffalo bulletin*, 32(2):834-839

[24] Pande, R.S (1997). Fodder and pasture development in Nepal. Udaya Research and Development Services (P) Ltd. Kathmandu, Nepal, pp 5.

[25] Paneru U, Sharma M, Kolachhapati MR and Shrestha VS (2015) Evaluation of Productive Performance of Cattle in Dairy Pocket Areas of Chitwan and Nawalparasi Districts, *J. Inst. Agri C. Anim. Sci*, **33:** 207-212

[26] Pant, K R. (2017). Cattle farming in Nepal: Why farmers cry. Retrieved from https://www.dairyglobal.net/Markettrends/Articles/2017/9/Cattle-farming-in-Nepal-Why-farmers-cry-183891E/

[27] RAN (2015). Benchmark survey on quality of raw and processed milk in Nepal,,final report, RFP no.: NDDB/C/RFP/2072-73/01, Right to Access Nepal

[28] Santra, S. (2018). Challenges Faced by The Indian Dairy Sector. Retrieved from: https://www.franchiseindia.com/wellness/challenges-faced-by-the-indian-dairy-sector.11159

[29] Sharma, B. and Banskota, K. (2002). Smallholder Dairy Farming in Nepal: Characteristics, Constraints, and

Development Opportunities, Chapter 5, In: Smallholder Dairy in Mixed Farming Systems of the Hindu Kush-Himalayas. Issues and Prospects for Development, HKH, Publisher,73-92

[30] Shrestha, H.R., (2000). Small Holder Dairy Farming Systems at Milk Shed Area of Kathmandu Milk Supply Scheme, a study undertaken for Centre for Resource and Environment Studies (CREST) and presented to ICIMOD, Kathmandu, Nepal.

[31] Schingoethe, D. J. (2017). A 100-Year Review: Total mixed ration feeding of dairy cows. *Journal of dairy science*, *100*(12), 10143-10150.

[32] Shingh, S., Kamalvanshi, V., Ghimire, S., & Basyal, S. (2020). Dairy Supply Chain System Based on Blockchain Technology. *Asian Journal of Economics, Business and Accounting*, *14*(2): 13-19, DOI: https://doi.org/10.9734/ajeba/2020/v14i230189

[33] Thompkinson, D.K. and Sabikhi, L., (2012). Quality Milk Production and Processing Technology, New India publishing agency, pp.4.

[34] Timsina, K. and Regmi, P.P., (2009). Production and marketing dynamics of milk: A case of Chitwan district, Nepal. *Journal of Institute of Agriculture and Animal Science*, 30, 33-43.

[35] Tiwari, P., and Shingh, S., (2020). Gender Inequality in Nepalese Agriculture: Issues Concerning Sustainability and Food Security, *International Journal of Environment, Agriculture and Biotechnology*, 5(2):451-458, DOI: https://dx.doi.org/10.22161/ijeab.52.19

[36] Upadhyay, N.D., Timsina, K. P., Gairhe, S., Sapkota, S., Acharya, Y. and Khadka, S., (2017). Growth of livestock sector in Nepal: A perspective on agriculture perspective plan, proceedings of the 10th National workshop on livestock and fisheries research in Nepal, retrived from:file:///C:/Users/Hp/Downloads/GrowthoflivestocksectorinNepal.pdf

[37] Upadhyaya, R.M., Joshi, D.D & Thapa, T.B., (2001). History of dairy development in Nepal. National Dairy Development Board, Nepal.

[38] Upadhyaya, R.M., Singh, S.B., & Koirala, G.P., (2000). A Policy Review on Milk Holidays. Research Report Series No. 44. Kathmandu: Winrock International.

Phytoremediation: A way towards sustainable Agriculture

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***Abstract*— *Phytoremediation means utilizing the potential of a variety of plants to remediate soil, sludge, sediment and water (surface water and underground aquifers) contaminated with heavy metals at the point or non-point sources. Phytoremediation is solar energy-driven technology, eco-friendly and a cost-effective way of making soil and water pollutants free. It is a process of onsite remediation by using different biological processes of plants. Phytoremediation is known widely by different terms viz., green-remediation, botanic-remediation, agro-remediation, and vegetative-remediation, etc. Pollutants occur in different forms, like organic, inorganic, metallic and non-metallic, etc. Plants can be utilized for phytoremediation of heavy metal polluted soil and water resources. This review gives current understanding of the mechanism of heavy metal remediation by different plant species, therefore encouraging research and development in this area. Phytoremediation further needs a profound understanding of the underlying mechanism and requires pilot level as well as field level studies.*

***Keywords*— Phytoremediation, Sustainable agriculture, Heavy metals, Eco-friendly.**

I. INTRODUCTION

Phytoremediation is a dynamic process that eventually degrades or extracts pollutants in different proportions as they are hazardous and toxic to all living beings. The pollutants are degraded either through accumulation, filtration or dissipation. In the current scenario, the need of the hour is realizing the consequences of heavy metal pollutants in soil and water (Kaur, 2018). Heavy metal pollutants, the half-life is much more than that of organic pollutants like pesticides and petroleum by-products. Uranium in groundwater aquifers of Rajasthan is a great concern to environmentalists as in some or other ways it is affecting the natural occurrence of biogeochemical cycles (Daud *et al.*, 2018). As different phases of the industrial revolution have passed, a variety of remediation technologies have also come into the market to deal with a variety of pollutants. Out of these contaminants, heavy metals like Uranium pose a great threat to the surrounding environment (Papazogloua and Fernandob, 2017). Due to mining and milling, radionuclide contaminants is prevalent in subsurface sediments throughout India. Due to the overexploitation of underground water resources, the water level has declined beyond environmentally acceptable and recoverable levels. Subsequently, heavy metals have found its way to the subsurface level thus entered into water and soil, contaminated them as a result of waste-disposal practices (Bora and Sarma, 2020).

Table 1: Different Plants showing Phytoremediation Potential

Metal	Plant	Mechanism	Medium	Reference
Zn, Cd	*Thlaspi caerulescens*	Phytoaccumulation	Soil/Water	Robinson *et al.*, 1998
Trinitrotoluene (TNT)	*Myriophyllum aquaticum*	Phytoextraction	Hydroponic	Bhadra *et al.*, 1999

Ethanol blended Gasoline	*Weeping willow*	Rhizofiltration	Water	Corseuil and Fabio, 2001
TNT, Pyrene, Aroclor 1248	*Festuca arundinacea Schreb.*	Phytoextraction	Soil	Chekol and Vough, 2002
Polycyclic Aromatic Hydrocarbons (PAHs)	*Clover and Ryegrass*	Phytoextraction	Soil	Joner and Leyval, 2003
Polycyclic Aromatic Hydrocarbons (PAHs)	*Meliotus officinalis*	Phytoaccumulation	Soil	Parrish *et al*., 2004
Zn, Cd, Cu	Agricultural Crops, Woody Plants	Phytoaccumulation	Soil	Kayser *et al*., 2004
Se	*Brassica juncea* L.	Phytoextraction	Soil	Banuelos *et al*., 2005
Herbicides	Transgenic *Oryza Sativa*	Phytoextraction	Soil	Kawahigashi*et al*., 2006
Hg	Chloroplast Tobacco Transgenic	Phytoaccumulation	Soil	Hussein *et al*., 2007
Hg	*Polypogon monospeliensis*	Phytoextraction	Soil	Su *et al*., 2008
Pb	*Scrophularia canina*	Phytoextraction Phytostabilization	Mining Site	Cao *et al*., 2009
Pb, Zn, Cd	*Common sunflower*	Phytoaccumulation	Soil	Mukhtar *et al*., 2010
Radionuclides	*Wolffia arrhiza*	Rhizofiltration	Water	Louis *et al*., 2010
Cr	*Switch grass*	Phytoextraction	Soil	Li *et al*., 2011
Ar	*Ludwigia octavalvis*	Phytoextraction	Soil	Totah *et al*., 2012
Heavy Metals	*Salsola soda*	Phytoextraction Phytostabilization	Soil	Lorestani *et al*., 2013
Cd, Ni,Cu	*Indian hemp*	Phytoaccumulation	Soil	Girdhar *et al*., 2014
Cu,Zn	*Phalaris arundinacea* L.	Phytoextraction	Water	Polechonska and Klink, 2014
Co, Cr	*Pennisetum purpureum*	Phytoaccumulation	Soil	Lotfy and Mostafa, 2014
Heavy Metals	*Annual wageweed*	Phytostabilization	Soil	Lum *et al*., 2014
Cd, Pb, Zn, Cu	*Paulownia*	Phytoaccumulation	Soil	Tzvetkova *et al*., 2015
Pb, Ni	*Brassica nigra*	Phytoextraction	Soil/Water	Singh *et al*., 2015
Ar	*Pteris vittata*	Phytoextraction	Soil/Water	Lampis *et al*., 2015
Ar	*Lupinus microcarpus*	Phytoaccumulation	Soil	Diaz *et al*., 2016
Cd	*Tradescantia pallida*	Phytostabilization	Soil/Water	Areekijseree *et al*., 2016
U	*Carex nebrascensis*	Phytoextraction	Water	Gaikwad and Gavande, 2017

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Heavy Metals	*Cannabis Sativa*	Phytostabilization	Soil	Kumar *et al*., 2017
Landfill Leachate	*Lemna minor*	Phytoextraction	Soil/Water	Daud *et al*., 2018
Cu	*Bruguiera cylindrica* L.	Phytostabilization	Soil	Sruthi and Puthur, 2019
Heavy Metals	Native Herbaceous Macrophytes	Phytostabilization	Wetlands	Bora and Sarma, 2020

II. PHYTOREMEDIATION

The underlying methods encompassing phytoremediation are degradation, accumulation, dissipation, immobilization, etc. The different ways of phytoremediation are described individually in this review. The process of phytoremediation can be applied to a variety of potential pollutants, viz., petroleum hydrocarbons, organic contaminants, chlorinated compounds, heavy metals, radionuclides, agro-waste, pentachlorophenol (PCP), polycyclic aromatic hydrocarbons (PAHs), etc (Pivetz, 2001). The term hyper accumulator was coined and used for the first time by the late Professor, Robert Brooks (Brooks et al., 1980). Hyper accumulators as per Brooks and his co-workers are defined as plants that can accumulate different metalloids above the threshold concentration of 10,000 to 100 mg/kg (shoot dry weight) (Baker and Brooks, 1989; Brown *et al*., 1994). Moreover, a particular plant species will be called hyper accumulator, if it is able to accumulate more than 0.1 % of heavy metals of its dry weight (Kirkwood, 2002). However, the plant should show tolerance to heavy metals without having necrotic, chlorotic or any other cellular damage symptoms (Titah *et al.*, 2012; Kumar and Chauhan, 2016).

If plants accumulate more than 51 % of heavy metal of its dry weight overnight than it is considered as a potential agent for phytoremediation. The hyper accumulators are mainly documented from particular plant families, *viz*., Brassicaceae, Cunouniaceae, Caryophyllaceae, Asteraceae, Euphorbiaceae, Cyperaceae, Fabaceae, Lamiaceae, Violaceae, Poaceae, etc (Padmavathiamma and Li, 2007). A plant with phytoremediation potential has to have specific qualities, viz., high growth rate, high biomass accumulation, elongated and adventitious root system, high bioaccumulation coefficient, fantastic metal-accumulating strength, etc. Till now, around 500 plant species have been documented as hyper accumulators and recommended for phytoremediation of polluted soil and water resources (Bhaskar and Rajanna, 2018). Additionally, diverse plant species (crops as well as woody plants along with transgenic lines) showing phytoremediation capabilities are enlisted in Table 1.

Factors affecting Uptake Mechanism

1. Plant Species 2. Properties of Medium 3. Root Zone

4. Vegetative Uptake 5. Addition of Chelating Agent

III. PHYTOEXTRACTION

The literal meaning of phytoextraction is, "Phyto" meaning plant and "Extraction" meaning removing (Henry and Fabio, 2001) (as shown in Fig.1). The process of phytoextraction involves translocating the pollutants from the rhizosphere to different plant parts, viz., shoot, leaf, stem, flower, etc. Few plant species have the potential to extract both essential (Cu, Mg, Mo, K, Fe, Mn, Ni, P, and Zn) as well as non-essential metals (Se, B, Cd, Co, Cr, Ag, and Hg). Essential metals are those required by plants in optimum amounts for their growth and development, whereas non-essential metals are toxic even in low amounts (Tang *et al*., 2019; Gupta *et al*., 2020).

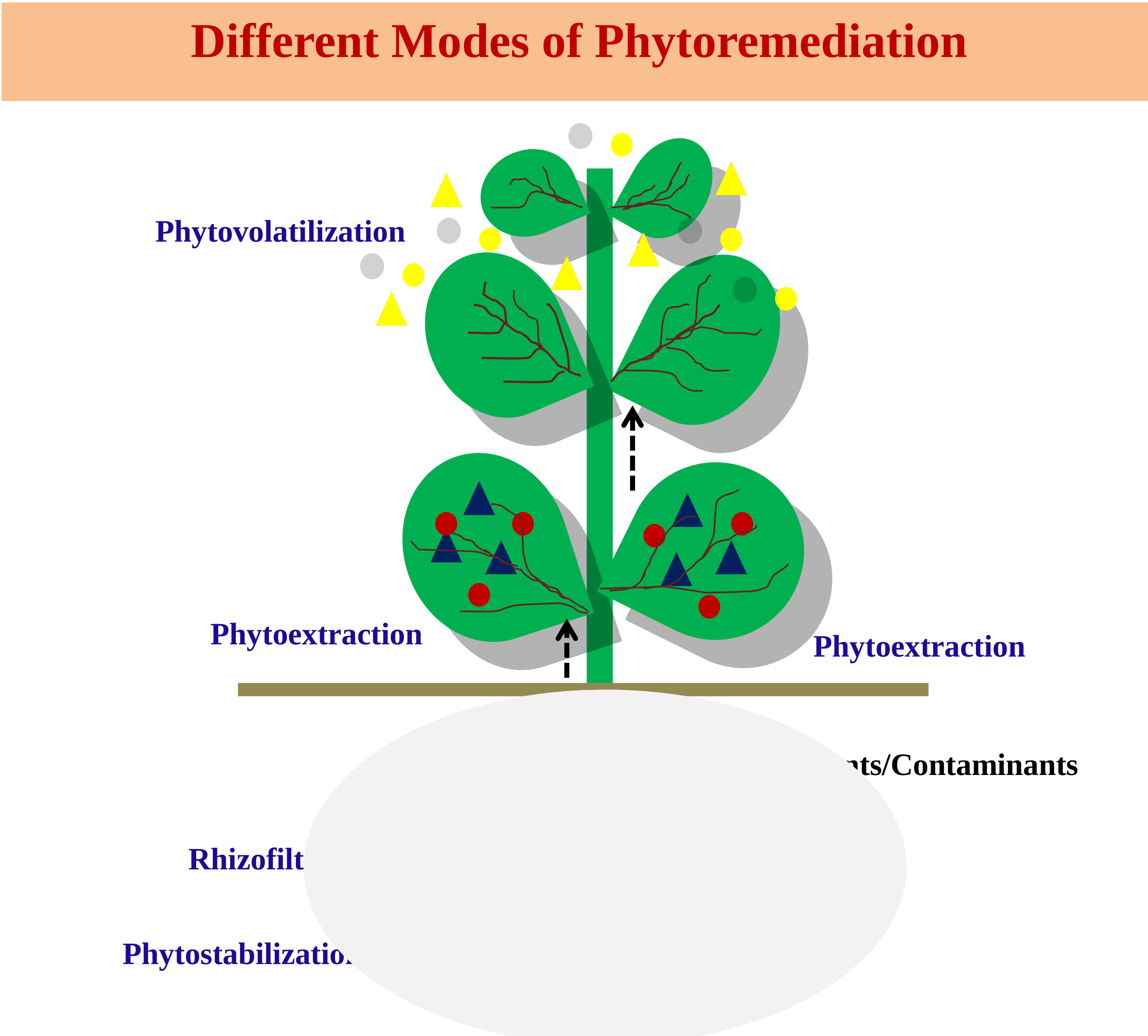

Fig.1: Different Modes of Phytoremediation

IV. RHIZOFILTRATION

The ability of plants to filtrate contaminated water aquifer, surface water, and wastewater with heavy metals, agri-waste (Pesticides and Insecticides) through a bunch of roots or adventitious root is known as Rhizofiltration (Akob *et al.*, 2007) (as shown in figure 1). Therefore, it is a modified phytoextraction method by using aquatic vegetation and adsorption of toxic elements primarily into the root zone (Beans, 2017). Initially, instead of soil, plants are grown in the hydroponic system and allowed to acclimatize in contaminated water under greenhouse conditions (Bibi *et al.*, 2016). Subsequently, plants are planted on the contaminated sites, where the adventitious roots will accumulate the toxins from the rhizosphere to roots and then transmission to the

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aerial parts (Cho and Choo, 2019). The factors affecting the rate of adsorption, concentrate, precipitate onto root surface are the concentration of hazardous elements and plants dry weight. A set of plants used for hemofiltration are sunflower, tobacco, spinach, rye and Indian mustard (Soliman and Sugiyama, 2016). The most widely used choice for hemofiltration is terrestrial plants as they possess fibrous roots and rapid growth rate. The process of hemofiltration can be applied to wetlands, ponds and constructed water tanks. The ultimate fate of rhizofiltered pollutants is rhizodegradation (degradation in roots) and then phytodegradation (degradation in aerial parts of the plant) (Gonzalez *et al.*, 2017).

V. PHYTOSTABILIZATION

It is the process in which plants store toxic metals at a particular site in a non-toxic metallic and immobile form, hence the metal is not able to mobilize to other organelles thus do not interfere with cellular metabolism (as shown in figure 1). Subsequently, the rate of migration of metals gets reduced (Oscar *et al.*, 2016). Therefore, the soil need not be free from contaminants and on-site phytoremediation can be done with potential plants whose roots are capable of growing under polluted soils and thus helps in metal immobilization through root adsorption, metal precipitation, complex formation or reduction (Barcel and Poschenrieder, 2003). Additionally, the metals are stabilized within plant cells from mobile and toxic to immobile form, for example, toxic Cr^{6+} gets transformed to Cr^{3+}, which is less mobile (James, 2001). The process of phytostabilization seems to be more efficient in the case of fine soils having high organic matter content (Berti and Cunningham, 2000).

VI. PHYTOVOLATIZATION

In this process, plants utilize transpiration to converts heavy metals from more toxic form to less toxic volatile form, thus eradicate pollutants from soil and water (as shown in figure 1). The metals that get volatized through transpiration are, Arsenic, Mercury (Hg, more toxic to Hg^{2+}, less toxic) and Selenium (Se, more toxic to $(CH_3)2Se$, 600 times less toxic), etc. Plant species that adopt the phytovolatilization process for removing contaminants are Arabidopsis thaliana and Musk grass.

VII. CONCLUSION

Phytoremediation of contaminated soil and water resources has proved to be a sustainable technology and emerged as one of the eco-friendly agriculture practices. Phytoremediation has a high potential when compared with other traditional and conventional approaches for heavy metal removal. A variety of plant species have shown high performance in hyper accumulation of heavy metals *viz.*, Cadmium, Copper, Mercury, Lead, Zinc, and Uranium, etc. Plants belonging to different families have different abilities to accumulate, detoxify and sequester a variety of heavy metals. However, the phytoremediation research studies are very few in number predominantly at field level. Hence, the need of the hour for phytoremediation research is on developing novel experimental design both at pilot as well as field level in polluted soil and water resources. Furthermore, the procedure for removal of heavy metals augmented biomass necessities to be additionally developed. Additionally, the current circumstances stresses on using the amalgamation and collaboration of traditional methods along with recent phytoremediation practices to deliver an advanced way of heavy metal remediation from both contaminated soil and water resources.

DISCLOSURE

The manuscript does not have any conflict of interest with any author, organization, institute, *etc*.

REFERENCES

[1] Akob DM, Mills HJ, Kostka JE. Metabolically active microbial communities in uranium contaminated subsurface sediments. FEMS Microbiol Ecol, 59: 95–107, 2007.

[2] Areekijseree M, Bunyakitjinda V, Euawong N, Srinark C. Effect of Cadmium accumulation on Purple Heart, (*Tradescantia pallida*): An investigation by SEI/EDX smiling program X-ray images. Veridian E-Journal, 3(6): ISSN 2408–1248, 2016.

[3] Baker A and Brooks R. Terrestrial Higher Plants Which Hyperaccumulate Metallic Elements, A Review of Their Distribution, E. Ecol Phytochem Biorecovery, 1, 1989.

[4] Banuelos G, Terry N, LeDuc DL, Pilon-Smits EAH, Mackey B. Field Trial of Transgenic Indian Mustard Plants Shows Enhanced Phytoremediation of Selenium-Contaminated Sediment. Environ Sci Technol, 39(6): 1771-1777, 2005.

[5] Barcel J, Poschenrieder C. "Phytoremediation: principles and perspectives," Contributions to Science, 2 (3): 333–344, 2003.

[6] Beans C, Science Writer. Phytoremediation advances in the lab but lags in the field. PNAS, 114 (29): 7475–7477, 2017.
[7] Bhadra R, Spanggord RJ, Wayment DG, Hughes JB, Shanks JV. Characterization of Oxidation Products of TNT Metabolism in Aquatic Phytoremediation Systems of *Myriophyllum aquaticum*. Env Sci & Tech, 33 (19): 3354-3361, 1999.
[8] Bhaskar K, Rajanna L. Phytosociological Analysis in Semi-Arid Region of India: A Case Study from Yadgir District, Karnataka. J Biodiversity, 9(1-2): 14-24, 2018.
[9] Bibi S, Jabeen R, Hayee AA. A Review: Phytoremediation: Using Aquatic Plants to Clean Up Polluted Water. Int J Current Research, 8 (8): 35561-35564, 2016.
[10] Bora MS, Sarma KP. Phytoremediation of Heavy Metals/Metalloids by Native Herbaceous Macrophytes of Wetlands: Current Research and Perspectives, 261-284, 2020.
[11] Brown SL, Chaney RL, Angle JS, Baker AJM. Phytoremediation Potential of *Thlaspi caerulescens* and *Bladder Campion* for Zinc- and Cadmium-Contaminated Soil. J. Environ Qual, 23: 1151-1157, 1994.
[12] Cao A, Carucci A, Lai T, Bacchetta G, Casti M. Use of native species and biodegradable chelating agents in the phytoremediation of abandoned mining areas. J Chemi Tech Biotechnol, 84 (6): 884-889, 2009.
[13] Chekol T,Vough LR. Assessing the Phytoremediation Potential of *Tall Fescue* and *Sericea Lespedeza* for Organic Contaminants in Soil. J Env Cleanup Costs, Tech, & Tech, 2002.
[14] ChoBW, Choo CO. Geochemical Behavior of Uranium and Radon inGroundwater of Jurassic Granite Area, Icheon,Middle Korea. Water, 11: 1278,2019.
[15] Daud MK, Ali S, Abbas Z, Zaheer IE, Riaz MA, Malik A, Afzal Hussain, Rizwan M, Zia-ur-Rehman M, Zhu SJ. Potential of Duckweed (*Lemna minor*) for the Phytoremediation of Landfill Leachate. J Chemistry, Article ID: 3951540, 2018.
[16] Gaikwad RS, Gavande S. Study of the Phytoremediation Process for Removal of Contaminants from Waste Water.Int J Sci and Res, 6, 2017.
[17] Gonzalez A, Gil-Diaz MM, Lobo CM. J Soils Sediments, 17: 1403, 2017.
[18] Gupta DK, Chatterjee S, Mitra A, Voronina A, Walther C. Uranium and Plants: Elemental Translocation and Phytoremediation Approaches. In: Gupta D., Walther C. (eds) Uranium in Plants and the Environment. Radionuclides and Heavy Metals in the Environment. Springer, Cham, 2020.
[19] Henry C, Fabio M. Phytoremediation potential of willow trees for aquifers contaminated with ethanol-blended gasoline. Water Res, 35: 3013-3017, 2001.
[20] Hussein HS, Ruiz ON, Terry N, Daniell H. Phytoremediation of Mercury and Organomercurials in Chloroplast Transgenic Plants: Enhanced Root Uptake, Translocation to Shoots, and Volatilization. Env Sci Technol, 41 (24): 8439-8446, 2007.
[21] Joner EJ, Leyval C. Rhizosphere Gradients of Polycyclic Aromatic Hydrocarbon (PAH) Dissipation in Two Industrial Soils and the Impact of *Arbuscular mycorrhiza*. Env Sci & Tech, 37 (11): 2371-2375, 2003.
[22] Kaur L. Accumulation potential of Indian mustard (*Brassica juncea var. arawali*) and fenugreek (*Trigonella foenum-graecum* L.) planted on Lead and Nickel contaminated soil. Tropical Plant Research, 5(2): 217–223, 2018.
[23] Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y. Phytoremediation of the Herbicides Atrazine and Metolachlor by Transgenic Rice Plants Expressing Human CYP1A1, CYP2B6, and CYP2C19. J Agricultural and Food Chemistry, 54 (8): 2985-2991, 2006.
[24] Kayser A, Wenger K, Keller A, Attinger W, Felix HR, Gupta SK, Schulin R. Enhancement of Phytoextraction of Zn, Cd, and Cu from Calcareous Soil: The Use of NTA and Sulfur Amendments. Env Sci Technol, 34 (9): 1778-1783, 2004.
[25] Kirkwood N. Here come the hyperaccumulators. Harvard Design Magazine Fall/Winter, 1–4, 2002.
[26] Kumar D, Chauhan DK. Seedling Morphology of two important Medicinal Plant Species of *Wrightia* R.Br. (Apocynaceae) and its Taxonomic Significance. ISSN 0376-5561, Geophytology 46(2): 157-162, 2016.
[27] Kumar S, Singh R, Kumar V, Rani A, Jain R. *Cannabis sativa*: A Plant Suitable for Phytoremediation and Bioenergy Production. In: Bauddh K, Singh B, Korstad J. (eds) Phytoremediation Potential of Bioenergy Plants. Springer, Singapore, 2017.
[28] Lampis S, Santi C, Ciurli A, Andreolli M, Vallini G.Promotion of arsenic phytoextraction efficiency in the fern *Pteris vittata* by the inoculation of As-resistant bacteria: a soil bioremediation perspective.6 (80), 2015.
[29] Li C, Wang QH, Xiao B, Li YF. Phytoremediation potential of switch grass (*Panicum virgatum* L.) for Cr-polluted soil. Int Symposium on Water Resource and Environmental Protection, 2011.
[30] Lorestani B, Yousefi N, Cheraghi M, Farmany A. Phytoextraction and phytostabilization potential of plants grown in the vicinity of heavy metal-contaminated soils: a case study at an industrial town site. Environ Monit Assess., 185 (12): 10217-23, 2013.
[31] Lotfy SM, Mostafa AZ. J Geochemical Exploration, 144: 367–373, 2014.
[32] Lum AF, Ngwa ES, Chikoye D, Suh CE. Phytoremediation potential of weeds in heavy metal contaminated soils of the Bassa Industrial Zone of Douala, Cameroon. Int J Phytoremediation,16(3): 302-19, 2014.
[33] Oscar D, Yasna T, Olivares P, Cazanga R, Segura M, Peredo R, Santiago. *Lupinus microcarpus* Growing in Arsenic-Agricultural Soils from Chile: Toxic Effects and It Potential Use as Phytoremediator Plant. J Env Prot, 07: 116-128, 2016.

[34] Padmavathiamma PK, Li LY.“Phytoremediation technology: hyper-accumulationmetals in plants. Water, Air& Soil Pollution, 184(1-4): 105–126, 2007.
[35] Papazogloua EG,Fernandob AL. Preliminary studies on the growth, tolerance and phytoremediation ability of sugarbeet (*Beta vulgaris* L.) grown on heavy metal contaminated soil. Industrial Crops and Products, 107 (15): 463-471, 2017.
[36] Parrish ZD, Banks MK, Schwab AP. Effectiveness of Phytoremediation as a Secondary Treatment for Polycyclic Aromatic Hydrocarbons (PAHs) in Composted Soil, Int J Phytoremediation, 6 (2): 119-137, 2004.
[37] Polechonska L,Klink A. Trace metal bioindication and phytoremediation potentialities of *Phalaris arundinacea* L. (reed canary grass). J Geochem Expl, 146: 27-33, 2014.
[38] Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH, Gregg PEH. The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant and Soil,203:47–56, 1998.
[39] Singh UV, Abhishek A, Bhaskar M, Tandan N, Ansari NG, Singh NP. Phyto-extraction of heavy metals and biochemical changes with *Brassica nigra L.* grown in rayon grade paper mill effluent irrigated soil. ISSN 0973-2063 (online), 0973-8894 (print), Bioinfo, 11(3): 138-144,2015.
[40] Soliman WS, Sugiyama SI. Phytoremediation and tolerance capacity of *Moringa olifera* to Cadmium and its relation to nutrients content. Poll Res., 35 (1): 23-27, 2016.
[41] Sruthi P, Puthur JT. Characterization of physiochemical and anatomical features associated with enhanced phytostabilization of copper in *Bruguiera cylindrica* (L.) Blume. Int J Phytoremediation, 21 (14): 1423-1441, 2019.
[42] Su Y, Han FX, Chen J, Sridhar BB, Monts DL.Phytoextraction and accumulation of mercury in three plant species: Indian mustard (*Brassica juncea*), beard grass (*Polypogon monospeliensis*), and Chinese brake fern (*Pteris vittata*). Int J Phytoremediation., 10(6): 547-60, 2008.
[43] Tang L, Hamid Y, Zehra A, Sahito ZA, He Z, Hussain B, Gurajala HK, Yang X.Characterization of fava bean *(Vicia faba* L.) genotypes for phytoremediation of cadmium and lead co-contaminated soils coupled with agro-production.Ecotoxicol Environ Saf, 30 (171):190-198, 2019.
[44] Titah HS, Abdullah SRH, Idris M, Anuar N, Basri H, Mukhlisin M. Arsenic Range Finding Phytotoxicity Test Against *Ludwigia octovalvis* as First Step in Phytoremediation. Research JEnv Toxicol, 6: 151-159, 2012.
[45] Tzvetkova N, Miladinova K, Ivanova K, Georgieva T, Geneva M, Markovska Y. Possibility for using of two *Paulownia* lines as a tool for remediation of heavy metal contaminated soil. J Environ Biol., 36: 145-51, 2015.

Production and Marketing of Ginger: A Case Study in Salyan District, Nepal

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***Abstract*— *Ginger is an important spice crop grown in the mid-hills of Nepal for cash income. 60 ginger producers were sampled using a simple random sampling technique. Primary data collection was done via questionnaire survey as well as via focus group discussion (FGD), key informant interview (KII) and rapid market appraisal (RMA). The collected data was analyzed using MS Excel and SPSS. Average area under ginger cultivation was 0.13 ha. Average cost of production and the productivity of ginger was NRs. 4,20,000/ha and 14.44 ton/ha respectively. The average selling price of fresh ginger was NRs. 29.34 whereas average selling price of dried ginger was NRs. 201.42. The Benefit Cost ratio of ginger production in the study area was 1.53. The major production related problem could be solved by making consulting services and input supplies more reliable and readily available. Development of the market information system may help in decreasing the dependency of farmers on middlemen. Establishment of farmer-run collection and processing centers in strategic levels could be a sustainable way to address various problems related to marketing of ginger in the study area.*

***Keywords*—Ginger production, Benefit Cost ratio, Production problem, Marketing problem, Nepal.**

I. INTRODUCTION

Ginger (*Zingiber officinale Rosc*.) is one of the valuable spice crops grown extensively in the mid hills of Nepal. It is one of the high value spice crops which can contribute to improve the socioeconomic status of rural people by raising their income (NSCDP, 2007).There are about 107 spices in use all over the world and more than 25 spices are commonly used in Nepal among which ginger and large cardamom have been exported from the country (GRP, 2017). Nepal is the world's fourth largest ginger producer after India, China and Indonesia with a production of approximately 270 thousand tons per year (Zoder, 2017). In Nepal, among the spice crops ginger occupies 1,262 ha area with production of 15650 tons in 2018 (MoAD, 2018). According to the Ministry of Commerce and Supplies (2010), ginger is one of the agriculture products having an export potential identified by Nepal Trade Integration Strategy. Nepalese government and development partners have identified ginger as a high potential sector for export, value addition and income generation. India is a vast and dynamic market for Nepali ginger (ITC, 2007). India accounts for close to 94% of Nepal's fresh ginger export and six percent of processed ginger (MoAD, 2018). Nepal exported 39581 tons of ginger in 2013 of which the share of fresh ginger was 96% in total exports and only 4% in dried form of which 0.02% was in powdered form (ANSAB, 2015). According to Sharma (2016), with substantial increments in yield, quality and volume, Nepalese Ginger has huge international trading potential. The top five ginger exporting countries in 2015 are China, Netherlands, India, Nepal and Thailand (Zoder, 2017).

Salyan (28° 22' 31.01" N, 82° 09' 42.01" E) is the major ginger producing district of Nepal. It lies in the mid-hill region at an altitude ranging from about 1530 meters above the sea level. The total area under ginger production in Salyan district in 2016/2017 was 2000 ha with a total production of 27564 tons (MoAD, 2018). The physical and biological condition of Salyan district favors the ginger production, but lack of technical knowledge during the period of sowing, poor practice of seed production, lack of storage facility, poor practice of cleaning and processing has reduced the quality of Salyan's ginger which affects the trade of ginger abroad. There is no proper way of recording on production costs as well as on profitability. There is farmers' lack of direct contact and access to market and establishment of local traders' is the only point for the transaction of the produce in Salyan District (Khanal, 2018). The objective of this study was to assess the economics of production and marketing status of ginger in Salyan district of Nepal.

II. MATERIALS AND METHODS

Selection of study site

The present study was conducted in two municipalities (Sarada Municipality and Bagchaur Municipality) and one Rural Municipality (Siddhakumakh) of Salyan district. These areas were purposively selected as major ginger cultivated area of Ginger/Turmeric zone under PMAMP project.

Selection of ginger farmers

Out of 568 ginger producing farmers, 60 farmers (10.56 % of the total population) were selected as samples for the survey. List of ginger growing farmers were obtained from the Ginger/Turmeric zone office. Altogether 5 traders were selected for an interview from Sharada Municipality. The study was conducted in the month of January, 2019.

Sources of data

Primary data were collected from ginger growers, traders, wholesalers and retailers. The information was collected through a household survey using an interview schedule. Observation, Focus Group Discussion (FGD), Key Informant Interview (KII) and Rapid Market Appraisal (RMA) were also used to triangulate the data.

The secondary sources of data were various sources like publication from GOs, NGOs, research articles from national and international journals along with the previous studies in the study area.

Data analysis

Raw data obtained from the field were analyzed to get to a certain conclusion. Both primary and secondary information collected from field surveys and other methods were coded, tabulated and analyzed by using Statistical Package of Social Science (SPSS) and Microsoft Excel. Different variables in this study were used on both quantitative and descriptive analysis.

Marketing margin

The difference between the farm gate price i.e. price obtained by the producer and retailer's price i.e. price paid by the consumer is known as marketing margin. It was calculated as:

Marketing margin = Retailer's price (P_r) - Farm gate price (P_f)

Producer's share

Producer's share is the price received by the farmer expressed as a percentage of the retail price, i.e. the price paid by the consumers. It was calculated by the following formula.

$$P_s = (P_f/P_r) \times 100$$

Where,

P_s = Producers' share

P_r = Retailers' price

P_f = Producers' price (Farm gate price)

Gross margin analysis

Gross margin is the difference between the revenue and cost of goods sold (production cost not including indirect fixed cost like office expenses, rent or administrative costs) for any enterprises. The gross margin of the ginger in this study was calculated as below:

$$\text{Gross margin} = \frac{\text{Revenue} - \text{cost of good sold}}{\text{Revenue}} \times 100$$

If the margin is x%, then x% of sales total is gross profit.

Benefit cost analysis

Cost benefit analysis was done after calculating the total variable cost and gross return from the ginger cultivation. Cost of production was calculated by summing all the variable cost items in the production process. For calculating gross return, income from product sales was accounted. So, the benefit cost analysis was carried out by using formula:

B/C Ratio = Gross return / total variable cost

Where,

Gross return = Total quantity of ginger marketed (Kg) × Price per unit of ginger (Rs)

Total variable costs = Summation of all variable cost items

Marketing channel

Marketing channels was drawn based on the information obtained from producer level to consumer level. All linkage and coordination among all levels i.e. input supplier, grower, collector, retailer and final consumer were analyzed for the marketing channel.

Problem prioritization

5-point scale: 1 for most problematic, 0.8 for second most problematic, following the order 0.6, 0.4 and 0.2 for least problematic one

$I_{imp} = \Sigma(S_i f_i/N)$

Where,

I_{imp} = Index of importance

S_i = i^{th} scale value (i= 1, 0.8, 0.6, 0.4, 0.2)

f_i= frequency of i^{th} importance given by respondents

N = total number of respondents

III. RESULTS AND DISCUSSIONS

Socio-demographic characteristics of ginger farmers

The total population of 60 respondent households was found to be 349 with an average family number of 6.58. The study showed that the average number of male and females in the family was 3.26 and 3.32 respectively. 53% of the respondents were male whereas 47% were female. It indicates that males relatively have a lead role in providing information about the household rather than females. The respondents belonged to six different ethnic groups Chhetri(61.67%), Dalit (13.33%), Janajati (8.33%), Sanyasi (8.33%), Brahmin (5%) and Thakuri (3.33%). Respondents were categorized into five education levels i.e. illiterate, primary level, lower secondary level, secondary level and higher studies. 13% of the respondents have had higher education, 24% of the respondents were found to have a secondary level of education, 8% had attended lower secondary school, 9% had attended primary level, 23% of them were literate and 19% of respondents were illiterate. Most of the people (55%) in this area depend on agriculture for livelihood followed by abroad and agriculture (20%). Similarly, along with agriculture, 15% were involved in services and 10% in business. Age of the family members was categorized into three different groups namely below 15 years, 15 to 59 (economically active population), above 60 years. During the study it was found that the majority of the population (60.17%) was in the economically active age group.

Production situation

The average land holding size of people in this area was 0.80 ha. Among that average land, area under ginger cultivation was 0.13 ha (16.25% of average land holding size) with productivity 14.44 ton/ha. In a study done by Mahat (2019); the average productivity of ginger was found to be 22.07 ton/ha which is more than the productivity seen in this study.

Table 1: Production status of Ginger compared to total land area as above

Description	Minimum	Maximum	Mean	Standard Deviation
Total area (ha)	0.10	4.07	0.80	0.73
Ginger Cultivated area (ha)	0.02	0.61	0.13	0.10
Ginger production (ton/ha)	3.17	30.87	14.44	5.93

Cost of production

The cost of production includes input cost (seed rhizome, fertilizer, manure), labor (land preparation, intercultural operation, post-harvest operation, marketing) and other associated costs. According to the findings, the average cost of production (per hectare) in the study area was NRs. 4,20,000 *(USD 1= NRs.110)*

Table 2: Cost of ginger cultivation per hectare

S.N.	Description	Average cost of production per hectare (NRs.)	Contribution to total cost (%)
A	Inputs		
1	Rhizome	200,000	47.61
2	Fertilizer (FYM)	50,000	11.90
3	Chemical Fertilizer	8,575	2.04
B	Labor		
1	Land Preparation	40,000	9.52
2	Plantation	30,000	7.14
3	Weeding	30,000	7.14
4	Harvesting	40,000	9.52
5	Transportation	21,425	5.10
C	Grand Total	420,000	100
D	Average ginger production per hectare (ton/ha)	14.44	
E	Cost of Production per kg of Ginger (NRs)	32.06	

(USD 1= NRs. 110)

It was found that the highest cost was incurred for seed rhizome (47.61%). Similarly, on similar studies performed by Kumar (2017) and Poudel (2007), cost for seed was reported up to 41.90% in India and 65.1% in Palpa district of Nepal respectively. The cost of production per kg of ginger was NRs. 32.06.

Selling price of ginger

The finding of the interview with the ginger grower showed that farmer from study sites sold their ginger in different forms i.e. fresh ginger, dried ginger (sutho), seed rhizome, and mother rhizome (Bruni). The price of different forms of ginger is mentioned in the table below (Table 3).

Table 3: Price of different form of ginger (NRs/kg)(USD 1= NRs.110)

Forms of Ginger	N	Minimum	Maximum	Mean	Standard Deviation
Dried Ginger (Sutho)	7	90	250	201.42	53.67
Seed Rhizome	12	40	120	80.41	24.9
Mother Rhizome	31	30	120	68.87	19.48
Fresh Ginger	47	12	45	29.34	8.52

Quantity of different forms of ginger

On average, we found that the quantity of dried ginger (sutho) marketed was 2,434.04 kg/ha. The quantity of mother rhizomes sold was 2,294.72 kg/ha. Likewise, fresh ginger 5973.20 kg/ha, seed rhizome 1,961.85 kg/ha. The rhizome for next year was found to be 4,450.72 kg/ha.

Table 4: Quantity of different forms of ginger (kg/ha)

Variables	N	Minimum	Maximum	Mean	Standard Deviation
Sutho	7	841.02	5,895	2,434.04	1831.57
Mother rhizome	31	196.5	7,860	2,294.72	1402.81
Fresh ginger	47	982.5	13,755	5,973.20	3430.10
Seed rhizome	12	786	2,593.8	1,961.85	1080.75
Rhizome for next year	60	884.25	9,825	4,450.72	1703.65

Gross income from different forms of ginger

The gross income from various forms of ginger is given in Table 5. The sum of income from sales of each form of ginger per hectare was calculated for each farmer. Then, an average was calculated from this sum of all the sample population to determine average total income per hectare which was found to be NRs. 646,742.80*(USD 1= NRs.110)*

Table 5: Gross income from different forms of ginger (NRs. /ha)

Forms of Ginger	N	Minimum	Maximum	Mean	Standard Deviation
Sutho	7	176,850	491,250	330,824.6	122376.9
Mother rhizome	31	15,720	550,200	160,688.7	115668.5
Fresh ginger	47	20,632.5	550,200	169,549.2	111226.9
Seed rhizome	12	62,880	314,400	164,077.5	79723.98
Rhizome for next year	60	43,947.23	975,426	357,894.9	226035.5

Benefit cost ratio of ginger

Benefit cost analysis shows that farmers were making nearly NRs. 226,742.80 profit or gross return per hectare while cultivating the ginger.

Table 6: Benefit cost ratio of ginger

Cost of production for 1 ha	NRs. 420,000
Income from 1 ha	NRs. 646,742.80
Profit	NRs. 226,742.80
Benefit Cost ratio (B:C ratio)	**1.53**

It was found that the benefit cost ratio for ginger farming (all forms of ginger) was 1.53. Since the Benefit Cost ratio was more than unity, ginger cultivation can be considered as profitable business.

Market Channel

Ginger marketing includes all the activities involved in the transfer of farmer's product, either fresh or processed, to the consumers at both domestic and international level. Different channels were involved in the transfer of different forms of ginger from farmers to consumers. The type of channel involved varied on the basis of forms of ginger the farmer had and the location where the farm was present. Sutho was mainly sold to the traders in the Indian markets. Generally, almost all the producers sold ginger to the local collectors without any intermediaries. The ginger from Salyan were found to be transported to different domestic markets of Nepal, as well as to the traders in Indian markets operating on commission-basis. The common marketing channel found in the flow of ginger from producers of Salyan is presented in the figure below:

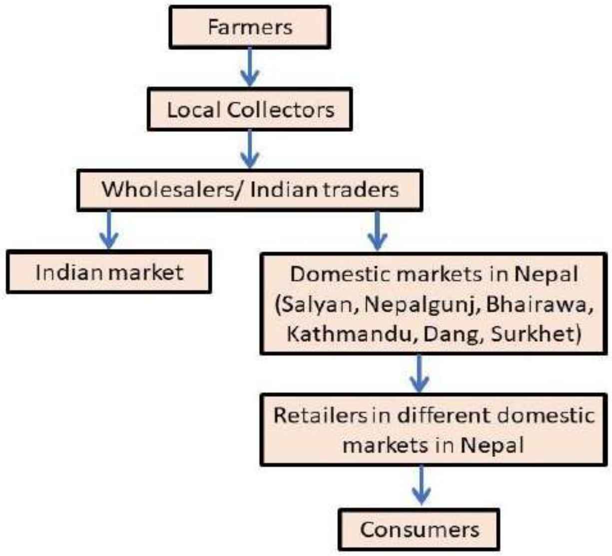

Fig 1: Marketing Channel of Ginger in Salyan District

Market margin and producer share of zone vicinity area

Market margin and producer share are important determinants for market efficiency. Lower marketing margin and higher producer share on retail price indicates an efficient market system.

Table 7: Market margin and producer share of zone vicinity area (Prices in NRs.)

Forms of Ginger	**Farm gate price (P_f) per kg**	**Retailer's price (P_r) per kg**	**Market margin (M_m)**	**Market margin (%)**	**Producer's share (P_s) (%)**
Fresh ginger	29.34	55	25.66	46.65	53.34
Dried ginger	201.42	270	68.58	25.4	74.6
Mother rhizome	68.87	90	21.13	23.47	76.52
Seed rhizome	80.41	110	29.59	26.9	73.1

The study revealed that the average price received by the farmers i.e. the farm gate price for fresh ginger was NRs. 29.34/kg and the price paid by the consumer i.e. the retailer's price was NRs. 55/kg with a market margin of NRs. 25 and producer's share of 53.34%. Calculated average price received by the farmer was only NRs. 29.34/kg which was less than the cost incurred for producing one kg ginger i.e. NRs. 32.06. Price of ginger depends entirely on demand in India and the whims of the Nepal-India border because of which the price of ginger is not stable and greater fluctuation is seen even within a year.

Dried ginger (sutho) is the major value-added product of ginger which is made from fresh mature rhizome by drying, so that it can be preserved for a longer period of time. Dried ginger required high labor cost for cleaning, drying and processing therefore, producers only prepared sutho if the market price for fresh ginger is comparatively low. Around 6 quintal fresh ginger is required to produce 1 quintal dried ginger (6:1 fresh ginger to dried ginger) so the price of dried ginger was high. The study revealed that the farm gate price for dried ginger and retailer's price was NRs. 201.42 and NRs. 270 respectively with a market margin of NRs. 68.58 and a producer share of 74.6%. The average farm gate price of sutho was less than the cost incurred for producing 1 kg of sutho (NRs. 224.56).

Harvesting of mother rhizomes was done 3-4 months after the plantation of ginger. Generally, growers harvested mother rhizomes when there was high demand in the market because at that time the price of the ginger was higher than at the main season of harvest. The study revealed that the average price received by the farmers from mother rhizome was NRs. 68.67/kg and the price paid by the consumer for 1 kg of mother rhizome (Retailer's price) was NRs. 90 with a market margin of NRs. 21.13 and the highest percentage of producer's share i.e. 76.52%.

Majority of the farmers used locally available variety from their own production as planting materials which they preserved in the soil pit. A few farmers purchased seed from the neighbors. During the study, it was found that the farm gate price for seed rhizomes was NRs. 80.41. The price paid by the consumer's i.e. retailer's price was NRs. 110 with a market margin of NRs. 29.59. Producer's share for seed rhizome was found to be 73.1%.

Production and Market problems

Each of the problems were given a weightage from 1 to 5 and then obtained frequencies were multiplied with the respective weightage. The obtained results were then added and then divided with the total number of respondents i.e. 60 multiplied by the highest weightage value i.e. 5 and then index value was obtained. The ranks were assigned in accordance with the obtained index value.

Table 8: Problems in ginger production as ranked by ginger producers in study area

S.N.	Problems	Frequency					Total	Weightage	Index	Rank
		P1	P2	P3	P4	P5				
1	Rhizome rot	48	9	2	0	1	60	56.6	0.943333	I
2	Dry rot	5	28	19	4	4	60	41.2	0.686667	II
3	Availability of quality rhizome	5	19	19	6	11	60	36.2	0.603333	III
4	Post-harvest loss	3	2	8	30	17	60	24.8	0.413333	IV
5	Input availability	1	3	11	19	26	60	22.8	0.38	V

Where, P= Priority level.

All 60 ginger producers were asked to rank the problems in ginger production as listed in the interview schedule. According to the priority ranking of the producers "Rhizome rot" was found to be most problematic while "Input availability" was ranked least problematic.

Marketing problems of ginger

Each of the problems were given a weightage from 1-5 and then obtained frequencies were multiplied with the respective weightage. The obtained results were then added and then divided with the total number of respondents i.e. 60 multiplied by the highest weightage value i.e. 5 and then index value was obtained. The ranks were assigned in accordance with the obtained index value.

Table 9: Marketing problems as ranked by ginger producers in the study area as above

S.N.	Problems	Frequency					Total	Weightage	Index	Rank
		P1	P2	P3	P4	P5				
1	Low market price of Ginger	11	26	12	10	1	60	60.8	1.013333	I
2	Lack of storage facilities	36	13	7	4	0	60	52.2	0.87	II
3	Lack of Transportation	5	10	14	17	14	60	31	0.516667	III

4	Lack of processing facilities	4	8	16	15	17	60	29.4	0.49	IV
5	Quality issue	4	3	11	14	28	60	24.2	0.403333	V

Where, P= Priority level.

The five different market problems were listed in the interview schedule and 60 ginger producers were asked to rank the problems in accordance with their perception. The first problem as ranked by the producers was "Low market price of ginger" followed by "Lack of storage facilities" and the "Quality issue" was ranked last indicating consumers preferred the prevailing quality of ginger.

IV. CONCLUSION

The climatic and geographic suitability of Salyan provides an ample opportunity to the farmers to grow ginger. Despite the opportunities, there was predominance of traditional practices in cultivation and processing of ginger prevailing in the district. Dried ginger (sutho) was the major value-added product and it was prepared in a very few households also in fewer quantities. Rhizome rot and low market price of fresh ginger were the major pressing constraints for production and marketing of ginger in the area respectively. Because there was no direct linkage of roads in major ginger growing areas, the farmers were compelled to sell their produce to the local traders and were minimally paid. Despite many problems, the ginger production in the study area was a profitable business (Benefit Cost ratio of 1.53) and gross margin was NRs. 226,742.80/ha. The cost of production per kg of ginger was NRs. 32.06 but the average selling price of fresh ginger was NRs. 29.34/kg. Farmers were not getting a fair price for fresh ginger. However, if the alternative approach is followed i.e. harvesting ginger in multiple forms (mother rhizome, sutho, seed rhizome), they will easily access the market, which may be more profitable than solely selling it in fresh form. In addition, the farmers do not account farm labor while calculating the cost of production. Due to this reason, even though the farmers think their enterprise as profitable, it actually is not. When labor cost was accounted, sales of ginger in the present price range would cause loss to the farmers. So, the farmers should be suggested to calculate the farm gate price of ginger by considering labor cost as well. The government should intervene in the pricing of ginger by either through subsidies to the farmers or by helping farmers to get a fair price for their product.

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REFERENCES

[1] Asia Network for Sustainable Agriculture and Bioresources. (2015). *A market study for Nepalese ginger and its derivatives in India and Bangladesh markets*.
[2] Ginger Research Program. (2017). *NARC Annual Report*. Kapurkot, Salyan, Nepal.
[3] International Trade Center. (2007). *Export Potential Assessment in Nepal*.
[4] Khanal, K. (2018). Factors Affecting and Marketing Chain of Ginger in Salyan District, Nepal. *Int. J. Appl. Sci. Biotechnol*, *6*(2). https://doi.org/10.3126/ijasbt.v6i2.xxxxx
[5] Kumar, D. (2017). Cost and return of ginger in Bidar district of Karnataka an economic analysis. *Journal of Pharmacognosy and Phytochemistry*, *6*(5), 472–475.
[6] Mahat, S., Sapkota, S., Sapkota, S., & Katuwal, K. (2019). Factors Affecting Ginger Production in Surkhet District, Nepal. *International Journal of Applied Sciences and Biotechnology*, *7*(2), 269–273. https://doi.org/10.3126/ijasbt.v7i2.24650
[7] Ministry of Agricultural Development. (2018). *Statistical Information on Nepalese Agriculture (2073/74)*.
[8] Ministry of Commerce and Supplies, G. of N. (2010). *Executive summary and action matrix*.
[9] National Spice Crops Development Program. (2007). *An Annual Report for 2007*. Khumaltar, lalitpur.
[10] Poudel, D. (2007). The trade scenario of Spice Crops of Nepal. In *Annual report of National Spice Crops Development Program*. Lalitpur, Nepal.
[11] Sharma, B. P. A. (2016). *Nepal Ginger Profile 2016 Research Report: An Assessment of Commercial Ginger Cultivated in Nepal*.
[12] Zoder, L. S. (2017). Ginger Sector in Nepal. *GIZ Nepal*.

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Aquaponics: prospects and challenges in Nepal

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***Abstract*— *Aquaponics is the integration of recirculating aquaculture and hydroponics in one production system. Nepal as an agricultural country has a higher prospect of this system. As the demand for fish and healthy vegetables and fruits are increasing in Nepalese market, aquaponics can be flourished as an alternative way for the optimum utilization of resources. With the increasing population, urbanization and industrialization, the land has been scarce for agriculture which leads to the higher scope of aquaponics to enhance the food security. Aquaponics is the sustainable, eco-friendly and intensive farming system that enhances the domestic food production or pre-urban and urban food security. Besides all the benefits, there are various challenges that limit the adoption of aquaponics system in Nepal. The major challenge is the high initial cost and requirement of adequate technical knowledge to maintain the balance of this system. The others are the lack of government policies and priority, lack of proper marketing for perishable products, load shedding etc. Hence, the government should give priority to the aquaponics system for the enhancement of food production in Nepal.*

***Keywords*— Aquaponics, hydroponics, agriculture.**

I. INTRODUCTION

Sustainable production, food and nutritional security has become a serious issue in the global market with the increase in human population. With the increasing urbanization, industrialization and land fragmentation, there will be the scarce land resources for the agricultural production to support the global population. In the context of Nepal, most of the people practice the traditional way of agriculture farming due to which, it is unable to meet the demand of agricultural products. In this scenario, fisheries and aquaculture seems to be the future towards food security as it is a crucial source of animal protein. Nepal is a developing country where agriculture is the main source of food, income and employment for the majority of people.According to the world bank collection of development indicators, agriculture supports 65% of total employment in Nepal and contributes 24.26% to the gross domestic product in 2019 (Statista, 2020). Similarly, the fishery sector contributes 4.18% to agricultural gross domestic product (AGDP) and 1.13% to gross domestic product (GDP) in fiscal year 2018/19. The annual production of fish has increased from 86,544 metric tonnes in 2017/78 to 91832 metric tonnes in 2018/19 with an increment of 6.11% (MoALD, 2020). However, the present aquaculture systems are facing challenges like water-quality management, diseases and traditional way of practicing which gives scope for the advanced technology to address these major challenges and boost up the production in quality and quantity. Similarly, the effluent from the production system has resulted in water pollution which has been a matter of concern for all the people. Hence, aquaponics is a sustainable and eco-friendly system for the enhancement of aquaculture production.

Aquaponics is the combination of two farming practices; aquaculture and hydroponics (Love et al., 2014). Aquaponics is a modern food production system which combines aquaculture and hydroponics (Raising of plants without soil beds) together symbiotically in a balanced recirculatory environment (Azad et al., 2016). Recirculatory environment signifies the reuse of the same water again and again through the biological filter to limit the toxic waste materials. It is a symbiotic relation between fish and vegetables where fish provides fertilizers to the plants and in return plants help to purify the wastewater as they use the nutrients where the fish live in (Roe and Midmore, 2008). This integration makes it much more profitable as compared to the conventional methods. The system is considered environmentally sustainable with the production of diversified high value products throughout the year.

History:

Aquaponics has an ancient root as its primal practice can be traced back to around 1000 AD where Aztec Indian raised plants on the rafts on the surface of a lake, known as Chinampas production system. Then , followed with the introduction of fish into paddy, rice fields in South-East

Asia about 1500 years ago (Coche, 1967). The term 'Aquaponics' was coined in 1970s .In the late 70s and 80s, researchers at the new Alchemy Institute North Carolina State University (USA) developed the basis of modern aquaculture (Love et al., 2014). The first closed loop system and first large-scale commercial facility started in the mid 1980s. After that, it has gained the momentum and interest among the global community.

In the context of Nepal, it is still in its rudimentary stage. It is only practiced in few cities like Syangja, Surkhet and Kathmandu. "Hope Nepal Bioponics", an agri-business private company, has been pioneering aquaponic food production in Nepal since 2006 (Adhikari et al., 2020). The Rotary Club of Patan, Nepal and the Rotary Club of Brussels with funding from Rotary International and technical support from the social enterprise, aquaponics UK had already run an aquaponics unit that supports a rehabilitation home for 20 children and mothers affected by HIV/AIDS (Mallypaty, 2012). The experimental evaluation in outdoor barrel aquaponics performed in IAAS, Paklihawa is a significant step towards the promotion of aquaponics in Nepal (Gyawali et al., 2019).

II. PRINCIPLE

Aquaponics is a freshwater ecosystem with the interaction between its biotic and abiotic components. The biotic components constitute fish, plants and bacteria while abiotic component constitutes water, air, grow beds and fish tank. The fish tank is set up and the fishes are provided with supplemental feeds. The nutrient rich water of fish tank is used to fertilize the grow beds.The feed supplied to the fish doesn't account for the total biological conversion. The uneaten feed undergoes decomposition and the waste released by fish both make the water foul and undesirable for fish. The fish takes 20-30 % of the N supplied by the feed (Schneider et al., 2005) and the rest is deposited in the culture media (Krom et al., 1995). This increases the toxic level in the tank affecting the fish growth. The nitrifying bacteria residing on grow beds convert the toxic ammonia and nitrite into less toxic form of nitrate. Nitrate is assimilated by the plants that enhances the nitrogen use efficiency (NUE), and thus creates a better environment for fish in the aquaculture tank when water is again re-circulated in the fish tank (Wongkiew et al., 2017).

III. MECHANISM

Aquaponics system relies on the natural sets of processes between the fish and bacterial community to produce the hydroponic plants. In this system, fish doesn't consume the overall supplied feed for its growth and metabolism. A small part (less than 5% (Yogev et al., 2016) is left uneaten and hence decomposed.This decomposition of uneaten feed and the faecal matter produced by the fish increases the nutrient load of the water leading to the consumption of dissolved oxygen and increasing the release of carbon dioxide and ammonia (Losordo et al,. 1998). This released ammonia is toxic to fish species even in minimal quantity whose toxicity increases with the increasing pH and temperature. To balance the nutrient cycle in this closed loop, the bacterial community has a significant role. They feed upon the uneaten feed, decayed matter and nutrient loaded fish waste. The beneficial bacterial community performs the nitrification process for the stabilization of nutrient cycle.

Nitrification is a two- step process where in first step the fish waste in the form of ammonia(NH_3) or ammonium (NH_4^+) is converted into nitrite(NO_2^-) by ammonia-oxidizing bacteria (AOB) like Nitromonas, Nitrosococcus, Nitrosospira. Likewise , in the second step of nitrification, toxic form of nitrite is converted into less toxic form of nitrate(NO_3^-) by nitrite-oxidizing bacteria (NOB) like Nitrobacter, Nitrococcus (Rurangwa and Verdegem 2013; Timmons and Ebeling 2013; Wongkiew et al. 2017). Nitrate is the form of nutrient which the plant can assimilate and are stripped by the plants from the water tank where the fish resides. When the nutrient system stabilizes, the water becomes clear and favorable for the growth of fish. In aquaponics, nitrate in excess is used for the valuable plant production instead of being removed in gaseous form in denitrification units (Van Rijn, 2013).In this process when there is a constant supply of water , the recirculation occurs in its own pace , supplying the required nutrients for the optimum growth of plants and creating sound habitat for the fish. Hence, it is a synergistic approach between fish, plants, bacteria and so on to create a sustainable and eco-friendly production system; which reduces the operational cost (Salam et al., 2013).

Suitable fish species in aquaponics system:

Fish is the component of aquaculture in an aquaponics system. It is the source of nutrient rich water for the hydroponic plants cultivated in grow beds. It can be reared in round, rectangular or elliptical tanks depending upon the design and cost of operation. Since, these tanks are the living space for the fish; various parameters like good water quality (adjustment of ammonia, nitrite and oxygen), adequate level of oxygen and the removal of waste should be maintained.

Selection of fish species should be done under various considerations. They should be able to live in dense population, thrive in less dissolved oxygen and should have a capacity to attain fast growth even in a confined environment. Several warm-water and coldwater fish species are adapted to recirculating aquaculture systems (Diver, 2006); including Tilapia (*Oreochromis niloticus*), Trout (*Oncorhynchus mykiss*), Perch (*Perca*), Arctic char (*Salvelinus alpinus*), Largemouth bass (*Micropterus salmoides*) (Diver,2006). Among these Tilapia is the fish species which has been selected in the study and practice. The experimental evaluation in outdoor barrel aquaponics was practiced in IAAS Paklihawa (Gyawali et al., 2019) with the stocking of Bighead carp (*Aristichthys nobilis*) and Common carp (*Cyprinus carpio)* which resulted in high survival rate and growth rate. The farmers of syangja district are growing Rohu, Naini, Silver and Common carp (Gurung, 2018).

Suitable Plants Species in aquaponics system:

Plants are the hydroponic component of the aquaponics system. They are cultured in the grow beds where the nutrient rich water is supplied through the fish tank. Since, aquaponics is a recirculating system, the waste water is regulated by the plants while stripping the excess nutrients in the form of nitrate. This results in their optimum growth. Therefore, the selection of plant species adapted to hydroponic culture in aquaponic greenhouses is related to the stocking density of fish tanks and subsequent nutrient concentration of aquaculture effluent (Diver, 2006). Both leafy vegetables and fruits can be harvested from aquaponics (Azad et al., 2016). Leafy vegetables like lettuce, spinach, chives, basil with low to medium nutritional requirements along with cabbage, carrots, okra (Salam et al,. 2013; Azad, 2015) are adoptive in the aquaponic system. Fruits like tomato, bell pepper and cucumber have a high nutritional requirement and perform better in a heavily stocked, well developed aquaponics system (Diver 2009). With further advancement in the system, it increases the potentiality for the production of flowers and medicinal plants. The experimental evaluation in outdoor barrel aquaponics was practiced in IAAS Paklihawa (Gyawali et al., 2019) with the cultivation of Broad leaf mustard of different varieties which showed the high yield. The farmers of Syangja district are growing garlic, cucumber, tomatoes, carrot, onion and coriander on stones while spinach, chilly, strawberry, potatoes and mint on PVC pipes and broccoli and Cauliflower in thermocol (Gurung, 2018).

Prospects of Aquaponics in Nepal:

As the income and livelihood of majority of Nepalese farmers depend on both the farming of vegetables and fruits, there is a high prospect of aquaponics in Nepal. In the context of Nepal, though it is practiced in some cities, it is still in its lagging phase, but a positive growth and expansion can be expected because of its various benefits and adoptive nature. Nepal is still under privileged in terms of proper dietary, food and nutritional security. Nepal is unlikely to achieve the daily standard requirement of 30 gram of fish or meat protein any time soon (Gurung, 2016). As a result of less intake of proper diet, many people are facing nutritional deficiency disease. Fish is an excellent source of omega-3 fatty acids, protein, vitamins, iron,zinc and fat soluble vitamin D. The fish production per capita in 2017/18 was 3.10 kg and fish availability per capita was 3.39 kg in Nepal (CFPCC, 2018). As seen there is a gap between the production and available amount of fish which is filled by the import from other countries. The fish was imported in a huge amount of 10,756 metric tonnes in 2017/18 (CFPCC, 2018). These imported fish lack the quality and are not beneficial in terms of health. To ensure the good health, proper diet, food and nutritional security Nepal needs to be self -sustained in terms of fish production. Similarly, vegetables are profound in Vitamins like A and C, iron, zinc, potassium etc. In Nepal, there is an increasing demand of vegetables with the increase in the healthy and conscious eating habit. The production of both fish and vegetables can go hand on hand to improve the economic and nutritional status of the country.

Furthermore, the rapid urbanization, industrialization and various other purposes has taken over the agricultural land and created much more pressure on the available natural resources. The lack of arable land in urban areas has given a new approach of rooftop farming, home gardening etc. The major city where there is scarce land and water resources has been adopting this aquaponics technology. Since, it can be practiced in the backyard or rooftop, it ultimately reduces the cost of transportation and ensures the quality of products. It accounts for the better health of the consumers since it provides the required nutrients like protein, vitamins without any growth stimulants and toxic chemicals. The consumers are concerned more about the organic food though they have to pay a little more amount. It is said that, a simple aquaponics system grows 6 times more per square foot than traditional farming and accounts for 70% less energy than the conventional method. The aquaculture and agricultural land of Nepal depends on the ground source of water. With the passing time these sources are being dried up as a result of which the agricultural production of Nepal has been affected to a great extent. While on contrary, the aquaponics system operating in a recirculatory mechanism saves about 95% of water (Dalsgaard et al., 2013); using only 10% of it. In this scenario, this aquaponic technique is a boon for the urban community.

Aquaponics is much more convenient than traditional farming practice as it does not require ploughing, mulching, tilling operations. This saves much more time and cost. Similarly, the yield that is reduced in soil culture due to soil borne pests is discarded in aquaponics which increase the production. The harmful effect of pesticides in both fish and plants are not pronounced in this technology, favoring better public health.

To combat the growing demand of vegetables and fish with increasing market value, the local production incorporating aquaponics could be handy for the future generations. Thus, the chemical-free, fresh, nutritious vegetables and good quality fish can be produced through the aquaponic system and thereby sound health can be maintained (Azad, 2015). This technology can serve a key point to maintain the healthy and sustainable food production and security in Nepal.

IV. CHALLENGES

Though aquaponics system tend to have enormous benefits, it is still in rudimentary stage and has not been widely accepted. It is a sophisticated technology; the farmers don't possess knowledge and awareness regarding the operation of this system. This has created a hindrance for the potential production of the system. Since, it covers multi -disciplinary areas, it requires vast knowledge regarding the management and monitoring of water quality parameters. The stabilization of pH and nutrient recycling are the major concern since the nitrification process directly changes the amount of pH affecting the nutrient assimilation by plants.

Along with technical challenges, it also faces the economic burden. The highly sophisticated technology requires a huge amount of money for the initial setup. Nepal, faces the problem of load shedding, this power cut in an enclosed system even for a short system disturbs the balance between the entities both abiotic and biotic resulting in a loss. To co-operate with this problem the farmers need to install inverters or generators in the farm, which causes a financial burden, increasing the cost of operation.

The nutrient loaded water when becomes turbid and unfavorable, may increase the susceptibility of fish towards diseases like fin rot and white spots. This problem can be managed by avoiding the overstocking of fish which signifies less fish during the harvest. However, there is a deficiency of available research to encourage the development of economically efficient aquaponic systems in Nepal. This has also limited the implementation of aquaponics commercially on a large-scale. Nepal is still under the line of poverty where the adoption of this highly sophisticated technology requiring vast theoretical and practical knowledge, highly skilled manpower with huge capital investment is still a big thing for the developing third world country like Nepal. These socio-economic and technical challenges have been a hurdle in the way of expansion of aquaponics.

Recommendation:

- The knowledge and experience sharing programmes should be launched to increase the awareness of farmers towards the aquaponics.
- The local farmers should be provided the training sessions regarding the installment and operation of aquaponics.
- The prerequisites like fish seed, feed etc should be evaluated by the government and provided to the farmers.
- The government should establish a proper market for the farmers to sell their harvest at a reasonable price.
- Government should provide funds and subsidies to the farmers in order to encourage their work.
- Governmental policies and research and extension works regarding this innovative system should be focused and given utmost priority.
- The local government should work in co-ordinance to form a congenial plan for the promotion and upliftment of aquaponics; to make the cities self -sustained on the production of fish and vegetables.

V. CONCLUSION

Nepal is an agricultural developing country where agriculture should be given as a functional priority by the government to succeed in the days ahead. Food security is the major problem faced by the modern world. To reduce this problem the modernization and commercial farming system should be introduced. One of the major food sectors to support food security in the present scenario is fisheries and aquaculture. The integrated system of the production of both plants and aquaculture species is best for the optimum utilization of input resources. In this regard, aquaponics is the one of the emerging modern technologies to ensure the production of plants like vegetables, fruits etc. and aquaculture species like fish in the same area under symbiotic relationship.

As aquaculture is increasing rapidly in Nepal with the increasing rate of 6.11% in fiscal year 2018/19, there are high prospects of practicing aquaponics system. The involvement of farmers in fish farming is increasing in Nepal and also many youths are attracted to the modern technology system of farming. Aquaponics can be the opportunity for the Nepalese youths to generate income and employment. Besides these prospects and various benefits of the aquaponics system, there are many challenges faced by farmers as it is a sophisticated system. Lack of adequate technical knowledge leads to failure of this system and also the initial cost of this system is high due to which farmers are afraid of rapid adoption of this system. Lack of proper research and government policies, lack of technical knowledge, economic constraints due to poverty of farmers, load shedding as it requires continuous electricity supply are the major challenges in the context of Nepal. Hence, for the further expansion of aquaponics in Nepal the government should make the policies and give priority to the research, extension and training related to the aquaponics system.

REFERENCES

[1] Adhikari, R, Rauniyar, S, Pokhrel, N, Wagle, A, Komai, T and Paudel, S.R., 2020. Nitrogen recovery via aquaponics in Nepal: Current prospects and challenges. A case study published on SN Applied Sciences, 2020, article number:1192.

[2] Azad K.N., 2015. Comparative study of okra production using different bedding media in aquaponic system. MSC thesis, Department of Aquaculture, Bangladesh Agricultural University.

[3] Azad, KN, Salam M. A, Azad, K.N. (2016) Aquaponics in Bangladesh: current status and future prospects. Journal of Bioscience and Agriculture Research,2016. Vol. 07, Issue 02:669-677.

[4] Statistical information on Nepalese Agriculture, 2020.Ministry of Agriculture and Livestock Department; Planning and development cooperation coordination division, statistics and analysis section: Singhadurbar, Kathmandu, Nepal. www.MoALD.gov.np

[5] CFPCC, 2018. Annual book. Balaju, Kathmandu: Government of Nepal; Ministry of Agriculture and livestock Department, Department of livestocks. Central Fisheries Promotion and Conservation Centre.

[6] Coche, A.G.,1967. Fish culture in rice fields: a worldwide synthesis. Hydrobiologia 1967, 30(1): pp 1-44

[7] Dalsgard J., Lund I., Thorarinsdotti R., rDrengstig A., Arvonen K. and Pederson P.B., 2013. Farming of different species in RAS in Nordic countries: current status and future perspectives. Aquaculture Engineering; 53,2-13.

[8] Diver S (2009) Aquaponics-Integration of Hydroponics with Aquaculture. ATTRA.

[9] Gurung Mohan, 2018. Syangja farmer using aquaponics to vegetables. https://myrepublica.nagariknetwork.com/news/syangja-farmer-using-aquaponics-to-grow-vegetables/

[10] Gurung TB, 2016. Role of inland fishery and aquaculture for food and nutrition security in Nepal. Agriculture food security 5 (1): 18.

[11] Gyawali, S, Chaudhary, P, Chaudhary, M, Gurung, S and Pathak R (2019) Comparative evaluation of Broad Leaf Mustard (BLM) under different boron concentrations in outdoor barrel aquaponics at IAAS, Paklihawa. International Journal of Applied Science and Biotechnology 7(3):359–364

[12] Krom MD, Ellner S, Rijin JV and Neori A (1995). Nitrogen and phosphorus cycling and transformations in a prototype ‘non-polluting’ integrated mariculture system, Eilat, Israel. Marine ecology progress series 118: 25-36.

[13] Losordo TN, Massen MP, Rakocy J, 1998. Recirculating aquaculture tank production systems: an overview of critical considerations. SRAC No. 451, pp 18-31.

[14] Love et al., 2014. International survey of aquaponics practitioners. Plos one, 9(7), e102662.

[15] Mallapaty S., 2012. Nepal sees potential in aquaponics. https://www.scidev.net/global/biotechnology/news/nepal-sees-potential-in-aquaponics.html

[16] Nepal: distribution of Gross Domestic Product (GDP) across economic sectors from 2009-2019. Statista, 2020.https://www.statista.com/statistics/425750/nepal-gdp-distribution-across-economic-sectors/

[17] Roe and Midrmore, 2008. Sustainable aquaponics. Centre for plant and water science. CQ university, Pockhampton, Queensland, Australia (Issue 103; hydroponics and greenhouse)

[18] Rurangwa and Verdegem MCJ, 2013. Micro-organisms in recirculating aquaculture systems and their management, review. Aquaculture 7: 117-130.

[19] Salam, M.A., M. Asadujjaman and M.S. Rahman(2013). Aquaponics for Improving High Density Fish Pond Water Quality Through Raft and Rack Vegetable Production.World Journal of Fish and Marine Sciences, Volume 5 (3): 251-256.

[20] Schneider O, Sereti V, Eding EH and Verreth JAJ, 2005. Analysis of nutrient flows in integrated intensive aquaculture system. Aquaculture Engineering 32: 379-401.

[21] Timmon MB, Ebeling JM, 2013. Recirculating aquaculture, Ithaca publishing, Newyork.

[22] Wongkiew S, Huz, Chandran K, Lee JW, Khanal SK, 2017. Nitrogen transformations in aquaponic systems: a review. Aquaculture engineering 76: 9-19.

[23] Yogev U, Barnes , Gross A, 2016. Nutrients and energy balance analysis for a conceptual model of a three loops off grid, aquaponics water 8.

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Growth performance and Carcass Characteristics of broiler Chickens fed Graded levels of differently processed Rubber Seed Meal based Diets

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***Abstract*— *High cost of conventional feed ingredients has force many poultry farms to ford-up therefore call more researches in the use alternative feedstuffs which rubber seed meal is one. This experiment was therefore carried out to evaluate the growth performance and carcass characteristics of broiler chickens fed processed rubber seed meal (RSM) based diets in a six weeks straight feeding trial in completely randomized design with two by four factorial arrangement i.e. two processing methods (roasted rubber seed meal and` Hot water soaked sample and four equi-protein replacement of soybean meal levels (0, 15, 25 and 35% levels). The results revealed that the final weight (2150.00-2243.07g), weight gained (2106.00-2213.60g), daily weight gained (50.14-52.01g) average feed intake (101.89 -108.46), average feed consumed (4177.67- 4447.00g) and feed conversion ratio (1.91-2.10) were not significantly ($p \geq 0.05$) affected by dietary treatments. The dressed and eviscerated weights of broilers fed HRSM based treatment were significantly ($p \leq 0.05$) higher than those that fed RRSM based treatment. Level interaction, treatment contrast and treatment and level interactions did not showed any significant ($p \geq 0.05$) variation in all parameters measured suggesting rubber seed meal as suitable replacement of soybean meal in broilers chickens ration.*

Keywords*— rubber seed meal (RSM); hot water rubber seed meal (HWRSM); roasted rubber seed meal (RRSM); soybean meal (SBM).

I. INTRODUCTION

Poultry products mostly meat, is a one meal size which did not require storage, it requires small space to rear, has short gestation period, can reach slaughter age within a short period. Thus, developing the poultry industry appears to be the fastest means of bridging the protein deficiency gap presently prevailing in Nigeria (Akinsanmi *et al*., 2017). Nigeria's feed production seem to be expanding rapidly in consistent with growth in the poultry industry. The business of raising poultry is cost-sensitive. Poultry feed accounts for higher percentage of the total feed produced in Nigeria (Agbede, 2019). The demand for poultry products has increased markedly over the past few years, due to the rapid growth specifically in the number of fast food restaurants featuring chicken menu in major urban areas (Akinnusotu *et al*., 2018). Profit of poultry farming mainly depends upon the economics of feeding the birds. Nigeria poultry industry is facing the problem of limited availability and high cost of conventional feed ingredients (Agbede, 2019). Therefore, search for alternative feedstuffs that can reduce cost of feed becomes a necessity. The use of unconventional feed sources like crop residues and agro-industrial by-products has been suggested to be the solutions to the problem of feed crisis in poultry production (Agbede, 2019; Igbasan, 2019). In fact, partial or complete replacement of the expensive conventional feed ingredients with cheaper non-conventional one has been suggested (Akinsanmi *et al*., 2020; Ijaiya *et al*., 2011). Over the years, different researches have been carried out on the use of unconventional feed ingredients in which rubber seed meal was one. Rubber seed is cheaper than full fat soya beans, it is not normally used as food by man and is a good source of protein and energy (Akinsanmi *et al*., 2017; Ijaiya *et al*., 2011). The rubber tree plantation in Nigeria was estimated to cover 200,000 hectares of land producing about 20,000 tons of seeds per year and seed yield of 214.9kg/hectare of which only about 20% were used as seedlings and the remaining were fed on by rodents or wasted (Noordin *et al*., 2012). Research has shown that the nutrient composition of rubber seed meal (RSM) were; metabolizable energy (1828.65- 2675.61kcal/kg), crude protein (22.16- 40.36 %,); ether extract (12.41 to 55.67%); crude fibre (2.65 - 3.62 %,); ash (2.20%- 6.57%) and carbohydrate (14.09 - 41.48%) (Akinsanmi *et al*., 2017; Eka *et al*., 2010). The range of metabolic energy of RSM (1828.65- 2675.61kcal/kg) fall within the range of metabolizable energy (ME) in other pulses used in feeding livestock. The values of 2750, 2460 and 2069 ME (Kcal/kg) for groundnut cake, soybean cake and cotton seed cake respectively (Olomu, 2011). The high energy and protein values of RSMs show that the meal could also be used as energy source and plant protein if it is properly processed (Oyewusi *et al*., 2007). though RSM is reported to be high in cyanide (18mg/100g) (Akinsanmi *et al*., 2018), those anti nutritional factors found in rubber seed are heat labile and are reduced to tolerable level while toasted (105^{o}C) or stored for 4-6 months before used (Akinsanmi *et al*., 2018). The seed meal or cake of rubber have been reported to have no any noticeable adverse effects on the health status of poultry birds (Akinsanmi *et al*., 2020) and contain higher contents of digestible nutrients than some conventional seed meals and are highly promising as future protein supplements in livestock and animal diets (Oyewusi *et al*., 2007).

Poultry birds can tolerate meal from undecorticated rubber seeds better than pigs because they have a gizzard to grind and help to digest the shell (Amaefule, *et al*., 2020). The crude protein content of rubber seeds and its products ranged between 22% in whole rubber seeds and 41% in commercial decorticated rubber seed meal (Eka *et al*., 2010).The utilization of rubber seed as a feed ingredient for monogastric animals has been limited by the presence of anti-nutritional factors especially hydrogen cyanide and the lack of proper processing methods that are cheaper and easy to adopt by the small scale rubber farmer or small scale poultry farmer living within the rubber producing zone of Nigeria (Akinsanmi *et al*., 2020) . This study was therefore carried out to assess growth performances and carcass characteristics of broiler chickens fed rubber seed meals based diets using two farmers' friendly processing methods and to establish the suitable incorporation levels of rubber seed meal in broilers feed.

II. METHODOLOGY

Sample collection and study site

This experiment was carried out at the Teaching and Research Farm of the Federal University of Technology; Akure which is located between 7.15^{o} North and 5.0^{o} East of the equator with the average annual rainfall of 1524mm and annual temperature of 28^{oc} to 31^{oc} and mean annual relative humidity of about 80% (Ajibefun, 2011). The rubber seeds used for this study were purchased from Ilusin rubber estate, Ilusin, Ogun state, Nigeria.

Sample processing and treatment

The seeds were washed with distilled water, sundried, dehulled and allowed to pass through the different processing methods: (a) soaking in hot water for 12 hours (HWRSM); (b) soaking in ash solution for 12 hours (ASRSM); (c) roasting for 15 minutes and cooled at room temperature (RRSM); (d) Stored at room temperature for thirty five days (STRSM), (e) Chemical de-fattened using Sohlext apparatus (DRSM), while the last sample was unprocessed (URSM). The samples were dried, milled and chemically analysed. Proximate analysis of the samples was carried out using the AOAC method (AOAC, 2000).

Experimental treatments/samples for experiment

Based on the result of the chemical compositions (Akinsanmi *et al*., 2018), the two samples with best chemical compositions (RRSM and HWRSM) were selected for the experiment. Two hundred and fifty (250) day-old broiler chicks of Hubbard strain were purchased from AFGRI BnotHarel hatchery, Ibadan, Oyo-State, Nigeria. Out of which two hundred and ten (210) were selected for the experiment. The chicks were weighed and randomly allotted to seven dietary treatments. Each dietary treatment contained 30 chicks which were divided into three replicates of 10 chicks each. The birds/replicate was housed in floor of 3m x

3m x 2.5m. The floor was covered with wood shavings as litter material. Each pen was equipped with feeding troughs and drinkers. The chicks were electrically brooded for two weeks. The experimental design for this experiment was completely randomized design with 2 by 4 factorial arrangement of two methods of processing (roasted rubber seed meal and hot water soaked rubber seed meal) and four levels of inclusion (0,15, 25 and 35%). Seven straight diets were formulated with equi-protein replacement of soybean meal with rubber seed meal (RSM) at graded levels. Diet 1 served as the control while diets 2, 3 and 4 contained roasted rubber seed meal (RRSM) at graded levels 15, 25 and 35% respectively, diet 5,6 and 7 contained hot water soaked rubber seed meal (HWRSM) at 15, 25 and 35% respectively (Table 1). The birds were offered their respective diets during the period and drinking water of the experiment *ad libitum*. The chicks were given all routine vaccinations and necessary medications. The initial weights of the individual birds were taken at the commencement of the study and subsequent weight at weekly intervals to determine the weekly weight gain using cumulative analysis and final weight. So also the daily feed intake per replicate were recorded for the calculation of total feed intake and feed conversion ratio.

Data analysis

The results were used to assess the profitability of the test treatments. Data generated were analysed using SPSS ((16.0) software version, differences among the means were determined by Duncan's multiple range tests of the same package

Table 1: Gross composition (%) of broiler chickens diets with graded levels of differently processed rubber seed meals

Ingredient	Control		RRSM			HWRSM	35%
replacement level	0%	15%	25%	35%	15%	25%	
Maize	53.00	50.90	49.51	48.11	49.73	47.56	45.38
Wheat offals	7.00	7.00	7.00	7.00	7.00	7.00	5.00
Soybean meal	28.00	23.80	21.00	18.20	23.80	21.00	18.20
RRSM (28.02% CP)	0.00	6.30	10.51	14.69	0.00	0.00	0.00
HWRSM (23.63% CP)	0.00	0.00	0.00	0.00	7.47	12.44	12
Groundnut cake	0.00	0.00	0.00	0.00	2.00	2.00	2.00
Fishmeal	4.00	4.00	4.00	4.00	4.00	4.00	4.00
DCP	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vegetable oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values							
ME (Kcal/kg)	3126.90	3092.00	3068.80	3045.53	3116.78	3019.45	3067.56
Crude Protein	20.43	20.15	20.13	20.09	20.62	20.42	20.23
Crude Fibre	3.52	3.40	3.32	3.25	3.34	3.28	3.21
Calcium	1.13	1.13	1.12	1.12	1.13	1.13	1.13

Av. Phosphorus	0.73	0.71	0.69	0.67	0.69	0.67	0.65
Lysine	1.38	1.58	1.72	1.86	1.66	1.84	2.01
Methionine	0.55	0.67	0.74	0.82	0.69	0.79	0.88
Analyzed values							
MC	9.00	8.44	9.50	9.00	7.91	7.93	7.93
Total Ash	9.56	9.97	9.23	9.99	8.56	9.27	8.95
Crude Protein	21.00	20.89	20.86	21.02	21.08	20.88	20.55
Crude Fibre	3.18	3.52	3.10	3.02	3.52	3.48	3.67
Fat	5.18	5.00	5.22	5.70	4.20	4.39	4.53
CHO	57.01	56.97	58.25	58.11	59.54	58.49	59.46

Keys: HWRSM; Hot water rubber seed meal, RRSM; Roasted rubber seed meal, Premix contained the following: (Univit. 15 Roche) 1500 I.U., Vit. A, 1500 I.U., Vit. D: 3000 I.U., Vit.E, 3.0 g; Vit, B2, 0.3 g; Vit. B6, 8.0 mg; Vit. B12, 8.0 g; Nicotinic acid, 3.0 g; Ca-Pantothenate; 5.0 mg; Fe, 10.00 g; Al, 0.2 g; Cu, 3.5 mg;Zn, 0.15 mg; I, 0.02 g; Co, 0.01 g/kg

III. RESULTS AND DISCUSSION

Growth performance of the birds fed graded levels of differently processed rubber seed meal (RSM) as shown in Table 2 revealed there were no significant ($p \geq 0.05$) differences in all the parameters measured at all levels of inclusion. The final weight (2150.00-2243.07g), weight gained (2106.00-2213.60g), daily weight gained (50.14-52.01g) average feed intake (101.89 -108.46), average feed consumed (4177.67- 4447.00g) and feed conversion ratio (1.91-2.10) were not significantly ($p \geq 0.05$) affected by dietary treatments. Suggesting that soybean meal (SBM) and processed RSM have similar influences on the growth performance of broiler chickens. The fact that there were no significant variations in the treatments at all levels showed that rubber seed meal could be used to replace soybean meal in broiler chicken diets up to 35% equi-protein replacement level. This is agreement with earlier report on performance of pigs fed up to 30% replacement level of soybean meal with RSM (Eka *et al*., 2010). Treatments interaction was not also significantly differed, this mean that the two processing methods have similar influences on the performance of the birds. Therefore, roasting or soaking of rubber seed in hot water can be used to process rubber seed for broiler chickens.

The range of average final weight (2150.00g-2243.07g) and average weight gained (2106.00g – 21213.60g) of birds on the test diets were more than 2kg recommended body weight for broilers chicken at six week (Noordin *et al*., 2012) which justified the inclusion of the processed RSMs in broiler chickens feed. Average daily feed consumed per bird (101.89 -108.46g) were lower than 111.90 – 335.34g/bird and 382.14g/bird reported for broiler chickens at 6-8 weeks fed defatted RSM respectively (Ijaiya *et al*., 2012; Olomu, 2011). The reduced feed intake might be due to high fat content in RSM based diets which might have resulted to high metabolizable energy (ME). Poultry birds eat to meet their energy requirement, so they eat less in high energy feed (Oloruntola *et al*., 2016). It has been reported that fat slow the rate of passage of feed in the alimentary tract thereby allow proper digestion and absorption of nutrients in feed (Jeffre *et al*., 2008).There is a positive relationship between dietary energy concentration and weight gain in broiler chicken (Onuh *et al*., 2010). Therefore, the used of full fat RSM in animal feed have some advantages which includes concentration of energy, increase growth rate and decreased feed intake. Rubber seed is a good potential feedstuff for livestock (Oyewusi *et al*., 2007).

Table 2: Performance of broiler chickens fed graded levels of differently processed rubber seed meals (RSMs) based diets.

Treatments	% replacement of SBM	Average Initial Weight (g)	Average Final Weight (g)	Average Weight gain (g)	Average Weight gain/day (g)	Average Feed intake (g)	Daily feed intake (g)	Feed conversion ratio
Level	0	44.07	2243.07	2213.60	52.01	4228.17	103.13	1.91
	15	43.78	2201.00	2157.18	51.36	4279.03	101.88	1.98
	25	43.83	2186.67	2142.28	51.01	4212.33	104.37	1.97
	35	43.78	2161.67	2117.82	50.42	4204.50	102.55	1.99
	SEM	0.13	28.49	26.17	0.75	38.13	1.06	0.03
	P value	0.93	0.92	0.85	0.67	0.70	0.89	.060
Treatment								
RRSM		43.71	2161.78	2118.00	50.43	4240.67	103.43	2.00
HWRSM		43.89	2204.44	2160.19	51.43	4223.24	103.01	1.96
SEM		0.11	25.07	22.04	0.69	32.89	25.92	0.02
P value		0.93	0.92	0.85	0.67	0.70	0.89	0.68
Treatment x	Level							
Control	0	44.07	2243.07	2213.60	52.01	4228.17	103.13	1.91
RRSM	15	43.17	2228.00	2184.17	52.00	4284.54	104.50	1.96
RRSM	25	43.93	2212.00	2166.97	51.60	4177.67	101.89	1.93
RRSM	35	43.97	2173.33	2129.43	50.70	4243.33	103.50	1.99
HWRSM	15	43.80	2174.00	2130.20	50.70	4409.33	107.54	2.07
HWRSM	25	43.73	2161.33	2117.60	50.42	4447.00	108.46	2.10
HWRSM	35	43.68	2150.00	2106.00	50.14	4365.67	106.48	2.07
SEM		0.01	36.22	34.86	0.90	50.84	0.24	0.06
P value		0.89	0.92	0.93	0.79	0.76	0.92	0.87
Significance								
Level		NS	NS	NS	NS	NS	NS	NS
Treatment		NS	NS	NS	NS	NS	NS	NS
Treatment x	Level	NS	NS	NS	NS	NS	NS	NS

Keys: URSM: Unprocessed rubber seed meal, HWRSM: Hot water soaked rubber seed meal, RRSM: Roasted rubber seed meal, DRSM: Defatted rubber seed meal, ASRSM: Ash soaked rubber seed meal, STRSM: Store at room temperature rubber seed meal. FCR; SBM: soybean meal; NS: not significant Means with the same superscripts in the same row are not significantly ($P \geq 0.05$) different.

Carcass characteristics of the birds as shown in Table 3 revealed that there were no significant [$P \geq 0.05$] difference in the live weight (1.93-2.15kg), dressed weight (% live weight) (86.23-89.53), eviscerated weight (% live weight) (73.25-76.57) at all levels of inclusion. Treatments contrast of dressed weight and eviscerated weight showed that broiler chickens fed HWRSM performed significantly ($P \leq 0.05$) better than their counterparts on RRSM based diet. Some parts weight (% eviscerated weight) and some relative organs weight (% live weight) measured as revealed in Table 4 and 5 respectively were not equally significantly ($P \geq 0.05$) affected by dietary treatments. The weight reduces gradually as the levels of equi-protein replacement of soybean with RSM increased, this might be due to the presence of residual anti-nutritional factors and variations in the quality of protein in the ingredients (Akinsanmi *et al*., 2018). The similarity observed in the performance of birds fed control and test diets indicates that processed RSM based diet promote similar growth and organs development as soybean meal based diet and can therefore be incorporated into poultry chicken feed to replace scarce and expensive soybean meal by Nigerian poultry farmers.

Table 3: Some carcass traits of broiler chickens fed graded levels of differently processed rubber seed meals (RSMs) based diets.

	% replacement of SBM	Live weight (kg)	Dressed weight (% live weight)	Eviscerated weight (% live weight)
Levels	0	2.08	88.83	73.05
	15	2.04	87.94	73.97
	25	2.08	87.05	72.81
	35	2.06	87.51	73.59
	SEM	0.20	1.27	1.51
	P value	0.81	0.86	0.70
Treatment				
RRSM		2.02	86.06^{b}	71.70^{b}
HWRSM		2.10	88.94^{a}	75.21^{a}
SEM		0.23	0.34	0.89
P value		0.13	0.03	0.02
Treatments x levels				
Control	0	2.08	88.83	73.25
RRSM	15	1..93	86.45	71.36
RRSM	25	2.10	86.23	72.32
RRSM	35	2.04	85.49	71.43
HWRSM	15	2.15	89.43	76.57
HWRSM	25	2.07	87.87	73.57
HWRSM	35	2.09	89.53	75.76
SEM		0.06	1.35	1.69
P value		0.16	0.76	0.45
Significance				
Level		NS	NS	NS
Treatment		NS	*	*
Treatment x level		NS	NS	NS

Keys: URSM: Unprocessed rubber seed meal, HWRSM: Hot water soaked rubber seed meal, RRSM: Roasted rubber seed meal, DRSM: Defatted rubber seed meal, ASRSM: Ash soaked rubber seed meal, STRSM: Store at room temperature rubber seed meal. SBM: Soybean meal, means with different superscripts in the same row are significantly (P ≥ 0.05) different, NS: Not Significant.* Significantly (P ≤ 0.05) different.

Table 4: Some parts weight (% eviscerated weight) of broiler chickens fed graded levels of differently processed rubber seed meals (RSMs) based diets.

Diets	% replacement of SBM	Head	Shank	Drumstick	Chest	Breast muscle	Abdominal fat	Thigh	Neck	Wing	Back
Level	0	3.36	2.25	5.35	16.89	6.98	1.50	6.154	5.66	4.98	15.29
	15	3.36	2.52	5.36	16.65	6.88	1.71	6.72	5.45	4.88	15.17
	25	3.35	2.47	5.39	16.75	6.72	1.75	7.32	5.54	4.86	15.09
	35	3.36	2.38	5.27	16.92	6.70	1.78	7.67	5.48	4.79	15.23
SEM		0.06	0.01	0.01	0.02	0.03	0.06	0.01	0.04	0.01	0.02
P value		0.76	0.76	0.65	0.72	0.54	0.43	0.45	0.55	0.62	0.34
Treatment											
RRSM		3.36	2.52	5.35	16.76	6.85	1.55	7.13	5.53	4.90	15.16
HWRSM		3.36	2.47	5.34	16.89	6.78	1.76	7.25	5.42	4.82	15.08
SEM		0.05	0.03	0.06	0.09	0.05	0.03	0.07	0.02	0.02	0.01
P value		0.92	0.89	0.88	0.76	0,79	0.77	0.89	0.69	0.87	0.56
Treatment	X level										
Control	0	3.36	2.25	5.35	16.89	6.98	1.50	6.15	5.66	4.98	15.29
RRSM	15	3.37	2.65	5.32	16.79	6.87	1.72	6.73	5.68	4.90	15.30
RRSM	25	3.35	2.52	5.42	16.56	6.86	1.75	6.80	5.58	4.97	14.99
RRSM	35	3.37	2.34	5.29	17.00	6.82	1.78	7.45	5.37	4.75	15.21
HWRSM	15	3.36	2.45	5.45	16.62	6.90	1.72	6.67	5.34	4.89	14.90
HWRSM	25	3.36	2.50	5.37	17.02	6.68	1.74	7.55	5.22	4.78	15.12
HWRSM	35	3.37	2.46	5.26	16.87	6.59	1.79	7.95	5.68	4.89	15.25
SEM		0.09	0.01	0.09	0.03	0.03	0.08	0.01	0.02	0.01	0.03
P value		0.32	0.42	0.45	0.78	0.64	0.56	0.35	0.34	0.64	0.32
Significance											
Level		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Treatment		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Treatment x	Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Keys: URSM: Unprocessed rubber seed meal, HWRSM: Hot water soaked rubber seed meal, RRSM: Roasted rubber seed meal, DRSM: Defatted rubber seed meal, ASRSM: Ash soaked rubber seed meal, STRSM: Store at room temperature rubber seed meal. SBM: Soybean meal, means with different superscripts in the same row are significantly (P ≥ 0.05) different, NS: not significant.

Table 5: Organs weight (% live weight) of broiler chickens fed graded levels of differently processed rubber seed meals (RSMs) based diets.

Diets	% inclusion of RSM	Liver	Heart	Kidney	Gizzard	Spleen	Pancreas	Lung
Level	0	1.02	0.21	0.10	1.12	0.06	0.10	0.23
	15	1.08	0.27	0.09	1.10	0.06	0.08	0.26
	25	1.09	0.29	0.09	1.01	0.05	0.09	0.23
	35	1.09	0.34	0.11	1.00	0.04	0.10	0.26
	SEM	0.06	0.01	0.01	0.06	0.01	0.01	0.02
	P value	0.66	0.14	0.46	0.48	0.36	0.26	0.62
Treatment								
RRSM		1.09	0.30	0.10	1.07	0.04	0.09	0.26
HWRSM		1.07	0.29	0.09	1.00	0.05	0.09	0.23
SEM		0.06	0.01	0.01	0.05	0.01	0.01	0.06
P value		0.84	0.94	0.32	0.35	0.24	0.70	0.17
Treatment x	Level							
Control	0	1.02	0.21	0.10	1.12	0.06	0.10	0.23
RRSM	15	1.05	0.29	0.09	1.22	0.06	0.09	0.29
RRSM	25	1.03	0.31	0.09	0.98	0.04	0.09	0.26
RRSM	35	1.09	0.38	0.13	1.01	0.03	0.09	0.24
HWRSM	15	1.10	0.26	0.09	0.98	0.05	0.076	0.23
HWRSM	25	1.04	0.29	0.09	1.04	0.050	0.08	0.21
HWRSM	35	1.06	0.32	0.08	0.99	0.05	0.11	0.25
	SEM	0.08	0.02	0.03	0.08	0.01	0.01	0.03
	P value	0.10	0.24	0.28	0.23	0.29	0.29	0.51
Significance								
Level		NS	NS	NS	NS	NS	NS	NS
Treatment		NS	NS	NS	NS	NS	NS	NS
Treatments x	Levels	NS	NSS	NS	NS	NS	NS	NS

Keys: SBM: soybean meal URSM: Unprocessed rubber seed meal, HWRSM: Hot water soaked rubber seed meal, RRSM: Roasted rubber seed meal, DRSM: Defatted rubber seed meal, ASRSM: Ash soaked rubber seed meal, STRSM: Store at room temperature rubber seed meal. SBM Soybean meal, NS: not significant.

IV. CONCLUSION

This study established the fact that equi-protein replacement of soybean meal in broiler chicken feed with graded levels of RSM from both processing methods [roasting and soaking in hot water] up to 35% did not have any noticeable adverse effects on the growth performance and carcass quality of the birds. This justify the call for the inclusion of processed RSM into the list of poultry feed ingredients as an alternative to scarce and expensive soybean meal.

AUTHORS' CONTRIBUTIONS

Conceptualization: S. K Akinsanmi, F.A. Igbasan

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Validation: F. A. Igbasan, J.O. Agbede

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Writing - original draft: S.K. Akinsanmi

Writing - review & editing: F.A. Igbasan, , J.O. Agbede, A. Akinnusotu

REFERENCES

[1] Agbede, J. O. Alternative feed resources: Key to profitable livestock enterprise in Nigeria Inaugural lecture series 109. Federal University of Technology, Akure. Nigeria. 2019; 1-15.

[2] Ajibefun, I. Akure city profile. www.wikipedia.org/wiki/Akure. Retrieved 5th May 2011.

[3] Akinnusotu A. Akinsanmi S.K, Aladekoyi G. and Adeniran O.A. Nutrients and Ant nutrients composition of three different poultry feeds in Ondo state, south western Nigeria. *J. Environmental and Agricultural Science* 2018;14:46-53

[4] Akinsanmi, S.K, Igbasan, F. A., Agbede, J.O and., Joachim, C.O. Effects of equi-protein replacement of soybean meal with graded levels of differently processed rubber seed meal on health status of broiler chickens. *Nig. J. Anim. Prod*. 2020; 47(1): 81- 89.

[5] Akinsanmi, S.K., Joachim, C.O., Sudik, S.D, Igbasan, F. A. and Agbede, J.O. Effect of processing on the nutrients qualities of rubber (*Havea brasilensis*) seed meal. Proc. 42nd Ann. Conf. Nig. Soc. Anim. Prod. 26-30th March, 2017. Landmark University, Omu- Aran. . 2017; 222-224.

[6] Akinsanmi, S.K., Joachim, C.O., Omotosho O.B, Igbasan, F. A. and Agbede, J.O. Effects of differently processed rubber seed meal based diets on performance and egg qualities of laying hens. *International Journal of Agricultural and Development Studies* (IJADS) 2018; 2(1): 87-94.

[7] Amaefule, R.A.; Etuk I.F; Iwuji, T.C; Ogbuewu I.P; Obikaonu, O.H and Amaefule K.U. .Haematological indices of grower pigs fed low protein and low energy diets supplemented with multi-enzyme. *Nigerian Animal Journal of Production.* 2020; 47(1):167-173.

[8] AOAC. Chemical method of Analysis. Association of Analytical Chemists. 20th edition Washington D.C. USA. 2000

[9] Eka, M. D., Tajul, A. A. and Wan Nadiah, W. A. Potential use of Malaysian rubber (*Hevea brasiliensis*) seed as food, feed and bio-fuel. *International Food Research Journal* 2010; 17: 527-534.

[10] Igbasan F.A. Poultry feed versus human food: Challenges and opportunities in sustaining poultry industry. Inaugural lecture series 116. Federal University of Technology, Akure. Nigeria 2019; 1-30

[11] Ijaiya, A. T., Alemede, L. C. and Erhuanga. R. A. Replacement value of rubber seed (*Hevea brasiliensis*) meal for full fat soya bean meal on performance, carcass characteristics and blood parameters of broilers. *Nig. J. of Anim. Prod*. 2011; 38 (2): 34-45.

[12] Jeffre, U. F., Kamyab, A. and Heinz, L. Comparison of fat sources in rations of broilers from hatch to market. *International Journal of Poultry Science* 2008; 7(12):1152-1155.

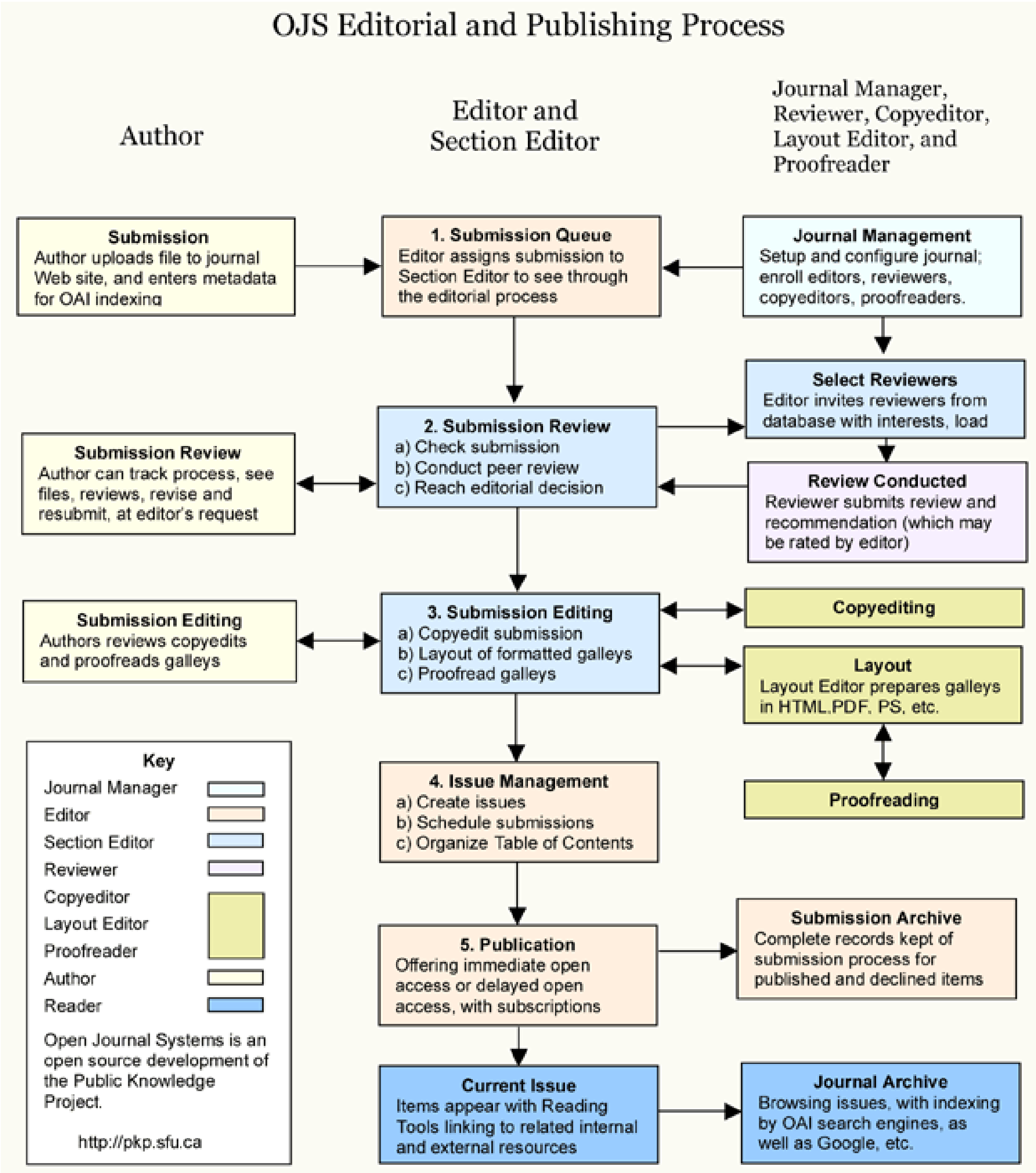
[13] Noordin, W. D., Shafar, J. M. and Che, F. I. Assessment of Selected *Hevea brasiliensis* (RRIM 2000 series) seeds for root stock production. *African Journal of Agricultural Research.* 2012; 7(21): 3209 – 3216.

[14] Onuh, P. N., Madubuike, F. N., Esonu, B. A. and Onu, O. O. The effect of wild cocoyam (*Caladium bicolor*) on the performance and internal organs weight of broiler finisher chickens. *Journal of Science Agriculture Food Technology and Environment*. 2010; 1(1): 19-24.

[15] Olomu, J.M. Monogastric animal production principles and practice. Jachem Publications, Benin City, Nigeria. 2011; 69-104.

[16] Oloruntola, O. D., Agbede, J.O., Onibi, G.E. and Igbasan, F.A.. Replacement value of rumen-liquor fermented cassava peels for maize in growing rabbit diets. *Archivos de zoo Technia.* 2016; 65(249):89-97.

[17] Oyewusi, P.A. Akintayo T.E., Olaofe O. The proximate and amino acid composition of defatted rubber seed meal. *International journal of food, agriculture and environment*, 2007; 5(3-5):115-118.*www.world-food.net*



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