IJOEAR

International Journal of Environmental & Agriculture Research

www.ijoear.com

ISSN
2454-1850

Volume-7, Issue-8, August 2021

Preface

We would like to present, with great pleasure, the inaugural volume-7, Issue-8, August 2021, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

Environmental Research:

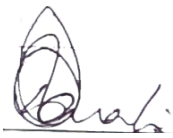
Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

Mukesh Arora
(Managing Editor)



Dr. Bhagawan Bharali
(Chief Editor)

Fields of Interests

Agricultural Sciences	
Soil Science	Plant Science
Animal Science	Agricultural Economics
Agricultural Chemistry	Basic biology concepts
Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
Crop Production	
Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
Pulse Crops: Peas (all types), field beans, faba beans, lentils, soybeans, peanuts and chickpeas.	Hay and Silage (Forage crop) Production
Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
Tree Nut crops: Hazlenuts. walnuts, almonds, cashews, pecans	Berry crops: strawberries, blueberries, raspberries
Sugar crops: sugarcane. sugar beets, sorghum	Potatoes varieties and production.
Livestock Production	
Animal husbandry	Ranch
Camel	Yak
Pigs	Sheep
Goats	Poultry
Bees	Dogs
Exotic species	Chicken Growth
Aquaculture	
Fish farm	Shrimp farm
Freshwater prawn farm	Integrated Multi-Trophic Aquaculture
Milk Production (Dairy)	
Dairy goat	Dairy cow
Dairy Sheep	Water Buffalo
Moose milk	Dairy product
Forest Products and Forest management	
Forestry/Silviculture	Agroforestry
Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
Mechanical	
General Farm Machinery	Tillage equipment
Harvesting equipment	Processing equipment
Hay & Silage/Forage equipment	Milking equipment
Hand tools & activities	Stock handling & control equipment
Agricultural buildings	Storage

Agricultural Input Products	
Crop Protection Chemicals	Feed supplements
Chemical based (inorganic) fertilizers	Organic fertilizers
Environmental Science	
Environmental science and regulation	Ecotoxicology
Environmental health issues	Atmosphere and climate
Terrestric ecosystems	Aquatic ecosystems
Energy and environment	Marine research
Biodiversity	Pharmaceuticals in the environment
Genetically modified organisms	Biotechnology
Risk assessment	Environment society
Theoretical production ecology	horticulture
Breeding	plant fertilization

Board Members

Dr. Goswami Tridib Kumar

Presently working as a Professor in IIT Kharagpur from year 2007, He Received PhD degree from IIT Kharagpur in the year of 1987.

Prof. Khalil Cherifi

Professor in Department of Biology at Faculty of Sciences, Agadir, Morocco.

Dr. Josiah Chidiebere Okonkwo

PhD Animal Science/ Biotech (DELSU), PGD Biotechnology (Hebrew University of Jerusalem Senior Lecturer, Department of Animal Science and Technology, Faculty of Agriculture, Nau, AWKA.

Prof. Özhan ŞİMŞEK

Agriculture Faculty, Department of Horticulture, Çukurova University, Adana, 01330 Turkey.

Dr. Anka Ozana Čavlović

Working as Professor in the department of Faculty of Forestry, University of Zagreb, Svetošimunska 25, Zagreb.

Dr. Rakesh Singh

Professor in Department of Agricultural Economics, Institute of Agricultural Sciences, Banaras Hindu University, Also Vice President of Indian Society of Agricultural Economics, Mumbai.

Dr. Sunil Wimalawansa

MD, PhD, MBA, DSc, is a former university professor, Professor of Medicine, Chief of Endocrinology, Metabolism & Nutrition, expert in endocrinology; osteoporosis and metabolic bone disease, vitamin D, and nutrition.

Dr. Ajeet singh Nain

Working as Professor in GBPUA&T, Pantnagar-263145, US Nagar, UK, India.

Dr. Salvinder Singh

Presently working as Associate Professor in the Department of Agricultural Biotechnology in Assam Agricultural University, Jorhat, Assam.

Dr. Salvinder received MacKnight Foundation Fellowship for pre-doc training at WSU, USA – January 2000- March 2002 and DBT overseas Associateship for Post-Doc at WSU, USA – April, 2012 to October, 2012.

Dr. V K Joshi

Professor V.K.Joshi is M.Sc., Ph.D. (Microbiology) from Punjab Agricultural University, Ludhiana and Guru Nanak Dev University, Amritsar, respectively with more than 35 years experience in Fruit Fermentation Technology, Indigenous fermented foods, patulin ,biocolour ,Quality Control and Waste Utilization. Presently, heading the dept. of Food Science and Technology in University of Horticulture and Forestry, Nauni-Solan (HP), India.

Dr. Mahendra Singh Pal

Presently working as Professor in the dept. of Agronomy in G. B. Pant University o Agriculture & Technology, Pantnagar-263145 (Uttarakhand).

Dr. Sanjoy Kumar Bordolui

M.Sc. (Ag.), PhD, FSTA, FSIESRP, Assistant Professor, Department of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia. W.B., India. He received CWSS Young Scientist Award-2016, conferred by Crop and Weed Science Society, received Best Young Faculty Award 2019 conferred by Novel Research Academy, received Innovative Research & Dedicated Teaching Professional Award 2020 conferred by Society of Innovative Educationalist & Scientific Research Professional, Chennai.

DR. Owais Yousuf

Presently working as Assistant professor in the Department of Bioengineering, Integral University-Lucknow, Uttar Pradesh, India.

Dr. Vijay A. Patil

Working as Assistant Research Scientist in Main Rice Research Centre, Navsari Agricultural University, Navsari. Gujarat- 396 450 (India).

Prof. Salil Kumar Tewari

Presently working as Professor in College of Agriculture and Joint Director, Agroforestry Research Centre (AFRC) / Program Coordinator in G.B. Pant University of Agric. & Tech., Pantnagar - 263 145, Uttarakhand (INDIA).

Dr. S. K. Jain

Presently working as Officer Incharge of All India Coordinated Sorghum Improvement Project, S. D. Agricultural University, Deesa, Gujarat.

Dr. Deshmukh Amol Jagannath

Presently working as Assistant Professor in Dept. of Plant Pathology, College of Agriculture polytechnic, NAU, Waghai.

Mr. Anil Kumar

Working as Junior Research Officer/Asstt. Prof. in the dept. of Food Science & Technology in Agriculture & Technology, Pantnagar.

Mr. Jiban Shrestha

Scientist (Plant Breeding & Genetics)

Presently working as Scientist (Plant Breeding and Genetics) at National Maize Research Programme (NMRP), Rampur, Chitwan under Nepal Agricultural Research Council (NARC), Singhdarbar Plaza, Kathmandu, Nepal.

Mr. Aklilu Bajigo Madalcho

Working at Jigjiga University, Ethiopia, as lecturer and researcher at the College of Dry land Agriculture, department of Natural Resources Management.

Mr. Isaac Newton ATIVOR

MPhil. in Entomology, from University of Ghana.

He has extensive knowledge in tree fruit orchard pest management to evaluate insecticides and other control strategies such as use of pheromone traps and biological control to manage insect pests of horticultural crops. He has

knowledge in agronomy, plant pathology and other areas in Agriculture which I can use to support any research from production to marketing.

Mr. Bimal Bahadur Kunwar

He received his Master Degree in Botany from Central Department of Botany, T.U., Kirtipur, Nepal. Currently working as consultant to prepare CCA-DRR Plan for Hariyo Ban Program/CARE in Nepal/GONESA.

Table of Contents

Effect of mixed *Gmelina* and *Moringa* leaf meal inclusion and sampling periods on haematology and serum biochemistry of growing Red Sokoto does

Okafor, E.C.[1*], Nwankwo, C.A.[2], and Ezejesi, H.C.[3]

Department of Animal Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
*Corresponding Author

Received:- 23 June 2021/ Revised:- 24 July 2021/ Accepted:- 5 August 2021/ Published: 31-08-2021

Abstract— *A study was carried out to investigate the effect of mixed Gmelina arborea and Moringa oleifera (GMMO) leaf meal inclusion and sampling periods on the haematology and serum biochemistry of Red Sokoto does fed Digiteria smutsii hay based diets. Twenty-eight (28) growing Red Sokoto does aged between 6 and 7 months with average weight of 14.71±0.09 kg were randomly assigned to four treatments balanced for weight with seven does per treatment in a completely randomized design. Experimental diets were offered at 4% of body weight. Haematological values shows that all parameters measured are significantly different. Significant (P<0.05) differences were observed cholesterol levels of the animals across the treatments. Cholesterol level was significantly higher (P<0.05) in 0% (107.90) and 10% (107.57) compared to other treatments. Effect of sampling periods on haematological parameters of growing does shows significant differences (P<0.05) in all the parameters measured except white blood cells count. There were significant differences (P<0.05) on total protein and globulin. Total protein ranged from 65.58 to 69.75 g/L at the end and middle of the experiment, respectively. It was significantly higher (P<0.05) at the mid than other periods. Values of globulin were statistically higher (P<0.05) at mid and end of the experiment than at the beginning. Values for all the parameters measured in this study were within the normal ranges for healthy goats. GMMO leaf meal inclusion and sampling periods did not have any adverse effect on blood profile of Red Sokoto does. It can be concluded that GMMO leaf meal can be included in the diets of Red Sokoto does up to 30% without detrimental effects.*

Keywords— Haematology, Serum biochemistry, Red Sokoto goats, Gmelina arborea and Moringa oleifera.

I. INTRODUCTION

Small ruminants are the principal domesticated animals in terms of total numbers and production of food and fibre products. This attribute may partly be due to their lower feed requirements compared to cattle, because of their body size (Okunlola *et al*., 2010). Lower feed and of course the lower capital requirement allows for easy integration into different farming systems (Hirpa and Abebe, 2008; Pollot and Wilson, 2009). Small ruminants have served as means of ready cash and reserve against economic and agricultural production hardship (Hamito, 2008). Goats are considered superior to other ruminant species in their utilization of poor quality and high fibre feeds (Oyeyemi and Akusu, 2005). Goats are by far the most important domesticated small ruminants in Nigeria (FAOSTAT, 2009).

Moringa is one of the most promising plants which could contribute to increased intake of some essential nutrients and health-promoting phytochemicals. It has a high crude protein content ranging from 20-26% CP in leaves (Ben Salem *et al.*, 2004 and Asaolu *et al.*, 2011) with negligible contents of anti-nutrients (Makkar and Becker, 1996). It produces leaves during the dry season and during times of drought, and is an excellent source of green vegetable when little other food is available (FAO, 2014). Moringa leaves can serve as feed for animals. They are valuable source of protein for ruminants but used in poultry, pigs, and fish diets in limited amount because of fibre and anti-nutrients.

Gmelina arborea Roxb. (Family verbenanceae) is a fast growing deciduous tree that can grow up to 40 m tall and 140 cm in diameter (Jensen, 1995). According to Little (1983), the leaves are harvested for fodder for animals and silkworm; the bitter-sweet fruits used to be consumed by humans. Gmelina fruit pulps, seeds and flowers have also been very useful in the feeding of livestock (Amata and Iwelu, 2012). Even though *Gmelina arborea* can shed some of its leaves when the dry season is approaching, the regrowth of new leaves could serve as animal feed during the dry season (Osakwe and Udeogu 2007). The anti-nutritional content of the leaves is low, implying that the overall nutritional value of the leaves will not be affected (Amata and Lebari, 2012).

Haematological studies represent a useful tool in the investigation of the extent of damage to the blood (Ogunbanjo *et al*., 2009). Etim *et al.* (2014) reported that it provides the opportunity to clinically investigate the physiological, nutritional and pathological status of an animal. Nutrition, age, sex, breeds, reproductive status, housing stress and environmental factors are known to affect the haematological and biochemical parameters in farm animals (Addass *et al.*, 2010, Daramola *et al.*, 2005). Iriadam (2007) reported that nutritional status and management practices can influence the physiological attributes and ability of animals to cope with stress.

The objective of this study was to investigate the effect of mixed Gmelina and Moringa (GMMO) leaf meal inclusion levels and sampling periods on haematology and serum biochemistry of growing Red Sokoto does fed *Digitaria smutsii* hay based diets.

II. MATERIALS AND METHODS

2.1 Experimental animals and diets

The experiments were conducted in the Experimental Unit of the Small Ruminant Research Programme of the National Animal Production Research Institute (NAPRI), Shika, Zaria, Kaduna State, Nigeria.

Twenty-eight (28) Red Sokoto does aged between 6 and 7 months with average weight of 14.71±0.09 kg were used with 7 animals per treatment. The animals were obtained from Small Ruminant Research flock, NAPRI. They were individually penned and given prophylactic treatment, consisting of Ivermectin® at 200µg/kg body weight (BWT) against endo- and ectoparasites and Terramycin long acting (LA)® at 20mg/kg BWT against bacterial diseases 7 days before the commencement of the experiment.

Fresh *Gmelina arborea* (GM) leaves were harvested within Ahmadu Bello University Main Campus and the leaves were allowed to air-dry for three days. Dried *Moringa oleifera* leaves were sourced from Sabon-Gari market, Zaria. *Digitaria smutsii* (Wooly finger grass) hay was sourced from the Feeds and Nutrition Research Programme of NAPRI. The dried leaves of the two browses and *D. smutsii* hay were ground with a hammer mill fitted with 2cm screen for easy mixing with other feed ingredients. The ground ingredients were packed in sacks and stored in a well-ventilated store.

Four isonitrogenous complete diets were formulated, with 40% *D. smutsii* hay base. The complete diets were compounded to contain 13% CP. *Gmelina arborea and Moringa oleifera* leaf meals were combined at 75 and 25% respectively. The mixed Gmelina and Moringa leaf meal was included at 0, 10, 20 and 30%. Each level of inclusion served as a treatment. Other ingredients in the complete diet include maize offal, cotton seed cake, salt and bone meal (Table 1).

TABLE 1
INGREDIENT COMPOSITION OF EXPERIMENTAL DIETS (%) FED TO RED SOKOTO DOES

Ingredients	Level of GMMO leaf meal inclusion (%)			
	T_1	T_2	T_3	T_4
75GM:25MO	0	10	20	30
Cottonseed Cake	23.40	20.00	16.00	12.30
Maize offal	34.60	28.00	22.20	15.80
Bone meal	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5
D.smutsii hay	40	40	40	40
Total	100	100	100	100
Calculated analysis				
% Crude Protein	13.01	13.06	13.02	13.00
ME (Kcal/kg)	2437	2407	2375	2357
Cost/kg feed (₦)	44.14	40.63	37.12	33.52

75 GM: 25 MO= 75:25% combination of Gmelina and Moringa leaf meal; ME=Metabolizable energy.

The diets were mixed fortnightly to maintain freshness and samples were taken to determine the chemical composition. Seven animals were randomly allocated to four treatments with each animal serving as a replicate in a completely randomized design.

At 8.00 hours, the animals were offered their daily ration of 4% body weight of the experimental diets. The animals had free access to clean drinking water. The experiment was carried out for a period of 60 days after 14 days' adjustment period.

2.2 Haematological Analysis

Blood samples of about 5ml were collected from the does at the beginning, middle and end of the experiment, through the jugular vein using a 5 ml syringe. Two (2ml) of the blood sample collected was transferred into a sampling bottle containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The blood samples collected were taken to the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria for determination of haematological parameters (Packed cell volume, haemoglobin, red blood cells and white blood cells).

2.3 Evaluation of serum Biochemical Constituents

The remaining blood sample of about 3ml was poured into plain bottle and allowed to clot at room temperature within 3 hours of collection. Plasma was separated by centrifugation at 3500 r.p.m. for 15 min and serum was thereafter frozen at -20ºC for the determination of total protein, albumin, total cholesterol, glucose, creatinine and urea nitrogen with the use of Elisa Multiplex Commercial Kits (Pfizer Animal Health, New York, NY) following the methods of the Manufacturers. The globulin values were obtained by subtracting the values of albumin from the corresponding values of total protein (Coles, 1974).

2.4 Statistical Analysis

All data generated were analyzed statistically using the General Linear Model (GLM) procedure of SAS, (2005). Significant differences between treatment means were determined according to Duncan's Multiple Range Test of SAS, (2005).

III. RESULTS AND DISCUSSION

3.1 Effect of Gmelina and Moringa leaf meal inclusion on hematological parameters of growing Red Sokoto does fed *Digitaria smutsii* hay based diets

The effects of Gmelina and Moringa leaf meal inclusion on hematological parameters of growing Red Sokoto does fed *D. smutsii* hay based diets are presented in Table 2. Significant differences were observed in all the parameters measured. The PCV, Hb and RBC were significantly ($P<0.05$) higher in animals fed 10% GMMO leaf meal compared to other treatments. White blood cell count was statistically ($P<0.05$) higher in animals fed 30% than those on 20% but comparable to values recorded in control and 10% respectively.

TABLE 2
EFFECTS OF GMELINA AND MORINGA LEAF MEAL INCLUSION ON HEMATOLOGICAL PARAMETERS OF GROWING RED SOKOTO DOES FED *DIGITARIA SMUTSII* HAY BASED DIETS

Parameters	Levels of Gmelina and Moringa leaf meal inclusion				SEM
	0%	10%	20%	30%	
PCV (HCT) (%)	35.89^{b}	41.11^{a}	33.78^{b}	34.44^{b}	2.46
Hgb(×10g/L)	11.93^{b}	13.69^{a}	11.22^{b}	11.46^{b}	0.82
RBC ($\times10^{12}$/L)	16.03^{ab}	16.96^{a}	15.72^{b}	15.71^{b}	1.16
WBC ($\times10^{12}$/L)	9.51^{ab}	9.93^{ab}	8.10^{b}	12.22^{a}	1.91

a,b, Mean values with different superscripts within a row differ significantly (P<0.05), SEM= Standard Error of Means. WBC = White blood cells; RBC = Red blood cells; Hb = Haemoglobin; PCV (HCT) = Packed cell volume (Haematocrit).

Etim *et al.* (2014) reported that haematological parameters are indicator of the physiological, nutritional and pathological status of an animal. The result showed that packed cell volume, haemoglobin, red blood cell and white blood cell were all within normal range for healthy goats (Latimer *et al.*, 2010). This may be due to the quality and adequacy of the treatment diets. Reports of Roberts *et al*. (2000) and Bello and Tsado (2013) indicated that diets containing poor protein affect the haematology and health of animals.

Table 3 shows the results of the effect of feeding of GMMO leaf meal on serum biochemistry of growing Red Sokoto does fed *D. smutsii* hay based diets. Significant (P<0.05) differences were observed cholesterol levels of the animals across the treatments. The cholesterol level decreased with increasing level of GMMO leaf meal inclusion. The cholesterol level was significantly higher (P<0.05) in 0% (107.90) and 10% (107.57) compared to other treatments. There were no significant differences (P>0.05) in other parameters measured.

TABLE 3
EFFECT OF MIXED GMELINA AND MORINGA LEAF MEAL ON SERUM BIOCHEMISTRY OF GROWING RED SOKOTO DOES FED *D. SMUTSII* HAY BASED DIETS

Parameters	Levels of Gmelina and Moringa leaf meal inclusion				SEM
	0%	10%	20%	30%	
Total protein (g/L)	66.67	66.44	68.22	67.33	5.09
Albumin (g/L)	38.00	32.56	35.11	34.44	9.46
Globulin (g/L)	28.67	33.89	33.11	32.89	9.94
Glucose (mg/dL)	61.67	70.11	59.56	61.78	9.42
Creatinine (µmol/L)	108.44	102.44	107.78	116.00	21.66
BUN (mmol/L)	5.97	5.32	5.28	5.49	1.69
Cholesterol (mg/dL)	107.90^{a}	107.57^{a}	86.28^{b}	81.07^{b}	10.53

a,b, Mean values with different superscripts within a row differ significantly (P<0.05), BUN= Blood Urea Nitrogen; SEM= standard error of mean.

The values of the parameters measured were within the normal ranges for healthy goats reported by Kaneko *et al.* (2008). This is in contrast with the work of Ologhobo *et al.* (2014) who reported no significant effect on most parameters measured for birds fed graded levels of *Moringa oleifera* leaf meal. The lower cholesterol level obtained in animals fed 20% and 30% GMMO leaf meal agreed with the work of Aderinola *et al.* (2013). This can be as a result of the hypocholesterolemic properties of Moringa leaf meal included in the diets (Olugbemi *et al*., 2010).

3.2 Effect of sampling periods on haematological parameters of growing Red Sokoto does fed levels of GMMO leaf meal in *D. smutsii* hay based diets

Table 4 shows the result of the effect of sampling periods on haematological parameters of growing Red Sokoto Does fed *D.smutsii* hay based diets supplemented with GMMO leaf meal inclusions. There were significant differences (P<0.05) in all the parameters measured except white blood cells count. Pack cell volume and haemoglobin were statistically similar at the beginning and middle of the experiment than at the end of experiment. Red blood cell was significantly (P<0.05) higher at the beginning of experiment than other periods.

TABLE 4
EFFECT OF SAMPLING PERIOD ON HAEMATOLOGICAL PARAMETERS OF GROWING RED SOKOTO DOES FED LEVELS OF GMMO LEAF MEAL INCLUSION IN *D. SMUTSII* HAY BASED DIETS

Parameters	Sampling Periods			SEM
	Beginning	Mid	End	
PCV (HCT) (%)	38.08[a]	35.33[ab]	33.17[b]	1.59
Hgb(×10g/L)	12.67[a]	11.36[ab]	10.91[b]	0.47
RBC ($\times10^{12}$/L)	12.10[a]	8.18[b]	9.55[b]	1.12
WBC ($\times10^{12}$/L)	16.28	16.29	15.75	0.36

a,b, Mean values with different superscripts within a row differed significantly (P<0.05), SEM= Standard Error of Means. WBC = White blood cells; RBC = Red blood cells; Hb = Haemoglobin; PCV (HCT) = Packed cell volume (Haematocrit).

All the values of parameters measured in this study fell within the normal range for healthy goats (Latimer, 2010). Reduction in the haemoglobin may be accompanied by a fall in the red blood cells count (RBC) and packed cell volume (haematocrit). Red blood cells are responsible for the transportation of oxygen and carbon dioxide in the blood and also involved in the manufacture of haemoglobin (Ologun and Ikeobi, 2006). Haemoglobin is an iron- containing compound found in the red blood cells, which transports oxygen around the body (Soetan *et al.,* 2006; Isaac *et al.,* 2013). Reduction in packed cell volume and red blood cell values are indicative of low protein intake or mild anemia but values are within the normal ranges for healthy goats.

3.3 Effect of sampling periods on serum biochemical parameters of growing Red Sokoto does fed levels of GMMO leaf meal inclusion in *D. smutsii* hay based diets

The results of effect of sampling periods on serum biochemical parameters of growing Red Sokoto does fed levels of GMMO leaf meal inclusion *in D. smutsii* hay based diets are presented in Table 5. There were significant differences (P<0.05) on total protein and globulin. The other parameters measured were not significant across the sampling periods. Total protein ranged from 65.58 to 69.75 g/L at the end and middle of the experiment, respectively. It was significantly higher (P<0.05) at the mid than other periods. The values of globulin were statistically higher (P<0.05) at mid and end of the experiment than at the beginning. The reduced total protein observed in this study may be as a result of the quality of protein fed. The parameters measured were within the ranges reported for clinically healthy goats (Kaneko *et al.,* 2008). This implied that the animals were in good health condition and were not stressed by the nutrition or environment. Reports of Olafedehan *et al*. (2010) found that blood acts as a pathological reflector of the status of exposed animals to diseases and other conditions.

TABLE 5
EFFECT OF SAMPLING PERIODS ON SERUM BIOCHEMICAL PARAMETERS OF GROWING RED SOKOTO DOES FED LEVELS OF GMMO LEAF MEAL INCLUSION IN *D. SMUTSII* HAY BASED DIETS

Parameters	Sampling Periods			SEM
	Beginning	Mid	End	
Total Protein	66.17[b]	69.75[a]	65.58[b]	1.35
Albumin	37.58	35.08	32.42	2.68
Globulin	28.58[b]	34.67[a]	33.17[a]	2.79
Glucose	62.83	62.17	64.83	2.92
Creatinine	95.92	118.08	112.00	11.53
BUN	5.66	5.61	5.28	0.49
Cholesterol	92.54	97.31	97.26	4.69

a,b, Mean values with different superscripts within a row differed significantly (P<0.05), SEM= Standard Error of Means; BUN= Blood Urea Nitrogen

IV. CONCLUSION

The study showed that mixed Gmelina and Moringa leaf meal inclusion and the sampling periods have no detrimental effects on in Red Sokoto does fed *D.smutsii* based diets. The animals were not stressed and were in healthy condition throughout the

period of the study. It was therefore concluded that mixed Gmelina and Moringa leaf meal can be included in the diets of Red Sokoto does up to 30% without adverse effects on them.

ACKNOWLEDGEMENT

The authors thank the Director, National Animal Production Research Institute, Prof. Gefu, J.O. for giving us the approval to carry out the research in the institute, and also to use the experimental animals and facilities. We are also grateful to the staff of the Small Ruminant Experimental Unit and the Central Laboratory of the Institute for their good work in the study.

REFERENCES

[1] Addass, P.A., Midau, A. and Babale, D.M. (2010). Haemato-biochemical findings of indigenous goats in Mubi, Adamawa State, *Nigeria Journal of Agricultural Sciences*, 6: 14-16.

[2] Aderinola, O.A., Rufiu, T.A., Akinwumi, A.O. Alabi, T.A. and Adeagbo, O.A. (2013). Utilization of *Moringa oleifera* leaf as feed supplement in broiler diets. *International Journal of food, Agricultural and veterinary Sciences*, 3(3): 94-102.

[3] Amata, I. A. and Lebari, T. (2011). Comparative evaluation of the nutrient profile of four selected browse plants in the tropics, recommended for use as non-conventional livestock feeding materials. *African Journal of Biotechnology*, 10 (64):14230-14233.

[4] Amata, I.A. and Iwelu, E.E. (2012). Changes in the proximate composition and anti-nutritional content of the fruits of Gmelina arborea tree during growth and development. *International Journal of Innovations in Bio-Sciences*, 2(3):126-129.

[5] Asaolu, V.O., Binuomote, R., Akinlade, J.A., Oyelami, O.S. and Kolapo, K.O. (2011). Utilization of *Moringa oliefera* fodder combinations with *Leucaena leucocephala*, and *Gliricidia sepium* fodders by West African Dwarf Goats. *International Journal of Agricultural Research, ISSN 1816- 4897/ DOI:IO. 3923/ ijar.2011.*

[6] Bello, A.W. and Tsado, D.N. (2013). Haematological and biochemical profile of growing Yankasa rams fed sorghum stover supplemented with graded levels of dried poultry droppings based diets. *Pakistan Journal of Biological Science*, 15 (24):1922-1928.

[7] Coles, E.H. (1974). *Liver function*. In: Saunders, W.B. (Editor) *veterinary clinical pathology* Second Edition. company Philadelphia. pp 202-215.

[8] Daramola, J. O., Adeloye, A. A., Fabola, T.A. and Soladoye, A.O. (2005). Haematological and Biochemical Parameters of West African Dwarf Goats", *Livestock Research for RuralDevelopment*, 17 (8).

[9] Etim, N.N., Williams, M.E., Akpabio, U. and Offiong, E.E.A. (2014). Haematological Parameters and factors affecting their values. Agricultural Science, 2: (1), 37-47.

[10] FAO (2014). Moringa. Traditional Crop of the Month. FAO.

[11] FAOSTAT (2009). http://faostat.fao.org/default.aspx

[12] Hamito, D. (2008). Preface. In: Sheep and Goat Production Handbook for Ethiopia, P. 5.

[13] Hirpa, A. and Abebe, G. (2008). Economic Importance of Sheep and Goats. In: Sheep and Goat Production Handbook for Ethiopia, Addis Ababa, Pp. 1-4.

[14] Iriadam, M. (2007). Variation in certain hematological and biochemical parameters during the peri-partum period in Kilis does. *Small Ruminant Research*, 73 (1): 54-57.

[15] Isaac, L.J., Abah, G., Akpan, B. and Ekaette, I.U. (2013). Haematological properties of different breeds and sexes of rabbits. In: *Proceeding of 32nd Annual Conference of Animal Science Association of Nigeria*, Pp 24-27.

[16] Jensen, M. (1995). *Trees Commonly Cultivated in South East Asia, Illustrated field guide*, *RAP* Publication: 1995/38 FAO Bangkok. Thailand, Pp 93.

[17] Kaneko, J., Harvey, J. and Bruss, M. (2008). *Clinical Biochemistry of Domestic Animals*. 5th Ed. Academic Press, New York, Pp 932.

[18] Latimer, K.S. (2010). Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th ed., Chichester (UK), Wiley-Blackwell, Pp 509.

[19] Little, E. L. (1983). Common fuel crops: a handbook for their identification. McClain Printing Company, Parsons, West Virginia.

[20] Makkar, H.P.S. and Becker, K. (1996). Nutritional value and anti-nutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Science Technology*, 63: 211-228.

[21] Ogunbanjo, S.O., Alemede, I.C., Adama, J.Y. and Abdullahi, J. (2009). Haematological parameters of Savannah brown does fed varying dietary levels of flamboyant tree seed meal In: *Proceedings of 34th Annual Conference of Nigerian Society for Animal Production*. Pp 88-91.

[22] Okunlola, O.O., Amuda, A.J. and Ayanwamide, P.M. (2010). Farmers Perception of Livestock Farming in Oyo State: A case study of small ruminant farmers. *Proceeding of 35thAnnual Conference of Nigeria Society for Animal Production* held 14- 17th March, University of Ibadan, Nigeria.

[23] Olafedehan, C.O., Obun, A.M., Yusuf, M.K., Adewumi, O.O., Oladefedehan, A.O., Awofolaji, A.O. and Adeniji, A.A. (2010). Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits. In: *Proceeding of the 35th Annual Conference of Nigerian Society for Animal Production*held 14- 17th March, University of Ibadan, Nigeria. Pp 427-433.

[24] Ologhobo, A.D., Akangbe, E.I., Adejumo, I.O. and Adeleye, O. (2014). Effect of Moringa oleifera leaf meal as replacement for oxytetracycline on carcass characteristics of the diets of broiler chickens. *Annual Research and Review in Biology*, 4 (2): 423-431.

[25] Ologun, A.G. and Ikeobi, C.O.N. (2008). Haematological parameters in rabbit breeds and crosses in humid tropics. *Pakistan Journal of Biological Sciences*, 9 (11): 2102-2106.

[26] Olugbemi, T. S., Mutayoba, S. K. and Lekule, F. P. (2010). Effect of moringa (Moringa oleifera) inclusion in cassava based diets fed to broiler chickens. *International Journal of Poultry Science*, 9 (4): 363-367.

[27] Osakwe, I.I. and Udeogu, R.N., (2007). Feed Intake and Nutrient Digestibility of West African Dwarf (WAD) Goat Fed *Pennisetum purpureum* Supplemented with Gmelina arborea. *Animal Research International*, 4 (3): 724- 727.

[28] Oyeyemi, M.O. and Akusu, M.O. (2005). Retrospective study on some diseases causing mortality in West African Dwarf (Fouta djallon) goats during the first year of life: an eight-year study. *Nigerian Journal of Animal Production*, 32 (1): 120–125.

[29] Pollot, G. and Wilson, R.T. (2009). *Sheep and goat for diverse products and profits*. FAO diversification booklet, 9/FAO Rome Italy.

[30] Roberts, K.M., Daryl, K.G., Peter, A.M. and Victor, W.R. (2000). *Mayer's Biochemistry*, 25th edition, McGraw Hill, New York, Pp 763-765.

[31] SAS (2005). Institute Inc. SAS/STAT user's guide. 6.03 Ed, Gray NC. USA.

[32] Soetan, K. O., Akinrinde, A. S. and Ajibade, T. O. (2013). *Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor*). *Proceedings of 38th Annual Conference of Nigerian Society for Animal Production*, Pp 49-52.

Review Article: Bacteriocin Production and its Application in Food and Pharmaceuticals

M. M. Nalwade[1], T. N. Gaikwad[2], R. S. Shinde[3], S. R. Nezkar[4], S. P. Owal[5*]

Department of Microbiology, Tulajaram Chaturchand College, Baramati, Dist – Pune, Maharashtra, India, 413102
*Corresponding Author

Received:- 28 July 2021/ Revised:- 05 August 2021/ Accepted:- 14 August 2021/ Published: 31-08-2021

Abstract— *Bacteriocins are ribosomal - synthesized antimicrobial peptides that inhibit the growing of pathogenic or deteriorating bacteria. Bacteriocin are a heterogenous group of bioactive bacterial peptides or proteins having variable biochemical properties. Bacteriocin is introduced to denote toxic proteins or peptide produced by any type of bacteria that is active on related bacteria but does not harm the producing cell. They are antimicrobial peptides which are ribosomally synthesized and produced by Lactic Acid Bacteria (LAB). Now days, there are various hazardous effect of chemicals. Instead of chemicals, bacteriocins are mostly effective in food technology which aims to extend food preservation time, treat pathogen diseases, cancer therapy & maintain human health. In food processing, lactic acid bacteria (LAB) shows numerous anti-microbial activities. This is mainly due to production of organic acid, but also of other compound, such as bacteriocin and various peptides. Bacteriocins are used as a potential drug for replacing antibiotics in order to treat multiple drug resistance pathogens. The important mechanism of bacteriocins is pore formation. Bacteriocins are used as food preservation against contaminating organism. It also used against anti-tumor drug in pharmaceutical. They play major role in prevention of human diseases such as cancer, inflammatory diseases, respiratory infection, intestinal disorders, etc. The species of Bacillus, Staphylococcus, Escherichia, Klebsiella, Lactobacillus, Pseudomonas, Proteus are successfully used. These are isolated from vegetables, dairy, cheese, meat and other products. Therefore, Bacteriocin may become a potential drug candidates for replacing antibiotics in order to treat multiple drugs resistance pathogens in the future. Bacteriocin become one of the weapons against micro-organisms due to the specific characteristics of large diversity of structure and function, natural resource and being stable to heat.*

Keywords— Bacteriocin, Lactic acid bacteria, Cancer therapy, Pore formation, Antimicrobial activity.

I. INTRODUCTION

Unlike chemical preservation and antibiotics, generally recognized as safe (GRAS) bacteriocins, such as nisin, promise safe use as food preservation in vegetables, dairy, cheese, meats and other food products, as they inhibit microorganism contamination during the production, process and storage.

1.1 Bacteriocins

There are many antibacterial substance produced by animal, plants, insect and bacteria, such as hydrogen peroxide, fatty acids, organic acid, ethanol, antimicrobial peptides (AMPs) or proteins produced by bacteria are categorized as bacteriocins. Nutrients in the environment trigger microbial production of a variety of bacteriocins for competition of space and resources.

Bacteriocins are abundant, have larger diversity, and the genes encode ribosomally synthesized antimicrobial peptides or proteins, which kill other related (narrow spectrum) or non-related (broad spectrum) microbiotas as one of the inherent defence system weapons of bacteria. They play major role in prevention, of human diseases such as cancer, inflammatory diseases respiratory infection, intestinal disorder, etc.

More than 99 % of bacteria can produce maximum one bacteriocins, most of which are not identified [1]. The Killing ability of bacteriocins is considered a successful strategy for maintaining population and reducing the no. of competitors to obtain more nutrients and living space in environments unlike most antibiotics, which are secondary metabolites bacteriocins are ribosomally synthesized and sensitive to proteases while generally harmless to the human body surrounding environment.

Modern society is more care an healthy of the importance of food safety, as many of the chemical additives used in food many toxic concern, therefore, it is beneficial to claim natural resources and health benefits of natural food without chemical additives have become more popular, and most commercially available preservation and antibiotics are produced by chemical synthesis, and long -term consumption and storage of such product can affect human body as they reduce the counts of bacteria is in a the gut. Moreover, the use of antibiotics or residues in food is illegal. Unlike chemical preservation and antibiotic, generally recognised as safe, (GRAS) bacteriocins, such as nisin, promise safe use as a food, preservation in vegetables, dairy, cheese, meats, and other food products as they inhibit microorganism. Contamination during the production time [**2].**

1.2 Classification of bacteriocins

Classification of bacteriocins from Gram-positive and Gram- negative bacteria.

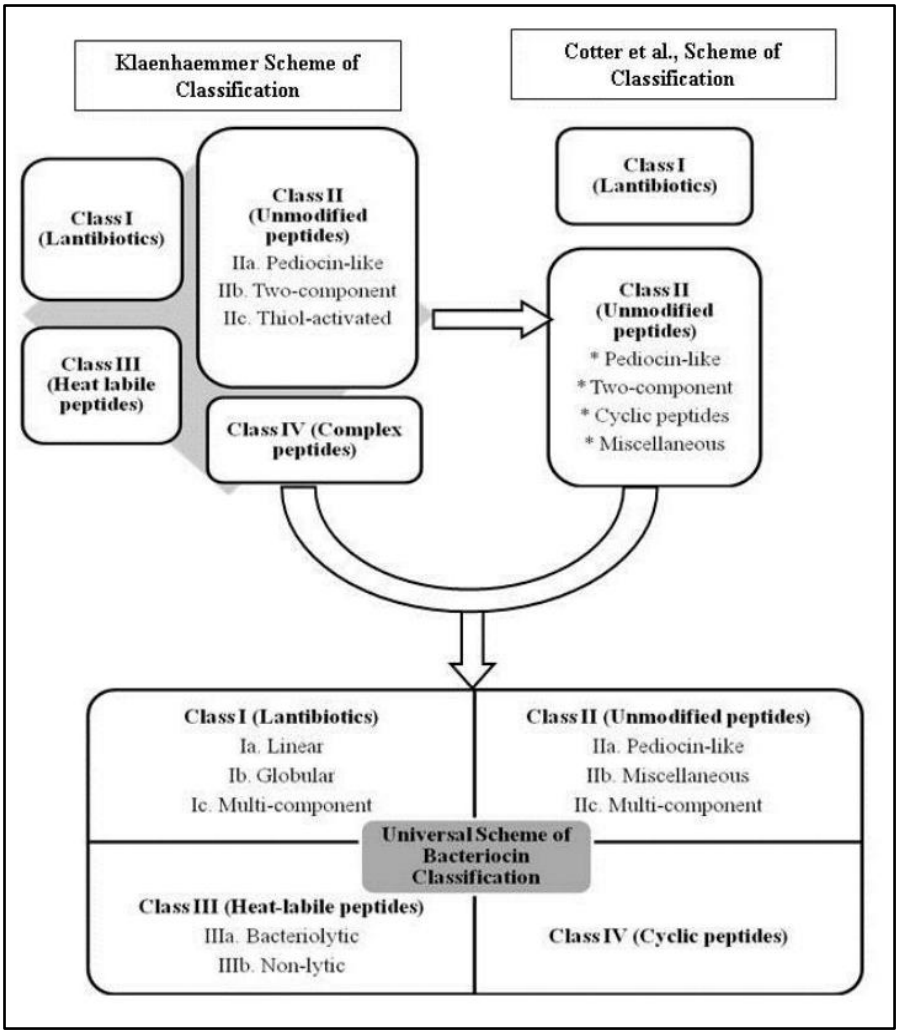


FIGURE 1: Classification of Bacteriocins

Several classification of bacteriocins have been proposed taking into consideration proposed by klaenhammer (1993).

Recently, in order to classify novel bacteriocins, presentation an adjusted classification scheme based on the biosynthesis mechanism and biological activity in accordance with other presentation.

They propose three major classes:-

Class 1:- Small post - translationally modified peptides.

Class 2:- Unmodified Bacteriocins

Class 3:- Heat labile peptide Bacteriocins.

Class 4:- Complex peptides.

1.3 Bacteriocins from Gram -negative bacteria

1.3.1 Colicin

Colicin are antibacterial proteins produced by bacteria, which can kill bacterial strains closely related to produced species.

Classification of colicins by different system :

TABLE 1
CLASSIFICATION OF COLICIN

Colicins	Antibacterial activity	Receptor	Translocators	Molecular weight (Da)	Producing strain
GROUP A					
A	Pore-forming	BtuB	OmpF, TolABQR	62989	*Citrobacter freudii*
E1	Pore-forming	BtuB	TolC, TolAQ	57279	*Escherichia coli*
K	Pore-forming	Tsx	OmpAF, TolABQR	59611	*Escherichia coli*
N	Pore-forming	OmpF	OmpF, TolAQR	41696	*Escherichia coli*
S4	Pore-forming	OmpW	OmpF, TolABQR	54085	*Escherichia coli*
U	Pore-forming	OmpA	OmpF, TolABQR	66289	*Shigella boydii*
28b	Pore-forming	OmpA	OmpF, TolABQR	47505	*Serratia marcescens*
E2	DNase	BtuB	OmpF, TolABQR	61561	*Escherichia coli* *Shigella sonnei*
E7	DNase	BtuB	OmpF, TolABQR	61349	*Escherichia coli*
E8	DNase	BtuB	OmpF, TolABQR	~70000	*Escherichia coli*
E9	DNase	BtuB	OmpF, TolABQR	61587	*Escherichia coli*
E3	16S rRNase	BtuB	OmpF, TolABQR	57960	*Escherichia coli*
E4	16S rRNase	BtuB	OmpF, TolABQR	ND	*Escherichia coli*
E6	16S rRNase	BtuB	OmpF, TolABQR	58011	*Escherichia coli*
DF13	16S rRNase	lutA	OmpF, TolAQR	59293	*Escherichia coli*
E5	tRNase	BtuB	OmpF, TolABQR	58254	*Escherichia coli* *Shigella sonnei*
GROUP B					
B	Pore-forming	FepA	TonB-ExbBD	54742	*Escherichia coli*
Ia	Pore-forming	Cir	TonB-ExbBD	69429	*Escherichia coli*
Ib	Pore-forming	Cir	TonB-ExbBD	69923	*Escherichia coli* *Shigella sonnei*
5	Pore-forming	Tsx	TolC, TonB-ExbBD	53137	*Escherichia coli*
10	Pore-forming	Tsx	TolC, TonB-ExbBD	53342	*Escherichia coli*
D	tRNase	FepA	TonB-ExbBD	74683	*Escherichia coli*
M	Peptidoglycanase	FhuA	TonB-ExbBD	29453	*Escherichia coli*

ND, not determined.

1.3.2 Microcine

Microcine are low molecular weight ribosomal synthesized hydrophobic antimicrobial peptides<10kDa. Which is distinguished by 25-80kda high molecular weight colicins protein.

TABLE 2
CLASSIFICATION OF MICROCINS.

Classification	Characteristics	Microcins	Molecular weight (Da)	Producing strain
Class I	Low molecular weight peptides (<5 kDa), post-translationally modified	B17	3094	*Escherichia coli*
		C7/C51	1177	*Escherichia coli*
		D93	<1000	*Escherichia coli*
		J25	2107	*Escherichia coli*
Class II	Larger (5–10 kDa) peptides, with or without post-translational modifications			
class IIa	Required more than one genes to synthesize and assemble functional peptides	L	8884	*Escherichia coli*
		V	8741	*Escherichia coli*
		N/24	7274	*Escherichia coli*
class IIb	Linear peptides with post-translational modifications or not at C-terminal	E492	7886	*Klebsiella pneumoniae*
		M	7284	*Escherichia coli*
		H47	4865	*Escherichia coli*

1.4 Mode of action of bacteriocins

The Proposed mode of action for bacteriocins is an initial binding to the bacterial membrane by electrostatic force between the negatively charged membrane.

Most of the bacteriocins break out the energy potential of sensitive cells, by a forming membrane pores. The best mechanism is pore formation.

1.5 Application of bacteriocins in Foods and Pharmaceutical

Bacteriocins are widely used in food science to food preservation [17], which inhibits the pathogen infection of animal disease [7] and pharmaceutical industry to treatment for cancer therapy against anti-tumor drug [16].

Bacteriocins are considered as natural product because they are peptides or protein produced by bacteria. In many fermented and non-fermented foods.

The main application of bacteriocins in:

1. Probiotics.
2. Cancer therapy.
3. Food preservation.
4. Drug resistance.

1.5.1 Probiotics

The Probiotic is generally considered to promote the balance of intestinal microbiota and increase the health benefits. The characteristics of Probiotics should include a group of strains beneficial to the host animals that can stably survive and have metabolic in intestinal environment. Probiotic demonstrate the capability of antimicrobial substance production, competitive exclusion of pathogen binding computation for nutrients and modulation of the immune system (FAO & WHO 2001).

Antimicrobial substance such as bacteriocins is produced by Probiotics for inhibiting gastrointestinal microorganisms or pathogens [14]. Bacteriocin produce Probiotic, can reduce the number of pathogen in animal model, such as mice, chikens, & pigs [8].

In many food product such as traditional European cheese, the milk is used in manufacturing process is easily contaminated with animal Excrement (waste matter).

1.5.2 Cancer therapy :-

Cancer has become serious problem and threat to human health. In cancer therapy some researches indicates that bacteriocin shows against tumor cells. Bacteriocin may be suitable as a potential anti -tumor drug. Some bacteriocin such as pore - forming colicin A and E1 inhibited the growth of human standard fibroblast line MRCs and 11 human tumor cell line [**9]**. In recent study [15] found the nisin had capability to prevent cancer therapy.

1.5.3 Food technology

Food preservation are incorporated into food to delay to microbial growth and possible corruption. Bacteriocins are produced by gram positive and gram negative gene encoded peptides or protein. Suitable to natural preservation in food products.

In food technology, nisinis produced by *Lactococcus lactic* and was the first antimicrobial peptides found in LAB [19]. Bacteriocins are used as food preservation against contamination by microorganism. It is any bacteriocin approved for utilization as preservation in many foods by the U. S food and Drug administration. Bacteriocins are used to extend food preservation time, treat pathogen diseases & maintain human health.

1.5.4 Drug Resistance

The first antibiotics penicillin was discovered in 1928 by Alexander Fleming. The problem with multiple drug resistance pathogens has become increasingly serious, regarding of antibiotics [18].

Bacteriocin is reported to inhibit important animal and plant pathogens such as shigo toxin producing *E coli, Staplococcusaurus* [12].

bacteriocins are mainly located in receptor binding of bacterial surface & through the membrane which cause bacteria cytoxicity. Bacteriocins are low-toxic peptides or proteins sensitive to protease, such as trypsin & pepsin [11]. Other bacteriocins, potential when applied to replace antibiotics in Poultry & other animal feeds.

II. CONCLUSION

Bacteriocins are considered as natural product because they are peptides or protein, which has different types, isolated from various sources, including plants. Among them, LAB is selected for bacteriocin production and their antimicrobial activity against microorganisms or pathogens i. e (*Bacillus coagulans, E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Proteus vulgaris*). Thus, bacteriocin has potential to inhibit the growth of pathogen or deteriorating bacteria. Thus, bacteriocin has been used to preserve food against contamination by microorganisms. Antimicrobial substances such as bacteriocin are produced by probiotics for inhibiting gastrointestinal microorganisms or pathogens. Thus, in disease control, bacteriocin can solve some of the most challenging problems of multi-drug resistant pathogens such as multifunctional bacteriocin, which are more powerful in functionality and germicidal range. As a result they can be widely used in food, animal husbandry and medicine.

ACKNOWLEDGEMENT

We all are thankful of honourable principal Dr. Chandrashekhar Murumkar, Dr. Sunil Pawar and Smt. Sheetal Owal, Tuljaram Chaturchand College, Baramati for support and guidance.

REFERENCES

[1] Riley, M. A., and Wertz, J. E. (2002). Bacteriocins: evolution, ecology, and application. Annu. Rev. Microbiol. [56, 117–137]. doi: 10. 1146/annurev. micro. 56. 012302. 161024.

[2] Deegan, L. H., Cotter, P. D., Hill, C., and Ross, P. (2006). Bacteriocins: biological tools for bio-preservation and shelf-life extension. Int. Dairy J. [16, 1058–1071]. doi: 10. 1016/j. idairyj. 2005. 10. 026.

[3] Settanni, L., and Corsetti, A. (2008). Application of bacteriocins in vegetable food biopreservation. Int. J. Food Microbiol. [121, 123–138]. doi: 10. 1016/j. ijfoodmicro. 2007. 09. 001.

[4] Burianek, L. L., and Yousef, A. E., (2000). Solvent Extraction of Bacteriocins from liquid cultures. Letters in Applied Microbiology. [31: 193-197].

[5] Ohmomo, S., Kobayashi, M., Yajima, M., Suyanandana, P., Budka, and Somchai, P., (1998). Screening of thermophilic lactic acid bacteria producing bacteriocins in the tropics. Retrieved it from http:// 2 Ohmomo.

[6] Martirani, L., Varcamonti, M., Naclerio, G., and De Felice, M., (2002). Purification and partial characterization of Bacillocin 490, a Novel Bacteriocin produced by athermophilic strains of Bacillus licheniformis. Microbial cell Factories [1: 1 – 5].

[7] Van Heel, A. J., Montalban-Lopez, M., and Kuipers, O. P. (2011). Evaluating the feasibility of lantibiotics as an alternative therapy against bacterial infections in humans. Expert Opin. Drug Metab. Toxicol. [7, 675–680]. doi: 10. 1517/17425255. 2011. 573478.

[8] Bernbom, N., Licht, T. R., Brogren, C. H., Jelle, B., Johansen, A. H., Badiola, I., etal. (2006). Effects of Lactococcuslactis on composition of intestinal microbiota: roleofnisin. Appl. Environ. Microbiol. [72, 239-244]. doi:10. 1128/AEM. 72. 1. 239244. 2006.

[9] Chumchalová, J., and Smarda, J. (2003). Human tumor cells are selectively inhibited by colicins. Folia Microbiol. (Praha) [48, 111–115]. doi: 10. 1007/BF029 31286.

[10] Grinter, R., Milner, J., and Walker, D. (2012). Bacteriocins active against plant pathogenic bacteria. Biochem. Soc. Trans. [40, 1498–1502]. doi: 10. 1042/BST20120206Lancaster, L. E.,

[11] Cleveland, J., Montville, T. J., Nes, I. F., and Chikindas, M. L. (2001). Bacteriocins: safe, natural antimicrobials for food preservation. Int. J. Food Microbiol. [71, 1–20]. doi: 10. 1016/S0168-1605(01)00560-8.

[12] Rogers, L. A. (1928). The inhibiting effect of Streptococcus lactis on Lactobacillus bulgaricus. J. Bacteriol. [16, 321–325].

[13] Vijai Pal, MarilinggappaJamuna and KadirveluJeevaratnam (2004). Isolation and Characterization of Bacteriocin producing lactic acid bacteria from South Indian special dosa (appam) Batter. Journal of culture collections, [4: 53-60].

[14] Dobson, A., Cotter, P. D., Ross, R. P., and Hill, C. (2012). Bacteriocin production: a probiotic trait? Appl. Environ. Microbiol. [78, 1–6]. doi: 10. 1128/AEM. 05576-11

[15] Joo, N. E., Ritchie, K., Kamarajan, P., Miao, D., and Kapila, Y. L. (2012). Nisin, an apoptogenic bacteriocin and food preservative, attenuates HNSCC tumorigenesis via CHAC1. Cancer Med. [1, 295–305]. doi: 10. 1002/cam4. 35

[16] Lancaster, L. E., Wintermeyer, W., and Rodnina, M. V. (2007). Colicins and their potential in cancer treatment. Blood Cells Mol. Dis. [38, 15–18]. doi: 10. 1016/j. bcmd. 2006. 10. 006

[17] Ghrairi, T., Chaftar, N., and Hani, K. (2012). "Bacteriocins: recent advances and opportunities" in Progress in Food Preservation, Chapter 23. eds R. Bhat, A. Karim Alias and G. Paliyath (Oxford: Wiley-Blackwell), [485–511].

[18] Joerger, R. D. (2003). Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poult. Sci. [82, 640–647]. doi: 10. 1093/ps/82. 4. 640

[19] Rogers, L. A. (1928). The inhibiting effect of Streptococcus lactis on Lactobacillus bulgaricus. J. Bacteriol. [16, 321–325].

Effect of Storage Method and Storage Duration on Chicken Egg Quality

J. C. Okonkwo[1*], Offiaukwu Chiamaka[2], I. F. Okonkwo[3], B.N Marire[4]

[1,2,4]Department of Animal Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[3]Department of Microbiology and Brewery, Nnamdi Azikiwe University, Awka, Nigeria
*Corresponding Author

Received:- 23 June 2021/ Revised:- 20 July 2021/ Accepted:- 02 August 2021/ Published: 31-08-2021

Abstract— *Poultry production is a fast growing industry and has become a dependable source of obtaining income for many farmers. There is therefore a growing need for technologies to preserve poultry products, in this case eggs, to prevent or reduce post-harvest losses. The aim of this present study is to evaluate the effect of storage method and duration on internal and external egg quality traits of eggs stored under three different storage conditions. A total of 190 eggs (from Isa brown breed hens aged 51 weeks) were used for the analysis. The storage methods to which the eggs were subjected included; 1) Cold Storage (M1), 2) Saw dust (M2) and 3) Control (M3) at a temperature range of 26°C- 32°C.The eggs were stored for 15 days, while the readings were taken at three day intervals. Albumen height, haugh unit, yolk index and egg weight have been found to be important parameters influenced by storage method and storage duration. Cold storage had the highest value for albumen height, haugh unit, yolk index and egg weight, while eggs stored under sawdust had no significant difference from those under control. Duration however had a deteriorating effect on important egg quality traits form D3 to D15 as could be observed in Albumen weight (41.14 to 36.37), Haugh unit (73.0 to 55.1), Yolk Index (36.99 to 26.61) and Egg weight (61.85 to 56.76). Thus, lower egg quality was recorded as storage time increased. Results from the first microbial analysis (freshly laid eggs) showed that no organism was isolated. Coliform bacteria, mold and yeast were isolated from eggs stored using cold storage and control conditions respectively. This study showed that eggs stored under cold storage retained both internal and external quality traits for longer time than those stored under sawdust and control, after the eggs had been stored for 15 days.*

Keywords— Egg, Egg quality, Storage method, Storage duration.

I. INTRODUCTION

The effect of storage methods and time on the external and internal egg quality traits of laying hens in different climate is going to examined. The parameters were: egg weight, albumen and yolk height, albumen and yolk width, albumen and yolk indices, Haugh unit, egg width, egg length and shape index.

II. MATERIAL AND METHOD

Eggs used in this study were collected from Department of Animal Science and Technology Teaching and Research Farm, and analyzed at the Departmental Laboratory, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria. Awka, the capital of Anambra state is located on Latitude: 6° 12' 45.68" N, Longitude: 7° 04' 19.16" E.

The eggs were collected from 51 weeks old Isa brown layers in less than 24 hours of lay. The first storage method (M1) is the cold storage (7°C), the second storage method (M2) is storage on saw dust at room temperature at the range of 26°C- 32°C, while the third storage method (M3) is the Control (Stored without any form of treatment). Standard methods were used to determine the internal and external egg quality trait.

III. RESULT AND DISCUSSION

3.1 Effect of storage method on internal egg quality

While albumen height, albumen index, albumen length, albumen weight, albumen width, yolk diameter, yolk height, yolk index, yolk ratio, yolk weight and Haugh unit were significantly different ($P<0.05$), Albumen ratio was not significantly ($P>0.05$) affected by method of storage. The Yolk height and Haugh unit were highest for eggs stored using cold storage and lowest for eggs stored using control.

This result agrees with that of Tabidi (2011) who found that albumen height was higher in eggs stored in refrigerator compared to that obtained from eggs which were subjected to storage at room temperature. Albumen index of eggs preserved using cold storage was higher, followed by those stored under sawdust, then those under control condition. The reduction in albumen index in sawdust and control could be attributed to the high room temperature compared to conditions in cold storage. Albumen length and Albumen width were lowest for eggs stored under cold storage. The highest Albumen length was recorded for eggs stored under control (9.62), while eggs stored under sawdust had the highest width (7.87). This result aligned with that of Scott and Silversides (2000) and Raji *et al*. (2009). The increase in length and width of the albumen could be as a result of high loss of CO_2 which occurs at room temperature, leading to rapid deterioration in albumen quality. Albumen weight had the highest value for eggs stored under cold storage, while control group gave the lowest value. This finding agreed with that of Jin *et al.*(2011) who attributed changes in egg quality to loss of moisture by evaporation through the egg shell pores and the escape of CO_2 from albumen.

3.2 Effect of storage duration on internal egg quality

Except for Yolk ratio, other internal egg quality traits were affected ($P<0.05$) by storage duration. Albumen height, Albumen index, Albumen ratio, Albumen weight, yolk height, yolk index and Haugh unit decreased with an increase in storage duration, while as duration increased, Albumen length, Albumen width, and yolk diameter tend to increase with time.

Yolk diameter increased with storage time. This result agrees with that of Okonkwo (2009), Raji *et al*. (2009) and Scott and Silverside (2000). The yolk of fresh eggs is round and firm. As the yolk ages it losses quality by absorbing water and increasing in size. According to literature, yolk wholeness is dependent on the strength of the vitelline membrane which is inversely related to the duration of storage (Jones and Musgrove, 2005; Li-Chan *et al.,* 2017). Kirunda and McKee (2000) also reported the decrease in the strength of the Vitelline membrane during storage making the yolk more susceptible to breaking. Yolk weight increased from 3.96 to 4.46 with an increase in time of storage, this result agrees with findings of Jin *et al*. (2011). The increase in weight could be as a result of the absorption of water by the yolk from thin albumin. Yolk index decreased with duration from 36.99-26.6. This present study agrees with Caner and Cansiz (2007), Fasenko *et al*. (1995), Monira *et al*. (2003), and Miles and Henry (2004) in their findings observed a decrease in yolk index with storage time. Yolk height decreased from 1.46 to 1.16 with time, this aligns with findings of Okoli *et al*. (2000) and Seidler (2003), who observed that as the egg ages due to prolonged storage, the vitelline membrane degenerates and water from the albumen moves into the yolk causing the yolk to have a flattened shape.

3.3 Effect of storage method on external egg quality

As observed from the Table 3, only the Average shell thickness and egg weight were significantly ($P< 0.05$) affected by storage method. The Average shell thickness of eggs stored using cold storage and Control were not significantly different from each other. Egg length, Egg width, Shape index and shell weight of the three storage methods were not significantly different ($P>0.05$).

Also the shell thickness was affected by both storage method and duration. For control it decreased from 0.80 at day 3 to 0.70 at day 15, in sawdust from 0.90 to 0.81, and cold storage from 0.80 to 0.70. This result disagrees with the result obtained by Tabidi (2011) who observed no difference in the egg shell thickness between the eggs stored at room temperature and those stored using a refrigerator. This could be as a result of the rate of loss of CO_2 form eggs in the three storage method due to season.

3.4 Effect of storage duration on external egg quality

Results on the effect of storage duration on external egg traits is presented in table 4.The effect of storage duration was significant ($P<0.05$) for Average shell thickness, Egg length, Egg weight, Shape index and Shell weight. There was no

significant difference (P>0.05) for Egg width and Shell ratio. For the Average shell thickness, the durations (D3-D12) slightly differ from each other, and the highest shell thickness was recorded in D15.

Results from the study shows that Average shell thickness and Egg weight were affected by the method of storage. All other external parameters were unaffected by the method of storage. Cold storage had the highest egg weight (61.30) while eggs under control had the lowest egg weight (58.18). The highest weight loss was recorded in control while the least weight loss was recorded in cold storage. This result agrees with the findings of Dudusola (2009) who found that weight losses differ with the different storage methods, where the eggs preserved by refrigeration had lower moisture loss compared to those in groups stored at room temperature (control). This reduction in weight could be due to high rate of moisture losses through the egg shell from the albumen due to high room temperature compared to temperature of cold storage.

Average shell thickness was affected by duration. According to Jibir *et al.* (2012), shell thickness or strength is the most commonly used parameter for measuring external egg quality. Storage duration also had an impact on Shell weight, this present finding agrees with that of Samli *et al*. (2005) and Jin *et al.,* (2011) who observed and reported significant changes in shell weight with storage time and temperature. Egg weight tends to decrease with duration, this report is in accordance with that of Samli *et al.* (2005), Akyurek *et al.* (2009), Walsh *et al.* (1995) and Siyar *et al.* (2007), who observed a decrease in egg weight with duration of storage.

IV. CONCLUSION

This study has shown the effect of using cold storage, saw dust and control at room temperature to store eggs over a time frame in order to determine the most suitable means of preserving it. Cold storage happens to be better than the other two methods, since it has values higher than the other two storage methods in terms of Albumen height, Haugh unit, yolk index, shell thickness and egg weight which are considered to be important parameters in the determination of egg quality. Storage on Saw dust still maintained external quality of the egg, even after internal deterioration had set off. Sawdust can be used to store eggs that would be consumed within very few days of storage. The qualities of egg, especially the internal quality deteriorated greatly with time. The yolk began to stick to the shells and the thick albumen became completely watery and immeasurable. Therefore, eggs should be consumed as soon as possible otherwise it should be preserved using cold storage.

REFERENCES

[1] Akyurek, H., &Okur, A. A. (2009). Effect of storage time, temperature and hen age on egg quality in free-range layer hens. *Journal of Animal and Veterinary Advances*, *8*(10), 1953-1958.

[2] Caner, C., &Cansiz, O. (2007). Effectiveness of chitosan-based coating in improving shelf-life of eggs. *Journal of the Science of Food and Agriculture*, *87*(2), 227-232.

[3] Dudusola, I. O. (2009). Effects of storage methods and length of storage on some quality parameters of Japanese quail eggs. *Tropicultura*, *27*(1), 45-48.

[4] Fasenko, G. M., Christensen, V. L., Bakst, M. R., &Petitte, J. N. (1995). Evaluating yolk membranes from short and long stored turkey eggs using transmission electron microscopy. *Poultry Sci*, *74*(Suppl 1), 44.

[5] Jibir, M., Garba, S., &Ribah, M. I. (2012). Relative importance of external quality attributes of shell eggs: A consumer preference approach. *International Journal of Applied Agriculture and Apiculture Research*, *8*(1), 31-38.

[6] Jin, Y. H., Lee, K. T., Lee, W. I., & Han, Y. K. (2011). Effects of storage temperature and time on the quality of eggs from laying hens at peak production. *Asian-Australasian Journal of Animal Sciences*, *24*(2), 279-284.

[7] Jones, D. R., & Musgrove, M. T. (2005). Effects of extended storage on egg quality factors. *Poultry science*, *84*(11), 1774-1777.

[8] Kirunda, D. F. K., & McKee, S. R. (2000). Relating quality characteristics of aged eggs and fresh eggs to vitelline membrane strength as determined by a texture analyzer. *Poultry Science*, *79*(8), 1189-1193.

[9] Li-Chan, E. C., Powrie, W. D., &Nakai, S. (2017). The chemistry of eggs and egg products. In *Egg science and technology* (pp. 105-175). CRC Press

[10] Miles, R. D., & Henry, P. R. (2004). Effect of time and storage conditions on albumen quality of eggs from hens fed vanadium. *Journal of applied poultry research*, *13*(4), 619-627.

[11] Monira, K. N., Salahuddin, M., & Miah, G. (2003). Effect of breed and holding period on egg quality characteristics of chicken. *Int. J. Poult. Sci*, *2*(4), 261-263

[12] Okoli, I. C., &Udedibie, A. B. I. (2000). Effect of oil treatment and storage temperature on egg quality. *Journal of Agriculture and Rural Development*, *1*, 55-60.

[13] Okonkwo, J. (2009). Effects of breed and storage duration on the beta-carotene content of egg yolk. *Pakistan Journal of Nutrition*, *8*(10), 1629-1630.

[14] Raji, A. O., Aliyu, J., Igwebuike, J. U., &Chiroma, S. (2009). Effect of storage methods and time on egg quality traits of laying hens in a hot dry climate. *ARPN Journal of Agricultural and Biological Science*, *4*(4), 1-7.

[15] Samli, H. E, Agma, A. and Senkoylu. N. (2005). Effect of storage time and temperature on eggquality in old laying hens. *Journal of Applied Poultry Research.* 14: 548-553.

[16] Scotts, T.A. and Silversides, F.G. (2000): The Effect of Storage and Strains of Hen on Egg Quality. *Journal ofPoultry Sciences and Technology* 79: 1725-1729.

[17] Seidler, E. S. (2003). Egg marketing. A guide for the production and sale of eggs. *FAO Agricultural Services Bulletin (FAO)*.

[18] Siyar H.S.A, Aliarabi, H., Ahmadi, A, and Ashori. N. (2007). Effect of different storage conditions and hen age on egg quality parameters. *Australian Poultry Science Symposium*. 19: 106-109.

[19] Tabidi, M. H. (2011). Impact of storage period and quality on composition of table egg. *Advances in Environmental Biology*, *5*(5), 856-861.

[20] Walsh T J., Rizk R.E and Brake, J. (1995) Effects of temperature and carbon dioxide on albumen characteristics, weight loss and early embryonic mortality of long stored hatching eggs. *Journal of Poultry Science* 74: 1403-1410.

Genotype × Environment Interaction and Stability Analysis for Selected Agronomic Traits in Cassava (*Manihot esculenta*)

Kumba Yannah Karim[1*], Prince Emmanuel Norman[2]

[1]Crop Science Department, School of Agriculture and Food Sciences, Njala University, Sierra Leone Tel. +23279877815;
[2]Sierra Leone Agricultural Research Institute (SLARI), Tower Hill, Freetown PMB 1313, Sierra Leone Tel. +23276618454;
*Corresponding Author

Received:- 03 August 2021/ Revised:- 11 August 2021/ Accepted:- 16 August 2021/ Published: 31-08-2021

Abstract— *Cassava (Manihot esculenta Crantz) is an important root and tuber crop worldwide. The crop is highly influenced by variations in production environments. A significant Genotype × Environment Interaction (GEI) presents challenges in the selection of superior genotypes. This study determined the magnitude of GEI and stability performances of 26 cassava genotypes for key agronomic traits across three multi-environments. The trial was laid out in a randomized complete block design during 2016/2017 cropping season. Genotype TR0288 had the highest starch content at Pendembu and Kambia, while TR1436 performed best at Njala. Genotype TR0768 had the highest fresh storage root yield at Pendembu, TR0455 at Kambia and TR0591 and TR0657 at Njala environments. For dry matter content, genotypes SLICASS4, TR0310 and TR0740 performed best at Njala, Pendembu and Njala, respectively. Genotype TR0455 had the highest fresh storage root yield across the three production environments, TR1436 for starch content and TR0310 for dry matter content. TR0310 was the most stable and favorable genotype based on mean dry matter content and stability performance across the three production environments. Harvest index was positive and significantly correlated with storage root ($r = 0.54^{***}$), fresh storage root yield was highly and positively correlated with number of storage root ($r = 0.61^{***}$) and harvest index ($r = 0.49^{***}$). The information generated is relevant for selection initiatives targeted at superior high yielding, high dry matter content and starch content cassava genotypes combining resistance to cassava mosaic in Sierra Leone*.

Keywords— Genotypic performance, multi-environment trial, stability analysis, trait correlation, cassava.

I. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important starchy root crop utilized for human consumption, animal feed and various industrial applications [1]. The starchy storage roots of cassava are important source of dietary energy in sub-Saharan Africa (SSA) as they provide more returns per unit of input than any other staple crop [2–4]. Cassava serves as food security and income generation crop for resource poor farmers due to its tolerance to climate changes such as erratic rainfall and poor soil fertility. In Sierra Leone, cassava is the second most important staple crop after rice. The cassava root production in the country has increased from 82,500 tons in 1970 to 4.59 million tons in 2019 growing at an average annual rate of 12.08% [5]. However, on-farm cassava yields are significantly lower than the potential yields of improved varieties estimated at $\geq$ 25 t ha^{-1} [6]. For instance, in 2019, 59,660 ha were cultivated to cassava by 101,021 households, producing 817,342 MT [6]. A wide yield variability ranging from 6.5 MT ha^{-1} to 33.9 MT ha^{-1} exists among genotypes, with an average yield (14.5 MT ha^{-1}) below 50% relative to yields obtained under good agronomic practices [6]. Cassava is cultivated in all regions of Sierra Leone due to its easy propagation, value of cultivation and utilization.

Despite its enormous significance, increased cassava productivity is limited by a number of biotic and abiotic factors [7]. For instance, cassava green mite attack can cause about 15 and 73% yield losses in resistant and susceptible genotypes of cassava, respectively Bellotti [8], whereas about 88% yield loss can be due to cassava mealy bug infestation in susceptible genotypes [9]. Low crop yields are also caused by low yielding varieties, environmental variability and poor environmental management or use of elite agronomic packages.

The performance of any character is a combined result of the genotype (G), the environment (E) and the interaction between genotype and environment (GE) [10]. The GE interaction (GEI) exists when the responses of two genotypes to different

levels of environmental stress are inconsistent. The GEI and yield-stability analyses have become increasingly important for measuring cultivar stability and suitability for cultivation across seasons and ecological zones [11]. Multi-environment trials (METs) have been found to be important in plant breeding for studying cultivar stability and predicting yield performance of cultivars across environments [12].

Several authors have noted the effects of GEI in cassava. Tumuhimbise *et al*. [13] reported a non-significant GEE effect for early fresh storage root yield (FSRY). Moreover, the effect of GEI on dry matter content (DMC) has been well noted [14,15]. In Sierra Leone, there is dearth of information of the GEI effects and stability performance of putative cassava genotypes developed for key agronomic traits (yield, disease resistance, root dry matter content, starch content and harvest index). A good understanding of GEI effects is useful to plant breeders for selection of suitable genotypes for specific environments. The determination of stability performance of genotypes across varying test sites requires use of specific tools and methods [10]. The univariate, bivariate and multivariate techniques are the common methods often utilized for stability analysis [16]. The additive main effects and multiplicative interaction (AMMI) multivariate analytical technique is the most widely used method for GEI assessment [10]. The AMMI method effectively captures a large portion of the GEI sum of squares [17]. The identification of yield-contributing traits and knowledge of GEI and associated yield stability are important considerations in breeding new cultivars with improved adaptation to the environmental constraints that prevail in target environments [18]. Thus, the objective of this study was to determine the magnitude of Genotype × Environment Interaction and stability performance of genotypes for its effective utilization to improve key agronomic traits in cassava.

II. MATERIALS AND METHODS

2.1 Experimental sites

The trials were conducted during 2016/2017 cropping season at three locations representing different agro-ecological zones in Sierra Leone. The mean monthly minimum and maximum temperatures, annual rainfall and soil attributes of the various sites are presented in Table 1.

TABLE 1
AGRO-ECOLOGICAL CHARACTERISTICS OF THE TRIAL SITES

Attribute	Location		
	Njala	Kambia	Pendembu
Coordinates			
Longitude	8°9′32.14″N	9°7′30.16″N	7°57′06″N
Latitude	12°25′54.05″W	12°55′5.38″W	-10°55′26″E
Elevation (m)	73	57	157
Agro-ecological zone	Transitional rainforest	Savannah	Rainforest
Weather and climate attributes			
Rainfall (mm)	2616.6	2456.0	2745.4
Temperature (min-max) (ºC)	21.5–31.2	20.9–32.4	21.0–31.9
Relative humidity (min-max) (%)	62.6–83.1	64.0–87.0	70.7–83.7
Soil attributes			
pH(H_2O) (1:1)	5.00	4.40	5.08
OC (%)	0.54	0.21	0.31
N (%)	0.14	0.14	0.15
Bray P (ppm)	5.43	4.31	6.84
K (Cmol/kg)	0.08	0.08	0.06

2.2 Plant material and experimental design

A total of 26 genotypes comprising 23 advanced clones from IITA, one local check (COCOA) and two released checks (SLICASS 4 and SLICASS 6). The trial was laid out in a randomised complete block design with three replications. Healthy stems of each genotype were cut into 25 cm length each and planted horizontally on ridges at a spacing of 1 × 1 m. Each plot measured 3 ×10 m comprising three rows of 10 plants each.

2.3 Data collection

A total of six agronomic traits were collected including cassava mosaic disease (CMD); storage root number per plant (SRN); fresh storage root yield (FSRY); harvest index (HI); starch content (SC); and dry matter content (DMC). The CMD was collected at six months after planting (MAP) using the scale of 1 to 5 (1 = no visible symptom of disease; 2 = mild; 3 = low; 4 = intermediate; 5 = high) as described by Fukuda *et al*. [19].

Starch content was determined according to the method described by Sofa-Kantanka and Osei-Minta [20]. The amount of dried starch obtained from 2 kg of fresh cassava storage roots was weighed and expressed as a percentage of the weight of fresh storage roots. The starch content was calculated as follows:

$$\text{Starch content} = \frac{X}{Y} \times 100 \quad (1)$$

Where X = dry weight starch extracted and Y=fresh weight of cassava storage roots.

2.4 Data analysis

The data were subjected to combined analyses of variance using the GLM procedure of Statistical Analysis System (SAS 9.4) to determine the magnitude of the main effects and interactions. For genotypic assessment of the selected agronomic traits across environment trials, prediction assessment was conducted using the AMMI method [21]. The AMMI model was as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \sum_{k=1}^{n} \lambda_k \gamma_{ik} \delta_{jk} + \varepsilon_{ij} \quad (2)$$

where, Y_{ij} = the yield of ith genotype in jth environment over all replications, μ is the grand mean, α_i is the ith genotype mean deviation,
β_j = the jth environment mean deviation, λ_k is the singular value for IPC axis k, γ_{ik} is the ith genotype eigenvector value for IPC axis k, δ_{jk} is the jth environment eigenvector value for IPC axis k, and ε_{ij} is the error term.

The eigenvalue (EV) stability parameter of AMMI was calculated based on the equation by Zobel *et al*. [22]:

$$EV = \mu + \alpha_i + \beta_j + \sum_{n=1}^{N} \frac{\lambda_{in}^2}{n} \quad (3)$$

In this formula, γ_{in} = the genotype eigenvector for axis n, and N = the number of IPCs retained in the AMMI procedure using different F-test.

The sum of IPCs scores (SIPC) parameter was determined according to Sneller *et al*. [23]:

$$\text{SIPC} = \sum_{n=1}^{N} \lambda_n^{0.5} y_{in} \quad (4)$$

Where, λ_n is the eigenvalue of the IPC analysis axis n. In this equation, N = 1 for SIPC1; and for SIPCF, N is the number of IPCs retained in the AMMI model.

The GGE Biplot was done to visually assess the GEI pattern of data using GGE-biplot software [24]. The GGE Biplot is based on two concepts including the Biplot concept [25] and the GGE concept [26]. Correlation of the various plant parameters was done using Pearson correlation coefficients [27].

III. RESULTS

3.1 AMMI analysis of the measured traits

The combined analyses of variance (ANOVA) across the three environments revealed that, highly significant differences (P<0.001) were observed among genotypes (G) for NSR, DM, and CMD, while non-significant differences were observed among genotypes for HI, SY and FSRY. Significant differences were observed among locations for all traits except for dry matter content. Also, genotype × location (G × L) interactions were significant for number of storage root, starch content, fresh storage root yield, dry matter content and cassava mosaic disease (Table 2). In the combined AMMI ANOVA, the genotype mean squares were highly significant (P < 0.001) for all the traits evaluated (Tables 2 and 3).

TABLE 2
MEAN SQUARES VALUES OF COMBINED ANALYSIS FOR THE 26 CASSAVA GENOTYPES EVALUATED FOR DISEASE, ROOT YIELD AND RELATED ATTRIBUTES IN THREE LOCATIONS

Source	DF	NSR	HI	SC	FSRY	DMC	CMD
REP	2	2376.594**	0.005^{ns}	12.851^{ns}	117.304^{ns}	18.979^{ns}	0.205^{ns}
GEN	25	1352.410**	0.0286^{ns}	36.155^{ns}	110.077^{ns}	25.618**	3.507**
LOCATION	2	58213.453**	1.716**	1338.760**	3294.339**	16.056^{ns}	40.936**
GEN*LOCATION	50	1823.573**	0.023^{ns}	18.079^{ns}	146.047**	14.700**	1.989**
ERROR	154	394.780	0.021	20.795	71.898	7.0522	0.850
CV		42.620	32.865	45.321	48.861	7.578	43.065

P < 0.01; NSR=Number of storage root; HI= harvesting index; SC= starch content, FSRY = fresh storage root yield; DMC= dry matter content and CMD= cassava mosaic disease

TABLE 3
MEAN SQUARES OF AMMI ANALYSIS OF VARIANCE FOR THE 26 CASSAVA GENOTYPES EVALUATED FOR DISEASE, ROOT YIELD AND RELATED ATTRIBUTES ACROSS THREE LOCATIONS

Source	DF	NSR	HI	SC	FSRY	DMC	CMD
Treatments	77	1317***	0.07***	58.3***	216.1***	18.28***	3.49***
Genotypes	25	3135***	0.03^{ns}	36.2*	110.1***	25.62***	3.51***
Environments	2	58213***	1.69***	1338.8***	3294.4***	16.06^{ns}	40.94***
Block	6	1988***	0.002***	26.1^{ns}	54.6***	20.18^{ns}	0.27^{ns}
Interaction	50	1824***	0.02^{ns}	18.1^{ns}	146***	14.70***	1.99***
IPCA1	26	2822***	0.024^{ns}	21.9**	150.8***	21.63***	2.78***
IPCA2	24	742^{ns}	0.021^{ns}	13.9^{ns}	140.9***	7.20^{ns}	1.14
Error	150	357	0.021	20.5	72.8	6.66	0.86
Total	233						

P < 0.05 and P < 0.001; NSR=Number of storage root; HI= harvesting index; SC= starch content, FSRY = fresh storage root yield; DMC = dry matter content and CMD= Cassava mosaic disease

The location mean squares were highly significant (P < 0.001) for storage root number; harvest index; fresh storage root yield; cassava mosaic disease and significant (P < 0.05) for starch content. Genotype × environment mean squares were highly significant (P < 0.001) for number of storage root; fresh storage root yield; dry matter content and cassava mosaic disease. The IPCA1 mean squares were highly significant (P < 0.001) for number of storage root; fresh storage root yield; dry matter content, starch content and cassava mosaic disease. Harvest index had non-significant IPCA1 mean squares while the IPCA2 mean squares were non-significant for all traits except for fresh storage root yield that was highly significant.

3.2 The GEI patterns of traits and genotypes based on GGE biplot analysis

The partitioning of GGE through GGE biplot analysis showed that PC1 and PC2 accounted for 45.13% and 34.06% of GGE sum of squares, respectively, for root yield, explaining a total of 79.19% variation (Figure 1). Genotype TR0455 had the highest average fresh storage root yield and TR0657 had the lowest fresh storage root yield across three environments. Stability of each genotype is explored by its projection onto the AEC vertical axis. Thus, genotypes TR0768, TR0458 and TR0488 were the least stable. TR0024 was the most stable genotype followed by SLICASS 4, SLICASS 6 and TR0541. However, considering mean yield performance, genotype TR0455 is regarded as the most stable.

The partitioning of GE interaction through GGE biplot analysis showed that PC1 and PC2 accounted for 51.16% and 30.43% of GGE sum of squares, respectively explaining a total of 81.59% variation of the starch content (Figure 2). Genotype TR1436 had the highest average starch content, and TR0657 had the lowest mean starch content across the three environments. Genotypes TR1436, TR0048 and TR0288 were the most stable genotypes, whereas for mean fresh root yield and stability performances, genotype TR1436 is the most favorable. The PC1 and PC2 accounted for 60.39% and 27.08% explaining a total of 87.47% variation of the dry matter content (Figure 3). The average dry matter content of genotypes across the three environments was estimated by projections on to the AEC horizontal axis. Thus, genotype TR0310 had the highest average dry matter content, and TR1716 had the lowest mean dry matter content across the three environments. Stability of each genotype is explored by its projection onto the AEC vertical axis. Genotypes TR0310 and TR0488 were the

most stable genotypes. Considering both mean dry matter content and stability performance, genotype TR0310 is regarded as the most favorable.

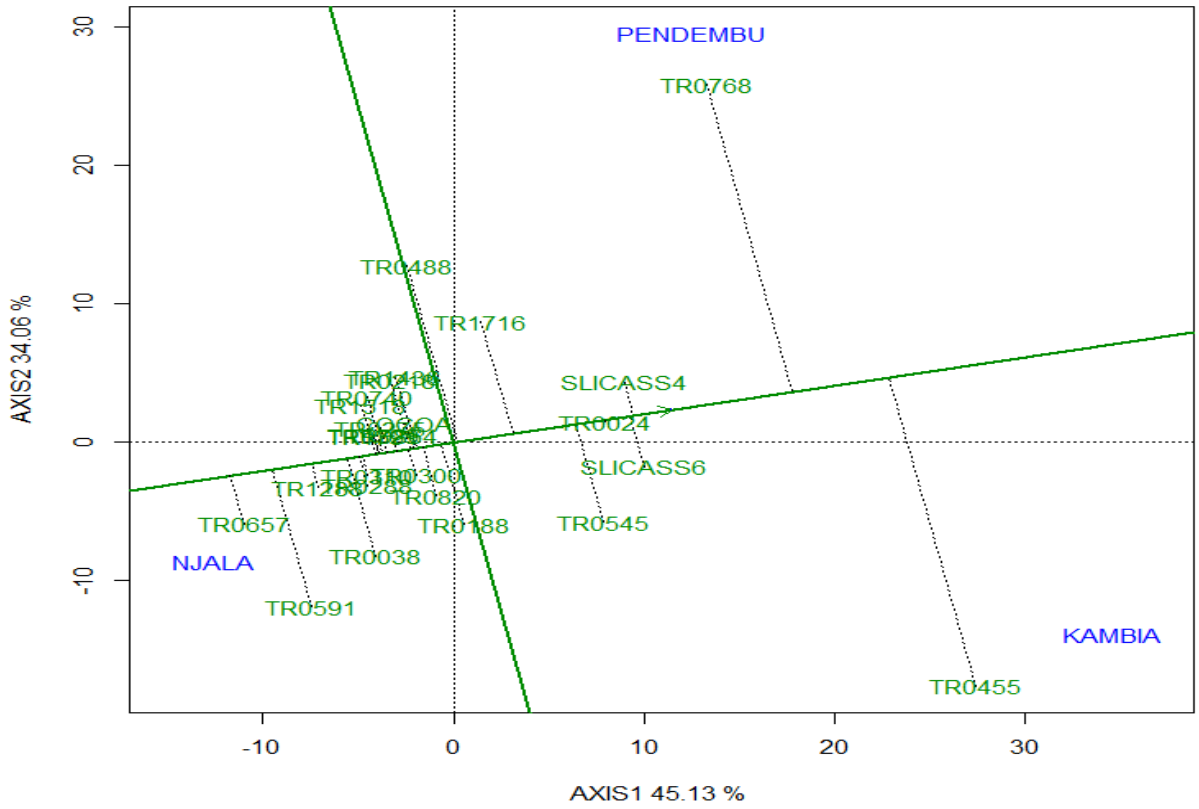


FIGURE 1: GGE biplot of mean and stability performance of 26 cassava genotypes for fresh root yield across three environments

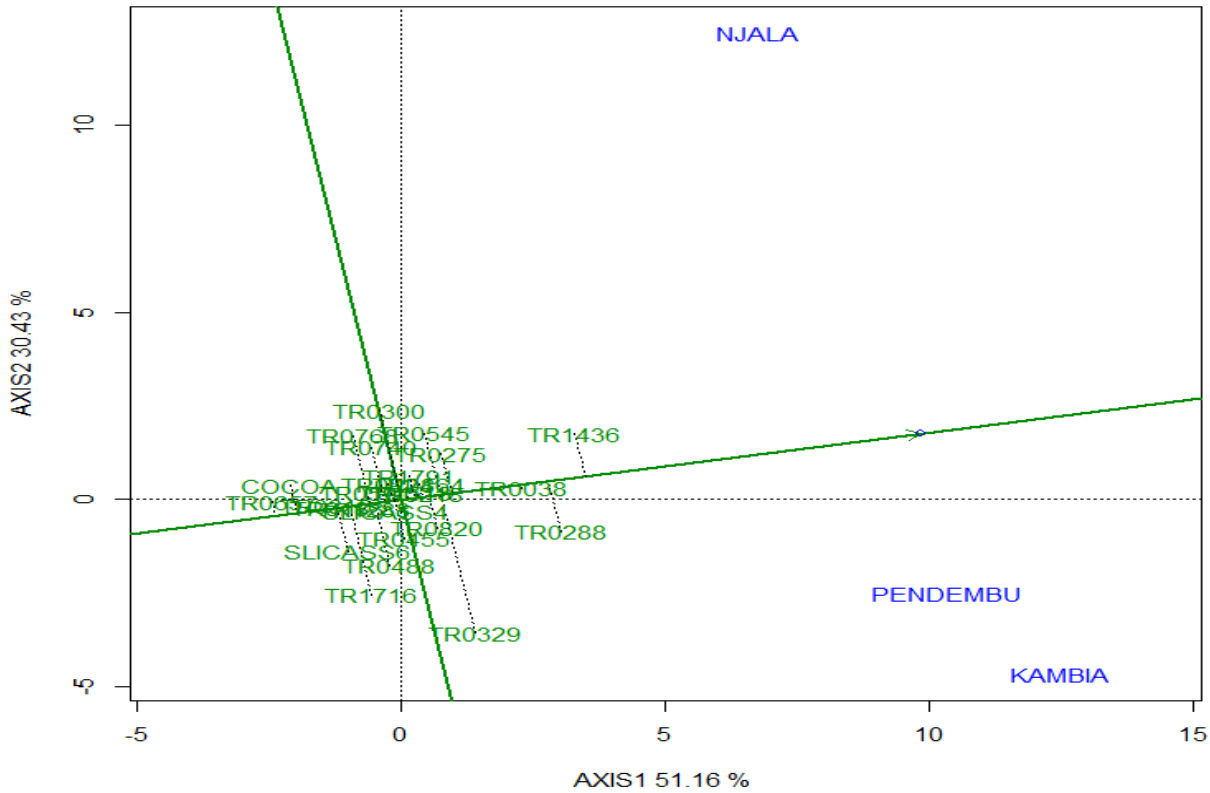


FIGURE 2: GGE biplot of mean and stability performance of 26 cassava genotypes for starch content across three environments

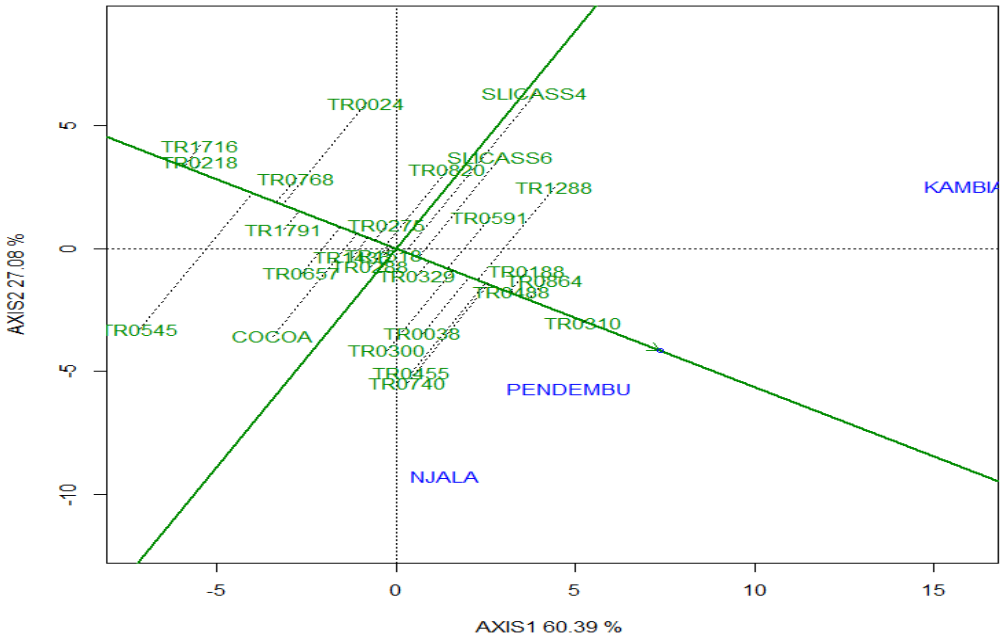


FIGURE 3: The GGE biplot showing mean performance and stability of 26 cassava genotypes for dry matter content

3.3 Ranking of genotypes based on measured traits

The average environment coordinate (AEC) view of the GGE biplot based on the genotype focused scaling, shows the best genotypes across the three environments**.** Genotypes SLICASS4 and TR0024 were ideal for Pendembu, whereas SLICASS6 and TR0024 were ideal for Kambia. Genotypes TR0455, TR0768 and TR0657 were high yielding, but unstable across the three locations (Figure 4). The comparison of the relative performance of all genotypes accross the environments is shown in Figure 5. Genotype TR1436 had higher average starch content in Njala environment while genotype TR0288 had the highest starch content for Pendembu and Kambia followed by TR0329. The relative performance of genotypes for dry matter content accross the Njala, Kambia and Pendembu test sites is shown in Figure 5. Genotype TR0310 had higher average dry matter content and was the most stable genotype accross the three environments while genotypes TR0218 and TR1716 showed lower dry matter content than average performance.

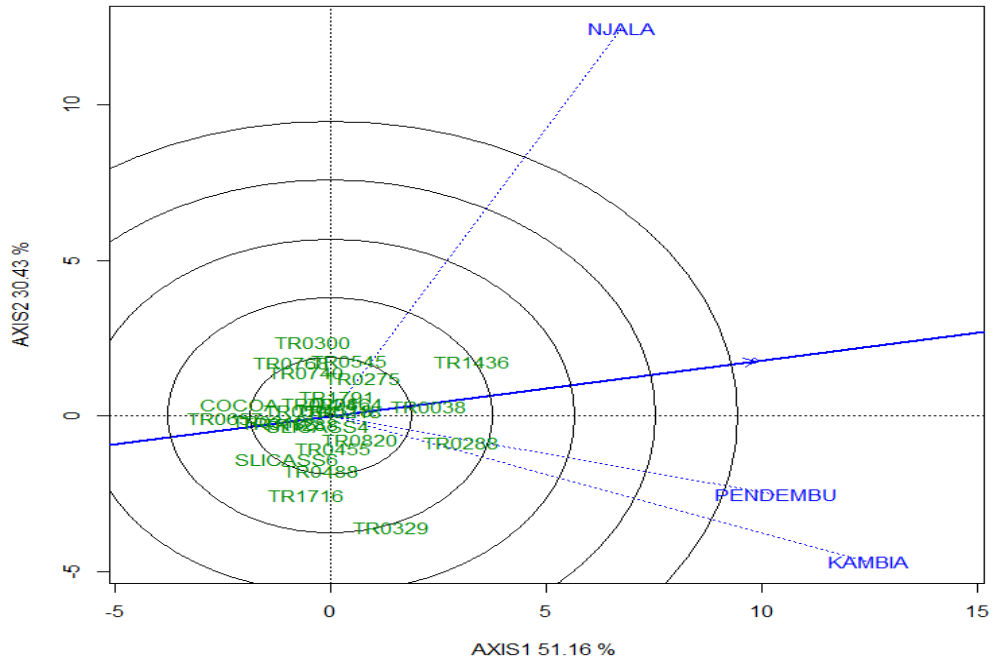


FIGURE 4: The average-environment coordination (AEC) view of genotypes relative to an ideal environment for starch content

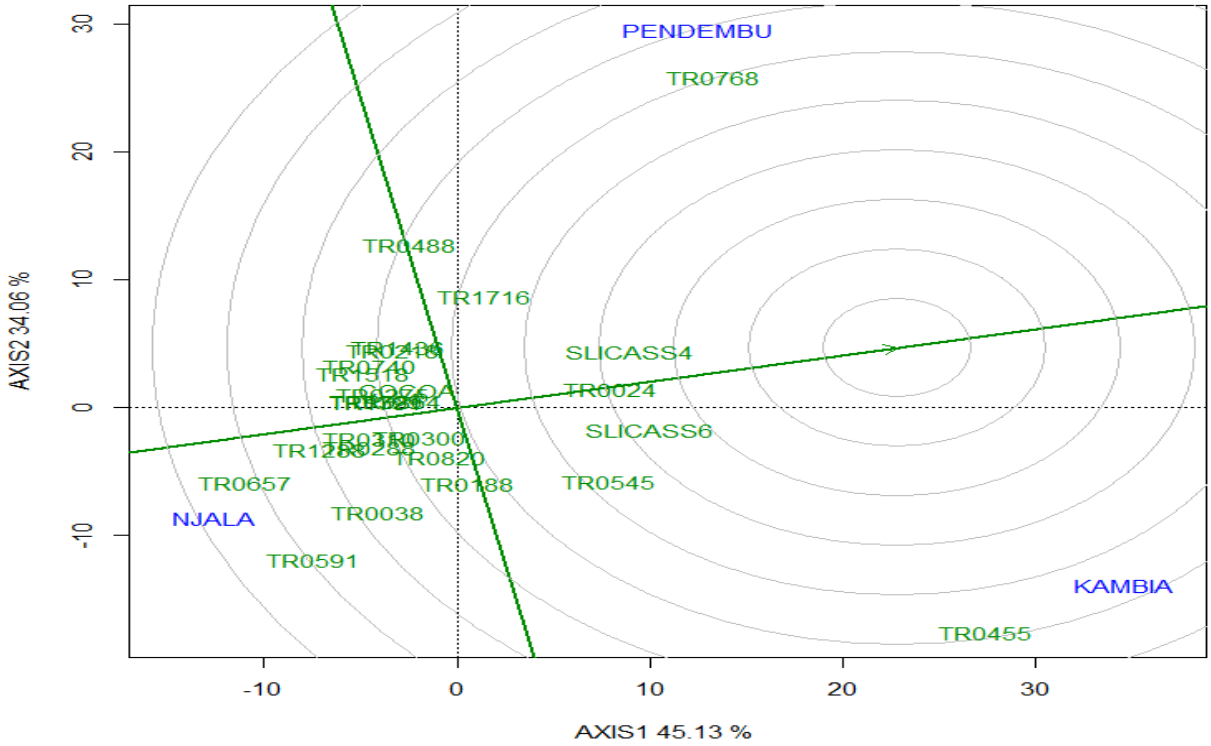


FIGURE 5: The average-environment coordination (AEC) view of genotypes relative to ideal environment for fresh storage root yield

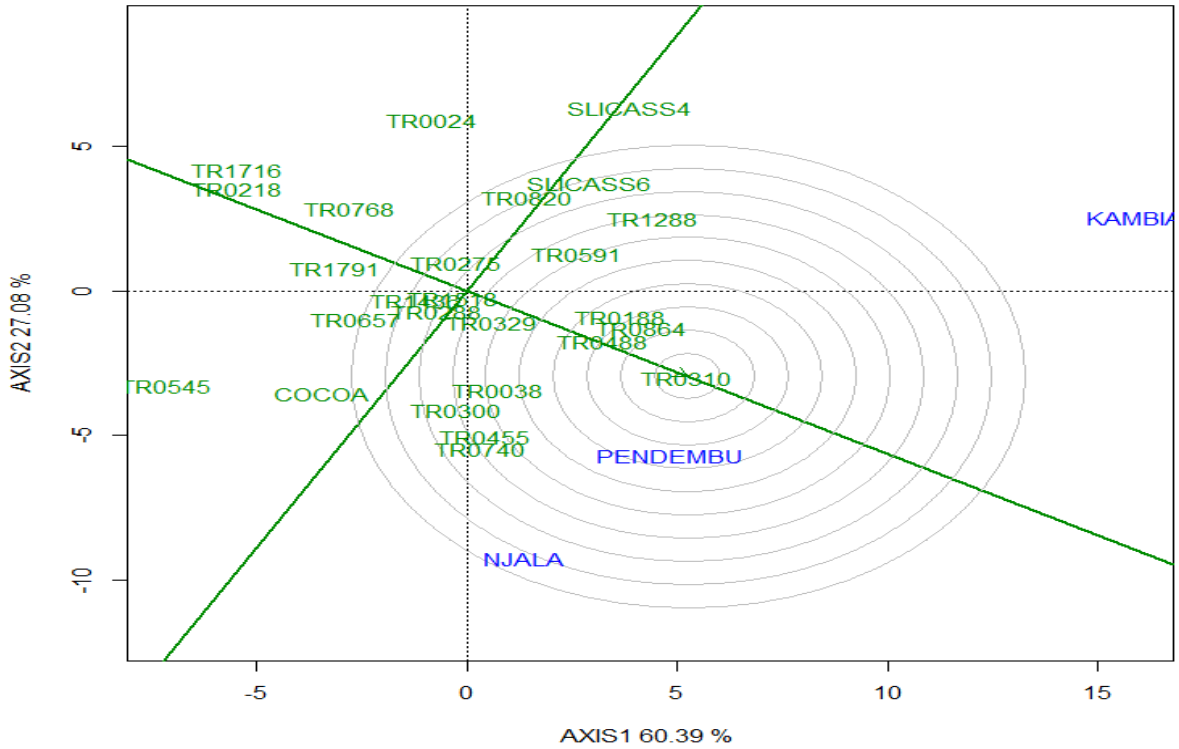


FIGURE 6: The average-environment coordination (AEC) view of genotypes relative to an ideal environment for dry matter content

The polygon view of cassava genotypes evaluated across Kambia, Pendembu and Njala environments is shown in Figure 7. The vertex genotypes TR0768, TR0488, TR455, TR0591 and TR0657 were the best performers or winning genotypes for storage root yield at the studied enironments because they are farthest away from the biplot origin. Genotype TR0768 is the winning genotypes in the Pendembu enviroment. The Kambia has TR0455 as the winning genotype. The Njala enviroment

has genotypes TR0591 and TR0657 as the winning genotypes. The genotype TR0488 performed poorly in all the three enviroments.

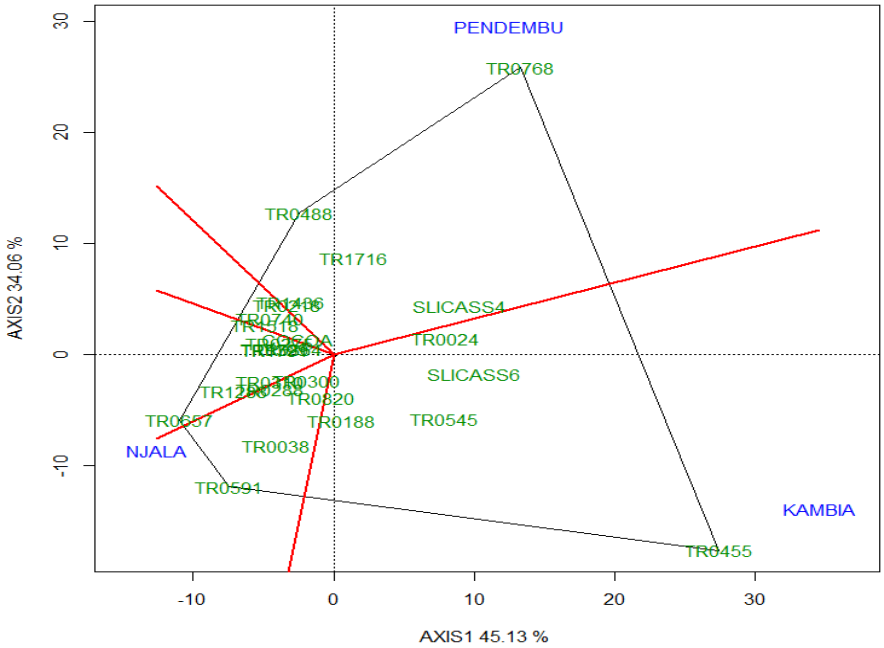


FIGURE 7: GGE biplot for best genotypes for fresh storage root yield across different environments

The polygon view of cassava genotypes across the three environments (Kambia, Pendembu and Njala) is shown in Figure 8. The vertex genotypes in this study are; TR0329, TR0288, TR1436, TR0300, TR0657 and TR1716. The first section contains Kambia and Pendembu environment with TR0288 as the winning genotype. The second section contains Njala enivironment with genotype TR1436 as the winning genotype. The other vertex genotypes TR0300, TR0657, TR1716 and TR0329 were not the top yielding genotypes in any of the three environments. The vertex genotype in each sector is the best genotype within the environments.

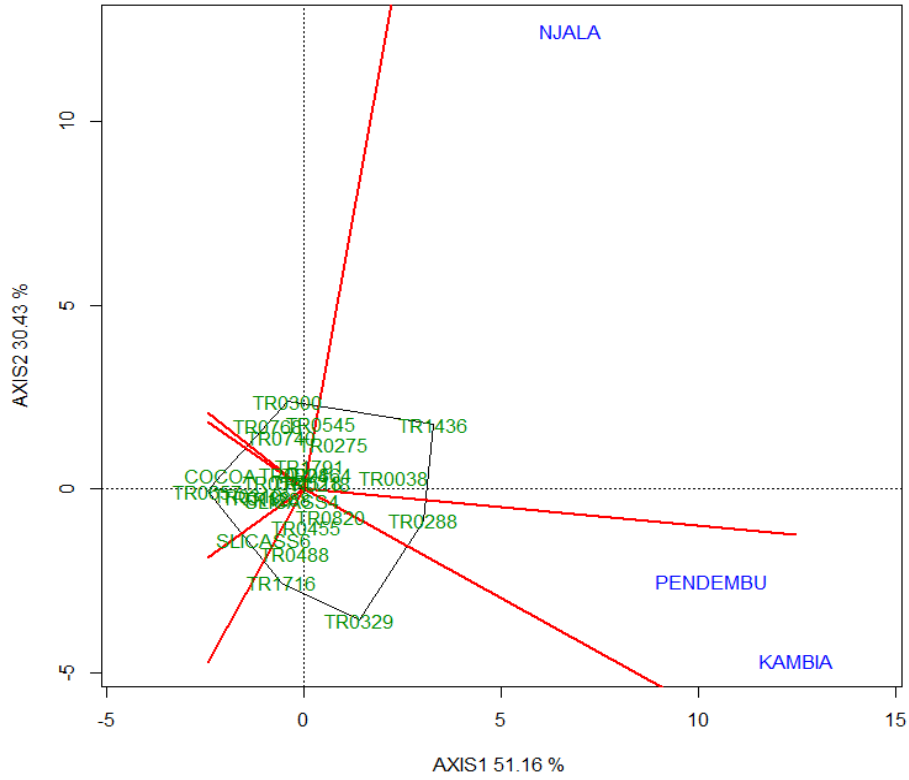


FIGURE 8: GGE biplot for the best performing genotypes for starch content evaluated across three production environments

The polygon view of cassava genotypes across the three environments (Kambia, Pendembu and Njala) for dry matter content is presented in Figure 9. The vertex genotypes in this study are SLICASS4, TR0310, TR0740, TR1716, TR0545 and TR0624. The Kambia environment has SLICASS4 as the winning genotype. The winning genotype in Pendembu environment was TR0310. The Njala environment had genotype TR0740 as the winning genotype The other vertex genotypes TR1288, TR0455, and TR1716 were not the top genotypes in any of the three environments.

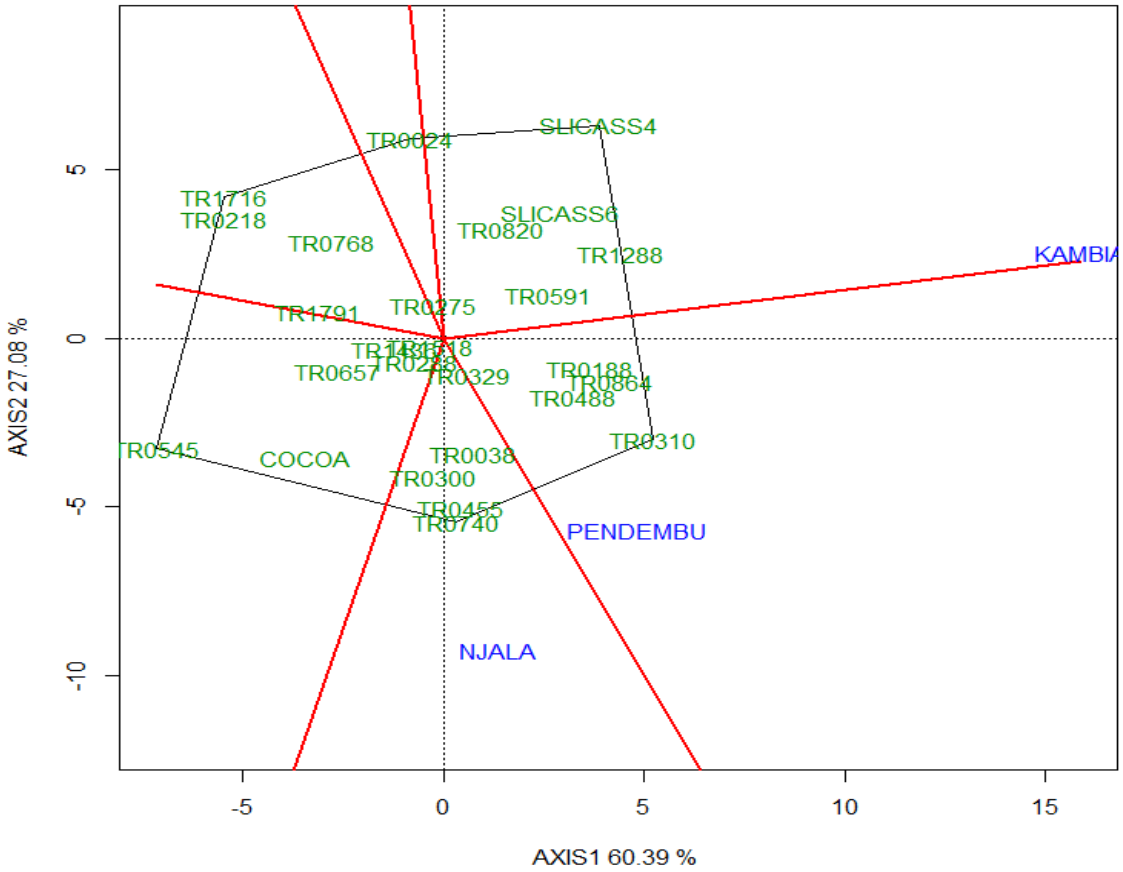


FIGURE 9: GGE biplot for best genotypes in different environments for dry matter content

3.4 Phenotypic correlations among measured agronomic traits

The phenotypic correlation among root yield and its related traits revealed that, number of storage root was positive and significantly correlated with harvest index (r = 0.54***), and fresh storage root yield root (r = 0.61***). The relationship between fresh storage root yield and harvest index (r =0.49***) was also was positive and significant (Table 4).

TABLE 4
PHENOTYPIC CORRELATIONS AMONG AGRONOMIC TRAITS

	NSR	HI	SC	FSRY	DMC	CMD
NSR	1					
HI	0.54***	1				
SY	0.33	0.20	1			
FSRY	0.61***	0.49***	0.23	1		
DMC	-0.06	-0.05	0.03	-0.06	1	
CMD	0.09	-0.01	-0.13	0.04	-0.06	1

NSR=number of storage root; HI= harvesting index; SC= starch content, FSRY = fresh storage root yield; DMC = dry matter content and CMD= cassava mosaic disease

IV. DISCUSSION

Genotypes should be evaluated based on both mean performance and stability across environments [28]. Genotype effects were significant for number of storage root, dry matter content and cassava mosaic disease. The significant location effects

for harvesting index, starch content, fresh storage root yield and cassava mosaic disease indicate that the overall mean performances of the genotypes in each location were significantly different for these traits. This variation underlines the need to conduct multi locational trials in order to identify both generally and specifically adapted genotypes with good performance for the traits. The location effect was the major source of variation for fresh root yield in this study. Akinwale *et al*. [29] also found higher location effects for fresh root yield in cassava.

Elite cassava genotypes that out-performed at specific locations were identified using AMMI analysis. In this study, the IPCA1 in AMMI captured interaction exclusively in a sequence that decreases from the first and largest component to the last and smallest component. This also indicated the response patterns of genotypes to changes in location; so that the genotypes could be evaluated in terms of their performances across the three locations. This agrees with the view that the significant IPCA1 scores sufficed in enabling visual assessment of the genotype and location performances and their interactions for the AMMI1 [30]. The AMMI has been effective in identifying cassava genotypes for specific locations [29, 30]. The significant GEI for dry matter content and cassava mosaic disease demonstrate the combined effects of environment and genotype on the expression of these traits. The significant G × E interaction effect of dry matter content in this study was similar to the finding reported by Ssemakula and Dixon [14], who reported the influence of environment on cassava dry matter content. In the case of starch content, location effects had the greatest impact on the variation of the trait, suggesting the need to evaluate genotypes for more than a year in different environments for reliable inferences to be made on genotype performance. Fresh storage root yield, dry matter content and starch content are yield related traits and therefore, subject to influence from the environment.

Although the performances of some genotypes were location specific, some genotypes performed best in more than one location. Genotype TR1436 performed best at Njala, while genotype TR0288 performed best at Pendembu and Kambia for starch content. For fresh storage root yield, TR0768 performed best at Pendembu, TR0455 at Kambia and TR0591 and TR0657 at Njala environments. For dry matter content SLICASS4, TR0310 and TR0740 performed best at Njala, Pendembu and Njala, respectively. The high impact of genotype on fresh storage root weight indicates that evaluation and selection can be done in different environments to distinguish genotypes with high and stable performance. The superior yielding genotypes across the locations had consistently high number of roots per plant and low CMD attack. These results agree with the report that fresh storage root yield of cassava increases with increasing number of roots [31]. The low starch content observed in this study may be due to physiological changes that starch undergoes during the growth cycle, a comprehensive study would have to factor in time of harvesting, climatic changes and it may require testing in diverse and multiple environments to identify genotypes with broad and specific adaptation due to the high impact of location and interaction. However, G × E interaction on the three traits (fresh storage root yield, dry matter and cassava mosaic disease) indicates that some genotypes may not respond positively.

The results of correlation analysis showed a highly significant correlation ($P < 0.001$) between fresh root yield and number of roots and harvest index. Harvest index and number of storage roots that showed a strong positive correlation with fresh storage root yield were also found as good indicators of root yield in cassava [32]. In the present study, the functional relationship between root dry matter content and fresh storage root yield is negative indicating that genotypes with high root dry matter content may exhibit low fresh storage root yield. The negative correlations between CMD and harvest index, CMD and starch content, CMD and dry matter content, indicate that severe attacks of these diseases contribute to low performance of the genotypes for these traits in cassava. These findings are partly consistent with Karim *et al*. [33], who found that severe attack of CMD contributes to poor growth, low storage root yields and dry matter content in cassava. Selection for high dry matter content, high storage yield, and starch content are among major breeding objectives of the crop [31, 34].

V. CONCLUSION

The high degree of variation within locations compared to the variation due to genotypic differences and GEI for the measured traits could be exploited for selection of genotypes possessing desired traits for the targeted production environment. The GEI was significant for harvest index and starch content indicating that the ranking of the genotypes for the traits varied across locations resulting in the identification of genotypes with specific adaptation. Although genotypes did not significantly interact with locations for starch content, there were changes in ranking of the genotypes at each environment. The biplot identified best genotypes in each location for all the traits studied. Genotype TR0288 had the highest starch content at Pendembu and Kambia, while TR1436 performed best at Njala. Genotype TR0768 had the highest fresh storage root yield at Pendembu, TR0455 at Kambia and TR0591 and TR0657 at Njala environments. For dry matter content,

genotypes SLICASS4, TR0310 and TR0740 performed best at Njala, Pendembu and Njala, respectively. Genotype TR0455 had the highest fresh storage root yield across the different environments, TR1436 for starch content and TR0310 for dry matter content. Findings of this study present an opportunity for the genetic improvement of cassava for target environments in Sierra Leone.

ACKNOWLEDGEMENTS

The authors are thankful to the cassava breeding teams at the International Institute of Tropical Agricultural (IITA), Nigeria and the Njala Agricultural Research Centre (NARC), Institute Agricultural Research Institute (SLARI), Njala, Sierra Leone for their technical support during the course of this study. This study was funded by the West Africa Agricultural Productivity Program Sierra Leone (WAAPP 1C SL) (IDA Grant H654-SL), Japan (PHRD TF099510- SL) and the International Development Research Centre (IDRC) under the IDRC/CORAF-WECARD/IITA sub-grant agreement for developing capacity for Agricultural Research in Sub-Saharan Africa (PJ-2126).

REFERENCES

[1] T. Sánchez, E. Salcedo, H. Ceballos, D. Dufour, G. Mafla, N. Morante, F. Calle, J.C. Perez, D. Debouck, G. Jaramillo, and I.X. Moreno. “Screening of starch quality traits in cassava (Manihot esculenta Crantz)”. Starch/Sta¨rke, 61:12–19.

[2] G.J. Scott, M.W. Rosegrant, and C. Ringler. 2000. “Roots and tubers for the 21st century: trends, projections, and policy options. No 31, 2020 vision discussion papers, International Food Policy Research Institute (IFPRI)”.
https://econpapers.repec.org/paper/fpr2020dp/ 31.htm

[3] N.M.A. Nassar. 2005. “Cassava: some ecological and physiological aspects related to plant breeding”. An article published online with Gene Conserve.

[4] K.Y. Karim, B. Ifie, D. Dzidzienyo, E.Y. Danquah, E.T. Blay, J.B.A. Whyte, P. Kulakow, I. Rabbi, E. Parkes, L. Omoigui, P.E. Norman, and P. Iluebbey. 2020a. “Genetic characterization of cassava (Manihot esculenta Crantz) genotypes using agro-morphological and single nucleotide polymorphism markers”. Physiol. Mol. Biol. Plants, 26(2): 317-330.
https://doi.org/10.1007/s12298-019-00740-x

[5] FAO 2020. “Food and Agricultural Organization (FAO) Accessed on 20/06/2021”. Available at:
https://knoema.com/FAOPRDSC2020/production-statistics-crops-crops-processed?tsId=1297390

[6] International Trade Centre (ITC) 2020. “The Status of cassava production and markets in Sierra Leone Preliminary version (October 2020). 42 p”. https://knoema.com/FAOPRDSC2020/production-statistics-crops-crops-processed?tsId=1297390

[7] K. Kintché, S. Hauser, N.M. Mahungu, A. Ndonda, S. Lukombo, N. Nhamo, V.N.E. Uzokwe, M. Yomeni, J. Ngamitshara, B. Ekoko, M. Mbala, C. Akem, P. Pypers, K.P. Matungulu, A. Kehbila, and B. Vanlauwe. 2017. “Cassava yield loss in farmer fields was mainly caused by low soil fertility and suboptimal management practices in two provinces of the Democratic Republic of Congo”. European J. Agron, 89: 107-123.

[8] A.C. Bellotti. 2002. “Arthropod pests. In: R.J. Hillocks JM, Thresh AC, Bellotti (Eds.). Cassava: Biology, production and utilization. London, UK: CABI Publishing, pp. 209-235”.

[9] A.C. Bellotti, C.H. Hershey, and O. Vargas. 1987. “Recent advances in resistance to insect and mite pests of cassava. In: Hershey, C.H. (ed.) cassava breeding: A multidisciplinary review, proceedings of a workshop held in Philippines, 4-7 March, 1985. CIAT, Cali, Colombia pp. 117-146”.

[10] P.E. Norman, P.B. Tongoona, A. Danquah, E.Y. Danquah, R. Asiedu, A. Agbona, and A. Asfaw. 2021. “Genetic parameter estimation and selection in advanced breeding population of white Guinea yam”. J. Crop Improv., (published open access online). https:// doi.org/10.1080/15427528.2021.1881012

[11] R. Howeler, N. Lutaladio, and G. Thomas. 2013. “Save and Grow: Cassava, FAO, Rome, Italy, 2013”.

[12] R. Tumuhimbise. 2013. “Breeding and evaluation of cassava for high storage root yield and early bulking in Uganda. Ph.D. Dissertation, University of KwaZulu-Natal, Pietermaritzburg, South Africa, 2013”.

[13] R. Tumuhimbise, R. Melis, P. Shanahan, and R. Kawuki. 2014. “Genotype × Environment Interaction effects on early fresh storage root yield and related traits in cassava”. The Crop J., 2: 329-337.

[14] G. Ssemakula, and A. Dixon. 2007. “Genotype x environment interaction, stability and agronomic performance of carotenoid-rich cassava clones”. Scientific Res. Essay, 2: 390-399.

[15] M. Benesi. 2005. “Characterization of Malawian Cassava Germplasm for diversity, Starch extraction and its Native and modified properties. PhD thesis, Free State University, South Africa”.

[16] A.B. Smith, B.R. Cullis, and R. Thompson. 2005. “The analysis of crop cultivar breeding and evaluation trials: An overview of current mixed model approaches”. J. Agric. Sci., 143: 449-462.

[17] M.G. Akinwale, B.O. Akinyele, A.C. Odiyi. and A.G.O. Dixon 2011. “Genotype × environment interaction and yield performance of 43 improved cassava (Manihot esculenta Crantz) genotypes at three agro-climatic zones in Nigeria”. British Biotech. J., 1: 68–84.

[18] H.G. Gauch, and R.W. Zobel. 1997. “Identifying mega-environments and targeting genotypes”. Crop Sci., 37: 311-326.

[19] W.M.G. Fukuda, C.L. Guevara, R. Kawuk, and M.E. Ferguson. 2010. “Selected morphological and agronomic descriptors for the characterization of cassava. International Institute of Tropical Agriculture (IITA): Ibadan (Nigeria)”.

[20] O. Sofa-Kantanka, and M. Osei-Minta. 1996. "Effect of cultivar and age at harvest on the dry matter, starch gelatinization properties and the cooking quality of cassava". Ghana J. Agric. Sci., 29: 81-89.

[21] K.R. Gabriel. 1978. "Analysis of meteorological data by means of canonical decomposition and biplots". J. Appl. Meteor., 11: 1072–1077.

[22] R.W. Zobel, M.J. Wright, and H.G. Gauch. 1988. "Statistical analysis of a yield trial". Agronomy J., 80(3): 388-393.

[23] M.C. Sneller, J. Wang, J.K. Dale, W. Strober, L.A. Middelton, Y. Choi, T.A. Fleisher, M.S. Lim, E.S. Jaffe, J.M. Puck, M.J. Lenardo, and S.E. Straus. 1997. "Clinical, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis". Blood, 89: 1341–1348.

[24] GGE-biplot 2009. "GGE-biplot software version 5.2. The Complete Biplot Analysis System: GGE biplot Pattern Explorer. Copy Right Weikai Yan, 2001-2009, USA".

[25] K.R. Gabriel. 1971. "The Biplot Graphic Display of Matrices with Application to Principal Component Analysis". Biometrika, 58: 453- 467.

[26] W. Yan, L.A. Hunt, Q. Sheng, and Z. Szlavnics. 2000. "Cultivar evaluation and mega-environment investigation based on the GGE Biplot. Crop Sci., 40: 597-605.

[27] R.W. Payne, S.A. Harding, D.A. Murray, D.M. Soutar, D.B. Baird, A.I. Glaser, S.J. Welham, A.R. Gilmour, R. Thompson, and R. Webster. 2011. "The guide to genstat release 14, part 2: statistics. VSN International, Hemel Hempstead, UK".

[28] W. Yan, and I. Rajcan. 2002. "Biplot analysis of test sites and trait relations of soybean in Ontario". Crop Sci., 42: 11-20.

[29] M.G. Akinwale, B.O. Akinyele, A.G.O. Dixon, and A.C. Odiyi. 2010. "Genetic variability among forty-three cassava genotypes in three agro-ecological zones of Nigeria". J. Plant Breed. and Crop Sc., 2: 104-109

[30] O.O. Aina, A.G.O. Dixon, L. Paul, and E.A. Akinrinde. 2009. "G×E interaction effects on yield and yield components of Cassava (landraces and improved) genotypes in the Savanna Regions of Nigeria". Afr. J. Biotech., 8(19): 4933-4945.

[31] P. Ntawuruhunga, P.R. Rbaihaho, J.B.A. Whyte, A.G.O. Dixon, and D.S.O. Osiru. 2001. "Inter-relationships among traits and path analysis for yield components of cassava: A search for storage root yield indicators". Afr. Crop Sci. J., 9: 599-606.

[32] L.S. Lain. 1985. "Selection for yield potential. In: Cork, J.H. and Reyes, J.A. (Eds.). Cassava: Research, Production and Utilization. Cali, Columbia: UNDP/CIAT".

[33] K.Y. Karim, P.E. Norman, D. Dzidzienyo, B. Ifie, P. Kulakow, I. Rabbi, L. Omoigui, and E. Parkes. 2020b. "Gene action analysis studies for agronomic traits in cassava (Manihot esculenta Crantz) genotypes developed using line by tester mating design". Afr. J. Agric Res., 16(10): 1471-1479. doi:10.5897/AJAR2020.14797

[34] K. Kawano, K. Narintaraporn, P. Narintaraporn, S. Sarakarn, A. Limsila, J. Limsila, D. Suparhan, V. Sarawat, and W. Watananonta. 1998. "Yield improvement in a multistage Breeding program for Cassava," Crop Sci., 38: 325-332.

Evaluation of Various Parameters in Mass Multiplication of *Beauveria bassiana* in Modified Method.

Mr. Prakash D. Vaghasiya[1*], Bhakti P. Chouhan[2], Anjali Nair[3]

Vise Innovation Solution Enterprise Pvt. Ltd, Vadodra
*Corresponding Author

Received:- 01 August 2021/ Revised:- 09 August 2021/ Accepted:- 15 August 2021/ Published: 31-08-2021

Abstract— *In the recent years, the environmental contamination caused by excessive use of chemical pesticides increased the interest in integrated pest management, where bio-pesticides are used to control plant pests and plant diseases. Present study deals with use of different media like SDA, rice bran, wheat bran, sorghum and to find their ability as substrates for mass multiplication of beauveria bassiana and creates effective production methodology which can be easily adopted. Biomasses of fungal grain media, organic media and non-synthetic media have been used for the production. Mass multiplication of Beauveria bassiana on different grain media, different temperature like with incubator and without incubator method and calculate biomass of fungus, microscopic examination. Development of SDA was the result which was considered as a best media for quickly growth of Beauveria bassiana and rice bran produced spore production which are most suitable for Beauveria bassiana.*

Keywords— Beauveria bassiana, chemical pesticides, SDA, rice bran, wheat bran, sorghum, fungal grain media, organic media and non-synthetic media.

I. INTRODUCTION

Biopesticide as defined by the United States Environmental Protection Agency, biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria and certain minerals.

In commercial terms, biopesticides include microorganisms that control pests, naturally-occurring substances that control pests, and pesticidal substances produced by plants containing added genetic material. The EPA separates biopesticides into three major classes based on the type of active ingredient used, namely, biochemical, plant incorporated protectants and microbial pesticides. Biochemical pesticides, which are naturally occurring substance that control pest by non toxic mechanisms. Though biopesticides cover about 1% of the total plant protection products globally, their number and the growth rate have been showing an increasing trend in the past two decades about 175 biopesticides active ingredients and 700 products have been registered worldwide. Regulatory system favorable to chemical pesticide and the gradual disappearance of multiple or mixed cropping, which is known to keep away the magic bullet chemical pesticide.

The main advantages of these biocontrol agents are their specificity to target pests, safety to the non-target organism, they do not cause ill effects on environment and human health and can be used against pests which develop resistance to the conventional insecticides, and they fit as ideal components in integrated pest management.

II. BIOPESTICIDE

B.bassiana, the white muscardine fungus belonging to class Deuteromycetes, is one of the important disease-causing pathogens in insects. B. bassiana formerly known as Botrytis bassiana is a widely distributed soil inhabiting fungus. Two fungi, B. bassiana and M. anisopliae are known to be pathogenic to the larval stage of the silkworm. A member of the hyphomycetes class of fungi, B. bassiana is categorized as a white muscardine fungus due to the white color of sporulating colonies. In culture, B. bassiana grows as a white mold. On most common culture media, it produces many dry, powdery conidia in distinctive white spore balls. The conidiogenous cells of B. bassiana are short, ovoid and terminate in a narrow apical extension called a rachis.

III. REVIEW LITERATURE

3.1 Historical development related to B. bassiana

The origins of microbial pest control date back to the early 19th century, when the Italian scientist Agrostino Bassi spent more than 30 years studying white muscardine disease in silkworms. He identified B.bassiana as the cause of the disease. Bassi himself recognized the potential to use organisms such as B.bassiana to control the insect pest (Bassi, 1836; Van Driesche & Bellows, 1996) and by the early 20th century ,field trials had been conducted with B.bassiana , B.brongniartii.v.

3.2 Mode of Action of B.Bassiana on Pests

The major issues involved in mass production and utilization of my pathogens are selection of effective strain, development of cost effective methods of mass rearing, development of effective methods for storage and shipment and creation of effective formulation. Environmental factors like temperature, humidity and sunlight play an important role in the field persistence of entomopathogenic fungi. One of the critical factors in the effective use of microbial agents as insecticides in their relatively short persistence on leaf surface. The commercial consideration such as identification of existing or novel isolation. Quality control of product and patent protection would benefit development of efficient strains.

The cutworm, *spodoptera litura* (fabricus) (Lepidoptera:Noctuidae), is a polyphagous sporadic pest with high mobility and reproducing capacity (Hollyways, 1989) that has about 150 host species (Rao et al. 1993). It is one of the most economically important insect pests in many countries including india. The efficacy of *B. bassiana* against *S. litura* was successfully studied by many scientists (Rangaswami et al., 1969: Robert and Marchal 1980 and Dayakar and Kanajujia 2001).

The mycoinsecticides based on *B. bassiana* have been reported to be useful to control *S.litura, Achaea janata* (Linn.) and *Euproctis fraternal* (Misra). As far as research is concerned, negligible work has been done in Gujarat regarding *B. bassiana*. There was a report of *B.bassiana* on *Helicoverpa armigera* infesting cotton at Junagadh by Baraiya(2003).

So considering the significance of *B. bassiana* in pest management, it is felt worthwhile to investigate various aspects of this insect pathogen under South Gujarat condition, where a humid atmosphere prevails throughout the year, providing a congenial environment for multiplication of the fungus. This will provide scientific information, for development strategies for various insect pests.

In the 1980s, the first insect pathogenic studies were carried out and their focus was to find the methods of disease management of the silkworm. Bassi in 1835, first time formulated the germ theory by the use of white muscardine fungus on the silkworm that was then named in his honor as *Beauveria bassiana*. Gilbert and Gill described that this silkworm disease gave the idea of using insect infecting fungi for the control of insect pest management. A group of fungi that kill an insect by attacking and infecting its insect host is called entomopathogenic fungi. The main route of entrance of the entomopathogen is through integument and it may also infect the insect by ingestion method or through the wounds or trache. Entomopathogenic fungi have a great potential as control agents, as they constitute a group with over 750 species and when dispersed in the environment, provoke fungal infections in insect populations. These fungi begin their infective process when spores are retained on the integument surface, where the formation of the germinative tube initiates, the fungi starting to excrete enzymes such as proteases, chitinases, quitobiases, Upases and lipoxygenases. These enzymes degrade the insect's cuticle and help in the process of penetration by mechanical pressure that is initiated by the appressorium, a specialized structure formed in the germinative tube. Once inside the insect, the fungi develop a hyphal bodies that disseminate through the haemocoel and invade diverse muscle tissues, fatty bodies, Malpighian tubes, mitochondria and haemocytes, leading to death of the insect 3 to 14 days after infection. Once the insect dies and many of the nutrients are exhausted, fungi start micelles growth and invade all the organs of the host. Finally, hyphae penetrate the cuticle from the interior of the insect and emerge at the surface, where they initiate spore formation under appropriate environmental conditions.

Fungi	**Target**
Beauveria bassiana (White muscardine fungus)	Colorado potato beetle, Corn rootworm, Citrus root weevil, Cotton bollworms, Coffee berry borer, codling moth, Japanese beetle, Pod borer, Mango mealy bug, Boll weevil, Cotton leaf hopper, Chinch bug, Yellow stem borer, Rice leaf folder, Brown plant hopper, etc

(Source: Pawar and Singh 1993 and Zimmermann, 1993)

Classification of B.bassiana : Steinhaus gave a brief idea of classification of insect pathogenic fungus including B.bassiana, according to him, insect pathogen fungi divided into four large classes.

1. Phycomycetes
2. Ascomycetes
3. Basidiomycetes
4. Deuteromycetes

Class Deutromycetes have an order Moniliales which include most of the insect pathogen fungus including B.bassiana.

Order: moniliales

Genus: Beauveria

Species: bassiana.

By the time so many modification came in classification finally at present B.bassiana is classification as:

Kingdome: Fungi

Phylum: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Cordycipitaceae

Genus: Beauveria

Species: bassiana

Biochemical name: Beuvaria bassiana.

3.3 Mass Multiplication of Beuvaria Bassiana:

3.3.1 Media for growth and sporulation of *B. bassiana:*

- **Solid medium:**

Pandey and Kanaujia recorded the highest number of conidia produced in Sabouraud dextrose agar medium. Santa et al. recorded that solid substrate (mixture of potato and sugarcane bagasse) gave the highest spore production due to better aeration, less compaction problems and greater surface for spore production. Subouraud dextrose medium with yeast extract was superior over all other media supported the maximum biomass , conidial count and viability of conidia , Rodriguez et al. studied that medium which contain glucose in the pre culture and sucrose and corn steep liquor in the culture medium produced highest spore.

- **Different grain substrates for their effect on sporulation and growth of B. bassiana:**

Among the liquid media, rice powder recorded higher spore production of b.bassiana. Rice and wheat wash water also supported the growth and sporulation of all the tested fungi. Alves and Percira tested different grain substrates for their effect on growth and sporulation of B.bassiana, clearly indicating that inoculation of rice media in plastic bags was highly successful in spore yield. Nirmala et al.notice that rice and its substrate was most suitable for productive growth of B.bassiana. Patel and Kanaujia found that sorghum grain medium was the best substrate for growth and sporulation of B.bassiana. Pandey and Kanaujia suggested that sorghum medium gave higher biomass and conidial count of B.bassiana. Sorghum grain yielded highest conidia/g of substrate due to the presence of rich source of carbon and nitrogen, essential for higher growth and sporulation

IV. METHODOLOGY

4.1 Mass Multiplication Method

1. Multiplication of *B. bassiana* on sorghum seed with incubator.
2. Multiplication of *B. bassiana* on sorghum seed without incubator.
3. Multiplication of *B.bassiana* on wheat bran with incubator.

4. Multiplication of *B. bassiana* on wheat bran without incubator.
5. Multiplication of *B. bassiana* on rice bran with incubator.
6. Multiplication of *B. bassiana* on rice bran without incubator.
7. Mass multiplication on A media given by vise innovative enterprise Pvt. Ltd to observe *B. bassiana* growth on different content of moisture.

4.2 Parameters:

4.2.1 CFU count by Hemocytometer

- Counting cells in a hemocytometer:

Total cells/ml = Total cells counted ×dilution factor/ no. of square × 10,000 cells/ml

So, for example , if you diluted your sample 1:1 with Trypan blue, and you counted 325 cells in 4 corner square plus the central big square.

Total cells per ml = 325 cells ×2(dilution factor)/5 square ×10,000 cells/ml = 130×10^4 cells/ml

If you want to know how many cells you have in your original sample, just multiply the cell concentration by total sample volume. For example, if your original sample volume is 5 ml , than your sample has a total = 130×10^4 cells/ml × 5ml = 650×10^4 cells.

4.2.2 BIOMASS calculation of Beauveria bassiana:

- ***Beauveria bassiana* growth with incubator:**

The dry weight of the fungus was calculated by using the following formula:

Dry weight = (weight of petri plate with mycelia) - (weight of petri plate)

- ***Beauveria bassiana* growth without incubator:**

The dry weight of the fungus was calculated by using the following formula:

Dry weight = (weight of petri plate with mycelia) - (weight of petri plate)

V. RESULT AND DISCUSSION

5.1 Zone of inhibition on different antibiotics dose on Beauveria bassiana:

100 ml dose of Different Antibiotic	Zone of inhibition on petri plate
Streptomycin	0.6 mm
Gentamicin	0.2 mm
Amoxicillin	0.3 mm
Chloramphenicol	0.5 mm
Fluconazol	0.9 mm
Dithen	0.7 mm
Mancozeb + Metalaxyl	0.8 mm

5.2 Biomass calculation of *Beauveria bassiana* with incubator and without incubator:

After 5-6 days with the incubator, it gives good results of fungal biomass calculate the dry weight of fungus give by below formula:

Dry weight = (weight of petri plate with mycelium) - (weight of petri plate)

- Biomass of B. bassiana with incubator = (7.017) – (6.4443) = 0.5727
- Biomass of B. bassiana without incubator = (7.2777) – (6.7057) = 0.5725

5.3 Effect of different grain substrate medium on sporulation of *Beauveria bassiana* with incubator:

B. bassiana maximum spore production on rice 5.8×10^8 CFU/gm. wheat bran and sorghum also produce good sore production.

Grain (20g)	Spore count ($\times 10^4$) (Series 1)	Spore count ($\times 10^6$) (Series 2)	Spore count ($\times 10^8$) (Series 3)
Rice	**7.9**	**6.5**	**5.8**
Wheat bran	**6.8**	**6.02**	**5.5**
Sorghum	**6.6**	**5.8**	**4.5**

5.4 Effect of different grain substrate medium on sporulation of Beauveria bassiana without incubator:

B. bassiana maximum spore production on rice 7.24×10^8 CFU/gm which is more than with incubator method. On wheat and sorghum spore production is less than with the incubator method.

Grain (20g)	Spore count ($\times 10^4$)	Spore count ($\times 10^6$)	Spore count ($\times 10^8$)
Rice	**8.87**	**7.89**	**7.24**
Wheat	**6.7**	**5.76**	**5.45**
Sorghum	**6.58**	**5.77**	**4.45**

5.5 Mass multiplication on A media given by vise innovative enterprise Pvt. Ltd to observe *B. bassiana* growth on different content of moisture:

Volume of Suspension	Growth of B.bassiana
4 ml	64%
6 ml	75%
8 ml	88%
9 ml	95%

VI. CONCLUSION

Using biological control agents such as entomopathogenic fungi can be used as a component of integrated pest management of many pests. Several fungal species such as Beauveria bassiana are being used as biocontrol agents for a number of crops, livestock and human nuisance pests. Various agriculture products and by-products such as grain, vegetable waste, seeds, rice husk, and sawdust were evaluated for mass multiplication. Here we take wheat bran, rice bran and sorghum for mass multiplication. Beauveria bassiana effect on rice weevil, Sitophilus oryzae.and rice leaf folder in rice grain.

REFERENCES

[1] A. Adane, K., Moore, D., Archer, S.A., 1996. Preliminary studies on the use of Beauveria bassiana to control Sitophilus zeamais (Coleoptera: Curculionidae) in the laboratory. Journal of Stored Products Research 32, 105–113.

[2] B. Bing LA, Lewis LC (1992b) Endophytic *Beauveria bassiana* (Balsamo) Vuillemin in corn: the influence of plant growth stages and *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Biocon Sci Technol 2:39–47.

[3] C. FAO. http//www.fao.org/news/story/en/item/131114/icode 2012.

[4] D. Ranga Rao GV, Rupela OP, Rameshwar Rao V, Reddy YVR. Role of Biopesticides in crop protection: Present status and future prospects. Indian Journal of Plant Protection 2007; 35(1):1-9

[5] E. Bravo A, Likitvivatanavong S, Gill SS, Soberón M.~ 473 ~ Journal of Entomology and Zoology Studies *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect Biochemistry and Molecular Biology 2011; 41:423-431.

[6] F. Rosell G, Quero C, Coll J, Guerrero A. Biorational insecticides in pest management. Journal of Pesticide Science. 2008; 33:103-121.

[7] G. de Faria MR, Wraight SP. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control 2007; 43:237-256.

[8] H. Menn JJ. Biopesticides: Has their time come? Journal of Environmental Science and Health. 1996; B31:383-389.

[9] I. Mazumdar D, Hallsworth, Magan, Puzari KC, Hazarika LK; Mass production of *Beauveria bassiana* and its potentiality on rice hispa. Indian Phytopathol. 1995; 48: 275-278.

[10] J. El Damir M; Effect of growing media and water volume on conidial production of *Beauveria bassiana* and *Metarhizium anisopliae*. J. of Biol. Sci, 2006;6(2): 269-274

[11] K. Sahayaraj S, Mamasivayam SKR;. Mass production of entomopathogenic fungi using agricultural products and by products. AfricanJournal of Biotechnology, 2008; 7(12): 1907: 1910.

[12] L. Pandey AK, Kanaujia KR; Effect of different grain media on sporulation, germination and virulence of *Beauveria bassiana* (Balsamo) Vuillemin against*Spodoptera litura* Fabricius larvae. J. Biol. Control,2005;19: 129-133.

[13] M. Feng MG, Paponsk TJ, Kbachachiurians GG. 1994. Production, Formulation and Application of the Entomopathogenic Fungus Beauveria bassiana For Insect Control. Biocontrol Sci. Technol. 4: 531-544.

[14] N. Gopalakrishnan C, Anusuya D, Narayanan K. 1999. In vitro Production of Conidia of Entomopathogenic Fungus.

[15] O. Puzari KC, Sharma DK, Saranka LK. 1997. Media for Mass Production of Beauveria bassiana. J. Biol. Contr. 11: 96-100.

[16] P. Raimbault M. 1998. General and microbiological aspects of solid substrate fermentation. In:International Training Course On Solid-StateFermentation. Proceedings... Curitiba, Brazil 1-20.

Implementation and analysis of diagnostic techniques for *Mycobacterium spp.* and *Francisella spp.* in granulomatous disease of fish in breeding and wild aquaculture of São Paulo/Brazil

Martins, A.M.C.R.P.F.[1*]; Hipólito, M.[2]; Catroxo, M.H.B.[3]; Dias, D.C.[4]; Tachibana, L.[5]; Ishikawa, C.M.[6]; Cassiano, L.L.[7]

[1,2,7]Interinstitutional Aquaculture Health Laboratory – Biological Institute, Brazil
[3]Electron Microscopy Laboratory / Biological Institute, Brazil
[4,5,6]Aquaculture Research Center, Fisheries Institute, São Paulo State, Brazil
*Corresponding Author

Received:- 20 July 2021/ Revised:- 04 August 2021/ Accepted:- 12 August 2021/ Published: 31-08-2021

Abstract— *Mycobacterium spp. and Francisella spp. bacteria have serious implications for Animal Health, Public Health and Agribusiness and yet, in Brazil, there is little knowledge about the best diagnostic techniques to detect and characterize them. Therefore, the occurrence of these bacteria was verified in 519 fish from fish farms (active collection), wild freshwater animals from the State of São Paulo (active collection), and in materials filed in our laboratory (passive collection), using the techniques in situ hybridization (IHS), immunohistochemistry (IHC), optical microscopy (MO) (H&E and (ZN) Ziehl Neelsen or Fite-Faraco), and negative staining for transmission electron microscopy (TEM). Histologically, granulomas were observed in 135 fish. By the ZN Faraco technique, Mycobacterium spp was found in 54 animals. By Immunohistochemistry and in situ Hybridization, 46 fish were found infected with Mycobacterium spp, 40 with Francisella spp and 30 with both bacteria. In one of the animals the presence of granulomas was found, although not caused by Mycobaterium spp or Francisella spp. TEM also showed the presence of other bacteria, protozoa, and viruses. The aim of this study was to evaluate the best diagnostic techniques for Mycobaterium spp or Francisella spp in fish fragments.*

Keywords— Francisella spp; Mycobacterium spp, fish disease.

I. INTRODUCTION

There are several etiological agents of acute, chronic, granulomatous, systemic, or focal diseases in animals worldwide. In aquaculture, in fish, reptiles, amphibians and crustaceans, bacteria have caused great losses in production, due to the death of the infected animals or the bad aspect of the sick ones, that makes commercialization unfeasible [1]. In addition, according to Miller and Neely [1], and Wang et al. [2], many of the bacteria pathogenic to aquatic animals are zoonotic, affecting the public health. The growing expansion of consumption in national and international markets since 1990 for various fish, especially tilapia, led to new cultivation strategies, focused especially on the increase of the stocking density and the use of formulated feeds, characterized by a marked reduction in natural food. Therefore, it is necessary to know the bacterial agents, their pathogenesis, which includes the parasite-host-environment relationship, so that new effective protocols can be used, to avoid the dispersion and circulation of these bacterial agents [3].

When tracking the first responses to bacterial infection, the immediate immune response, the knowledge comes that the macrophages residing in the tissue are the cells that first contact the infecting bacteria. In the case of *Mycobacteria* [4], the macrophages residing in the first response, phagocytize and eradicate mycobacteria, suggesting that, in order to establish a successful infection, the mycobacteria must escape from the initially infected resident macrophages, or cause macrophage apoptotic death and go to the monocytes thus permitting growth. Some cytosolic pathogens, to prevent antimicrobial autophagy, invoke specific mechanisms, such as altering its surface when recruiting proteins from the host, as is the case of

Francisella, a cytosolic pathogen that rapidly breaks down its phagosome and resides and successfully proliferates in the cytosol, for an extended period of time, without triggering an autophagy response [5].

1.1 *Mycobacterium* Spp.

Mycobacterium spp. is a pathogen capable of causing serious and costly diseases in many invertebrates such as crustaceans [6] and vertebrates, such as humans (tuberculosis, leprosy, Buruli ulcer), livestock (bovine tuberculosis) and ectothermic animals (reptiles, amphibians and fish) [7, 8, 9, 10, 11, 12, 13]. In recent years, due to the decrease in fishing activities, there has been an increasing interest in fish farming, and this increase in farms has favored the development of diseases such as mycobacteriosis and franciselosis [14].

Mycobacterium marinum, *M. fortuitum* and *M. chelonae* are the main agents of the disease, called mycobacteriosis or tuberculosis of wild or captive fish, and they are a part of 120 or more species of mycobacteria [15]. In marine ornamental fish and inland water fish these diseases are relatively common [3, 16, 17, 18]. It is a zoonosis because some species of fish mycobacteria are potentially capable of infecting humans [19, 20].

In fish, the severity of the infection varies from chronic, with no major tissue changes, in which a few fish die, to conditions of severe and acute infections, with high mortality [5].

In humans, M. marinum causes skin lesions such as pool granulomas [21, 22]. This disease is associated with aquatic activities such as swimming, fishing, managing aquariums, sailing, fish bites, fin wounds, and cleaning tanks and aquariums [21]. M. fortuitum can cause severe injuries such as lung diseases [23, 24].

Microscopically, we generally observe the formation of granulomas. Epithelioid granulomas, or immune granulomas, are characteristic of insoluble particles, typically microorganisms that can induce an immune response. Their center can be filled with caseous necrosis. Macrophages phagocytize such agents and present antigens to T lymphocytes. Their role is to prevent the spread of these agents, and they reveal them to the giant Langhans cell. Macroscopically we have a variety of lesions such as external ulcers, exophthalmos, weight loss or can even occur without symptoms, and this happens when the infection is acute. They mainly appear in the spleen, kidney and liver, in the form of whitish gray areas that can coalesce. As a result of all that, the granuloma is composed of macrophages, epithelioid cells, giant cells, and it is surrounded by T lymphocytes and, in some cases, plasmocytes. The older ones develop a fibroblast capsule and connective tissue. The mechanism of granuloma formation has not been fully clarified [4].

1.2 *Francisella Spp.*

Recently, the bacterium of the genus *Francisella spp*, an emerging pathogen and infectious agent extremely virulent for several animal species, has been found in marine and freshwater fish, amphibians, reptiles and even mollusks. It has been associated with massive tilapia mortalities in commercial farms in Taiwan, Hawaii and Costa Rica [25, 26, 27, 28], and, with breeding casualties between 5% and 80%, with an average of 50%. In 2005, this bacteriosis, initially confused with the disease caused by Piscirikettsia bacteria (common cause of septicemia in salmonids), decimated the tilapia stocks of one of the main producers and exporters of fresh fillets to the United States, Aqua Corporation in Costa Rica [27].

The *Francisella* genus, of the *Francisellaceae* family, is composed of non-mobile, gram-negative, strictly aerobic bacteria, and facultative intracellular coccobacilli [29] and comprises three widely known species, *F. tularensis, F philomiragia*, and *F. novicida*. Some authors consider the species *F. novicida* as a subspecies of *F. tularensis*, since it is being divided into three subspecies, *F. tularensis spp tularensis, holorctica* and *mediasiatica* [30, 31, 32]. The species *F. philomiragia* is divided into *F. noatunensis* spp *noatunensis* and *F. noatunensis spp orientalis* [33, 34]. Some authors do not consider it as a zoonotic factor and, therefore, apparently without the risk of people becoming contaminated [31]. Others consider *F. tularensis spp tularensis*, found mainly in North America, as the most virulent for animals and humans [30, 35]. Considering the apparent link between *F. tularensis* and aquatic environments, fish and amphibians have been considered likely reservoirs [36].

Clinical signs, not specific to this bacteriosis, include loss of appetite, pallor, lethargic behavior, and erratic swimming. Focal hemorrhagic areas, loss of scales, erosion of the epidermis, exophthalmos, renomegaly and splenomegaly can be observed [28].

Internally, a more specific sign of the disease is observed, which is the presence of many white nodules in the gills, with epithelial hyperplasia and whitish nodules in the spleen, kidney, and gonads. These nodules have occasionally been noticed also in the liver and the heart. The lesions contain a large number of cocobastonetes which accumulate in cellular cytoplasm and, therefore, the presence of focal and diffuse necrotizing vasculitis, particularly in the spleen and kidney, is common, resulting in chronic inflammation and granuloma formation [25].

The primary target cells for *Francisella spp* in vertebrates are phagocytes [37], epithelial cells and dendritic cells [38, 39]. Phagocytes are important in the initial control of infections by internalizing pathogens and in the formation of phagolysosomes that eventually degrade this content. Meanwhile, intracellular bacteria, including members of the genus *Francisella*, have developed resistance to this phagolysosome degradation. Golovliov et al. [20], Clemens et al. [5], Santic et al. [40] and Birkbeck et al. [41], noticed that the tilapia affected in fish farms in South America, showed intramuscular lesions, significantly affecting the processing of the carcasses. Up to 30% of the fish fillets from the affected stocks showed dark granulomatous lesions.

Other granulomatous diseases in fish can be caused by *Nocardia spp, Rhodococcus spp., Renibacterium salmoninarum, Citrobacter freundii, Photobacterium damselae, Vibrio spp., Seriola liquifaciens, Edwardsiella tarda, Piscirickettsia spp, and Flavobacterium spp.* [42].

The aim of this study was to investigate these diseases of fish and to evaluate the best diagnostic technique.

II. MATERIALS AND METHODS

From a total of 519 fish, both male and female, which were randomly collected from aquaculture farms (active collection), wild freshwater animals from the State of São Paulo (active collection) and from materials filed in our laboratory (passive collection), and with the use of *in situ* hybridization (IHS), immunohistochemistry (IHC), optical microscopy (MO) (H&E and Ziehl Neelsen or Fite-Faraco), and negative staining for transmission electron microscopy (TEM), we managed to identify the bacteria when they were present. Samples of spleen, hepatopancreas, kidney and gills were fixed in 10% neutral buffered formalin or frozen. The sampled fishes varied in length from 10 cm to 52 cm. Granulomas were macroscopically and histologically observed in the samples from 135 fish (1 and 1a).

We follow all the Ethical principles in Animal Experimentation adopted by the Brazilian Society of Science in Laboratory Animals(SBCAL / COBEA), by the National Council for the Control of Animal Experimentation (CONCEA) and the Brazilian Guideline for the Care and Use of Animals for Scientific and Didactic Purposes (DBCA): Protocol nº 169/20 about the Project: "Investigação de agentes bacterianos (*Mycobacterium* spp. e *Francisella* spp.) causadores de doenças granulomatosas em pisciculturas comerciais de água doce e marinha no sudeste do Brasil".

(https://www.sbcal.org.br/conteudo/view?ID_CONTEUDO=65;https://www.gov.br/mcti/pt-br; https://www.fc.unesp.br/Home/Pesquisa/diretriz-brasileira-para-o-cuidado-e-a-utilizacao-de-animais-para-fins-cientificos-e-didaticos.pdf); https://www.gov.br/mcti/pt-br) [43].

2.1 H-E Technique

Serial sections were prepared from the fixed material: fragments embedded paraffin. 5µm sections were cut using a microtome and adhered to the glass slides and stained by hematoxylin-eosin.

2.2 Fite- Faraco Ziehl-Neelsen technique (Z-N)

(We used Fite-Faraco staining protocol since the classic staining protocol of Ziehl Neelsen may result in false negatives). Serial sections were prepared from the fixed material: fragments embedded paraffin. 5µm sections were cut using a microtome and adhered to the glass slides. The sections will be de-paraffinize in a solution composed of two parts of xylol and one part of peanut oil (or almond oil) for 15 minutes. The sections are then washed in tap water to remove the remaining xylene / oil mixture. Filter on carbol fuchsin solution, DO NOT HEAT, for 20 min. Wash in running tap water. Differentiation will be done by means of 10% sulfuric acid for 2 minutes. Wash well in running tap water, rinse distilled water. Counterstain in 0.25% methylene blue for 20 seconds. Wash and blot dry. DO NOT DEHYDRATE IN ALCOHOL. Clear in xylene. Repeat the blotting-xylene treatment until section is clear. Mount in a DPX type mountan [44].

For transmission electron microscopy (TEM), a negative staining technique was used. The samples were suspended in 0.1M phosphate buffer pH 7.0 and placed in contact with metal grids previously coated with collodion and carbon film drained with filter paper. They were negatively contrasted with ammonium molybdate to 2% and pH 5.0 and observed using a Philips EM 208 TEM [45, 46].

2.3 Immunohistochemistry Technique

Sections of the organ fragments were submitted to Tris HCl 10% for melanin removal. 5 µm thick, were deparaffinized and rehydrated. Antigen retrieval was performed at room temperature, by applying 100 µl of Proteinase K (Dako - S3020) for each cut for 5 minutes, followed by a wash with distilled water. The blocking of endogenous peroxidase, aimed at the minimization of unspecific reactions, was obtained with 200 µl of hydrogen peroxide, 10 volumes at 3% in distilled water for 20 minutes. Sections were then rinsed with distilled water, followed by a wash with phosphate buffer (0.1 M PBS). Thereafter, 100 µl of primary antibody policlonal for anti-*Mycobacterium marinum* e anti-*Francisella* (Sinapse Biotecnologia Ltda), were diluted at 1:500. The Dako background reducing components (Code S3022) were applied at room temperature for each cut and incubated in a humid chamber for 18 hours in a refrigerator (2-8ºC). After this period, two washes with phosphate buffer (0.1 M PBS) for 1 minute were performed. The visualization system used was the LSAB® + System-HRP (Dako-code K0690), following the protocol recommended by the manufacturer. The incubation time was 20 minutes, at room temperature, for each of the reagents, alternated with two washes with phosphate buffer (0.1 M PBS) for 1 minute. The substrate chromogen system used was the Liquid DAB+ Substrate Chromogen System (Dako - code International. with 5 min incubation at room temperature, followed by a rinsing in distilled running water. The counterstaining was performed with hematoxylin. Negative checks were due to the lack of addition of antibody fragments of healthy fish gills, liver, spleen or kidney [47].

In situ* hybridization** The organ fragments were collected and fixed in 10% formalin for 36-48 hours, dehydrated with an increasing concentration of alcohols (70º, 80º, 95º and absolute), diaphanized with xylene and placed in a paraffin bath in a stove at 58ºC overnight. The 4 µm thick cuts were placed on marked slides and kept at room temperature. Prior to use, they were deparaffinized with xylene and rehydrated in decreasing concentration of alcohols (absolute, 95º, 80°, 70°) and distilled water. For antigen recovery, a pretreatment was performed, using a hot bath at 96º C and diluted buffer (Dako S1699) for 40 min. When the slides with cuts were cooled, the endogenous peroxidase was stopped at room temperature for 20 min. and, next, the enzymatic digestion of the tissues, with proteinase k (Dako) at room temperature for 5-15 min, was performed. The specific biotinylated probes were mixed, (Francisella spp***. [48]: Primers FLB16S180f: 5'-GCG-GATTAA- AGG-TGG-CCT-TTG-C-3' (forward primer) e FLB16S465r: 5'-CCT-GCA-AGC-TAT-TAA-CTC-ACAGG-3' (reverse primer). Modification of 5´ biotin (Invitrogen) e ***Micobacterium spp*** [49]: Primers specifically amplified: fragments 924-bp, T-39 (5´-GCGAACGGGTGAGTAACACG-3´) e T-13 (5´-TGCACACAGGCCACAAGGGA-3´ Modification of 5´ biotin (Invitrogen), including specific target DNA on the organ fragments, and a cover glass was laid on them. Samples from these fragments and probes were denatured and hybridized overnight (18 hours) in a Dako hybridization system (denaturation at 96ºC and hybridization at 37ºC). After stringency, a wash with TBST (Tris-buffered saline / Tween) was performed. The visualization system used was the primary streptavidin in a diluting buffer (Dako – Kit cod. K0690) for 30 minutes in a humid chamber, Biotinyl Tyramide reagent for 15 min, at room temperature and then the secondary streptavidin for 15 min. All the procedures were alternated with two washes of TBST buffer for 5 minutes. The substrate-chromogen system used was the Liquid DAB+ Substrate Chromogen System (Dako - code. K3468), and incubation was performed for 5 minutes at room temperature, followed by a wash in distilled running water. Counter-staining was performed with hematoxylin [50].

III. RESULTS

Of the 519 fishes examined, 54 were positives to *Mycobacterium spp* when stained using the Fite-Faraco Z-N technique (figure 2). In the H&E staining, animals presented numerous granulomas (figure 3a and 3b) of numerous sizes, with caseous necrosis in the center, eosinophilic cells and surrounded by inflammatory cells and fibroblasts. It was observed, thus, lymphocytes, neutrophils and heterophiles. Some macrophages alone or in groups, were filled with golden-yellow substance (melanomacrophage) next to the granulomatous or degenerative lesions. The most severe changes were observed in the

kidney that showed convoluted tubules in vacuolar degeneration or necrosis. Glomeruli were also visualized in degenerating, necrotic or deformed, hypo- or hyperplastic and presenting increased Bowman's space and nephrocalcinosis.

By immunohistochemistry and *in situ* hybridization, we found that 46 animals presented *Mycobacterium* spp, 40 presented *Francisella* spp, and 30 presented both bacteria (Figs 4, 5, 6 and 7). By TEM we observed the presence of mycobacteria in 28 fish and *Francisella* in 23 fish, out of the 79 fish in the active collection. Among these 79 fish, 17 presented both bacteria and one of the animals presented a granuloma, not caused by *Mycobaterium* spp or *Francisella* spp. TEM also showed the presence of other bacteria, protozoa, and viruses. (fig 8 and 9).

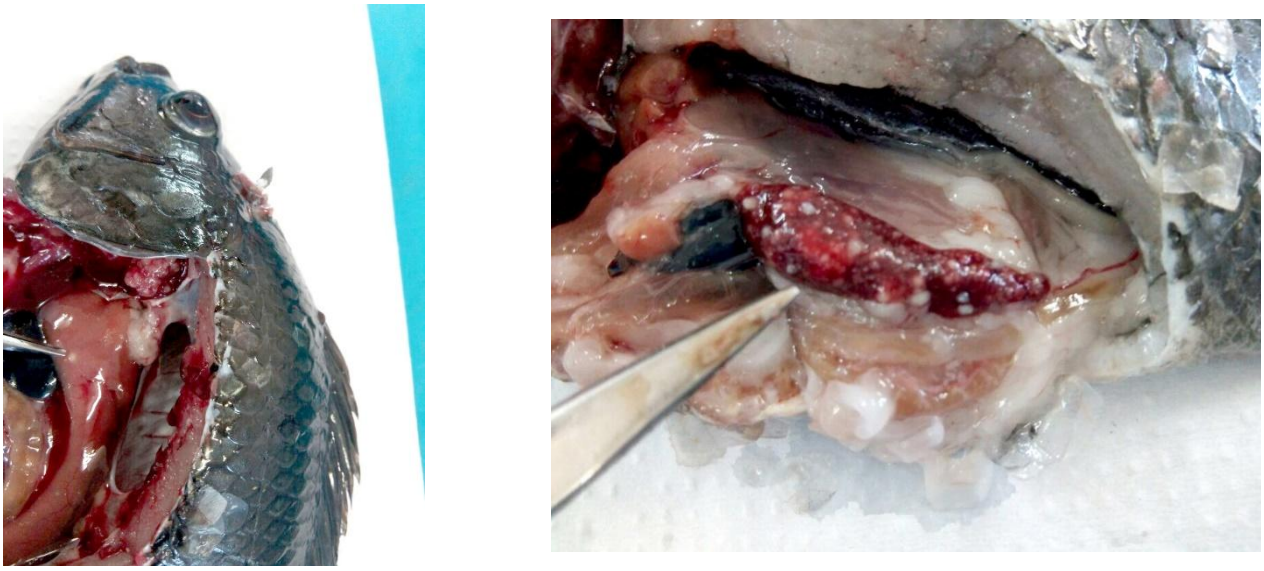


FIGURE 1: Presence of macroscopic granulomas indicated at the end of the forceps

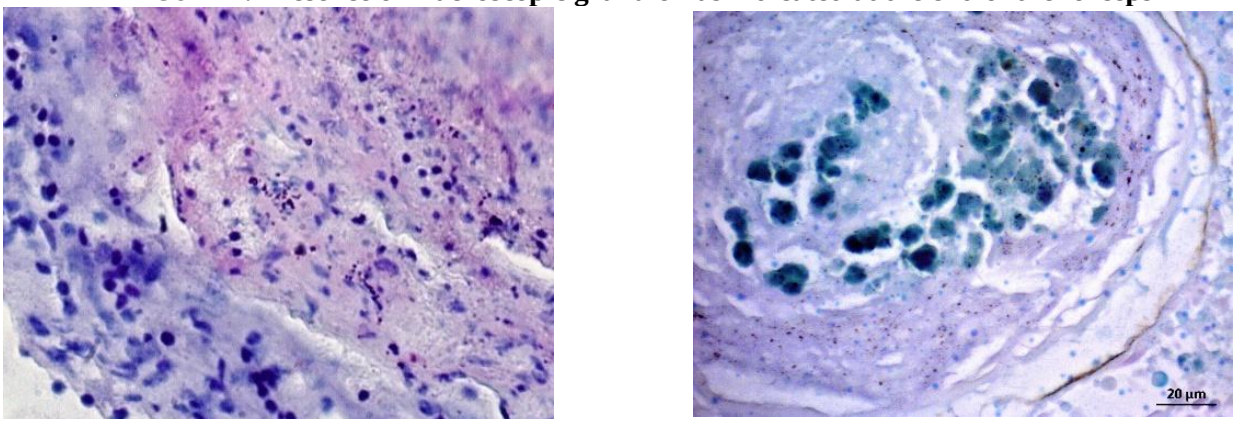


FIGURE 2: Hepatopancreas photomicrograph showing numerous red mycobacteria (small dots) in a granuloma using Fite-Faraco Ziehl Neelsen staining. X400, X200

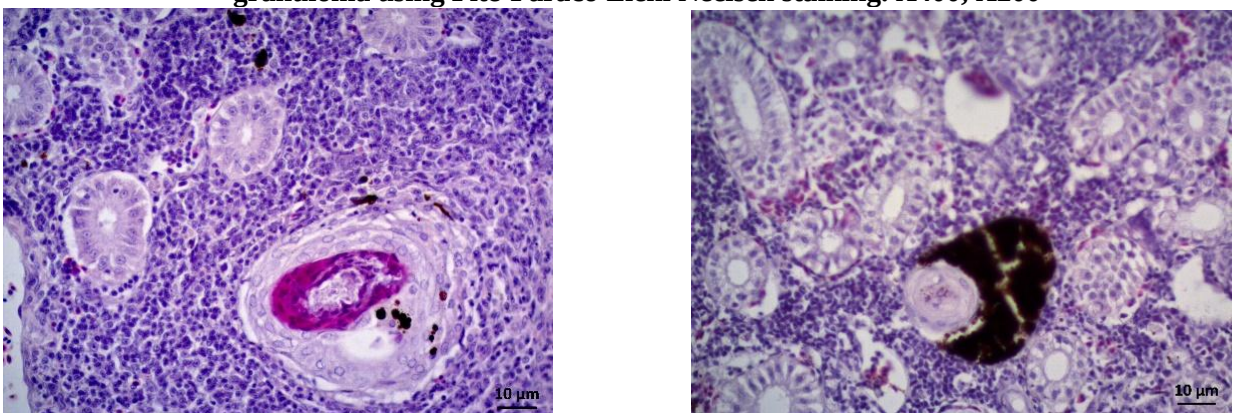


FIGURE 3: In the H&E staining, animals presented kidney granulomas (figure 3(a)) of numerous sizes. In figure 3b a melanomacrophage surrounding or encompassing a kidney granuloma X400

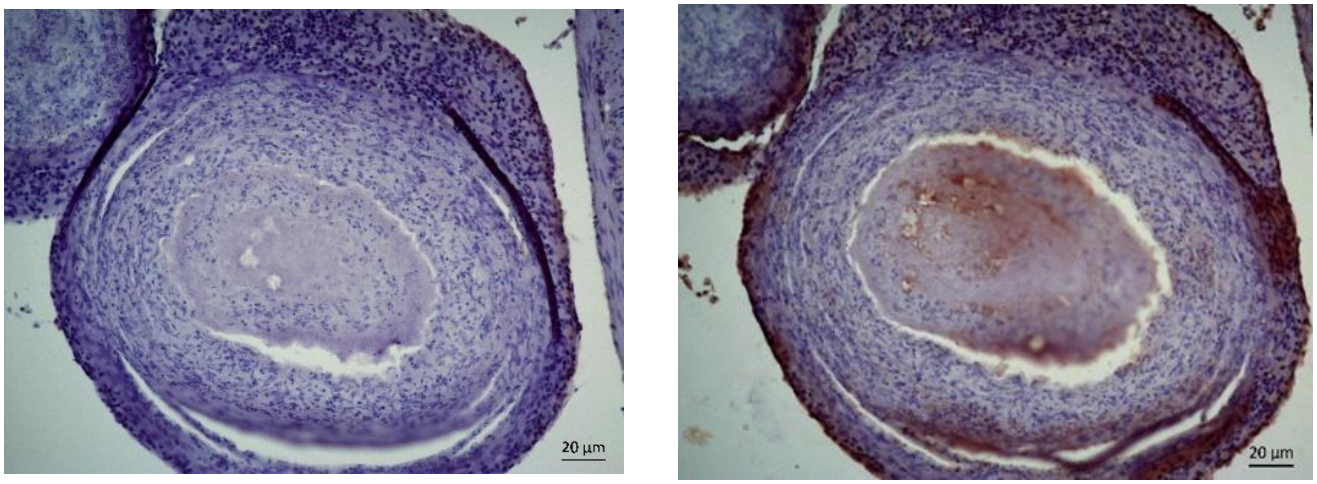


FIGURE 4: Slide photomicrograph by immunohistochemistry and negative for Mycobacterium spp. X200 and Figure 4a Positive immunohistochemistry for *Francisella* spp spp. X200

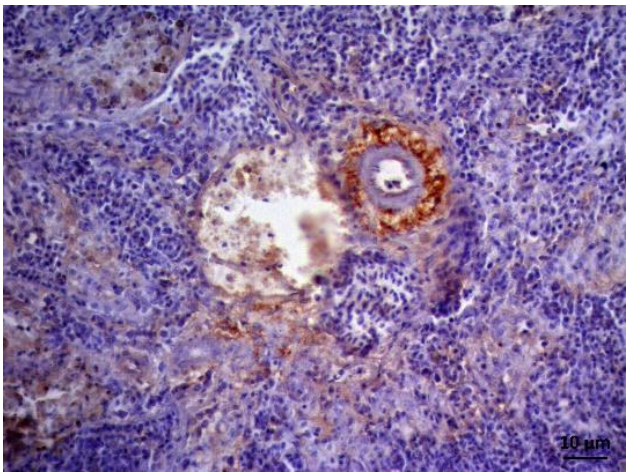


FIGURE 5. Photomicrograph of immunohistochemistry positive for *Mycobacterium* spp in kidney X630.

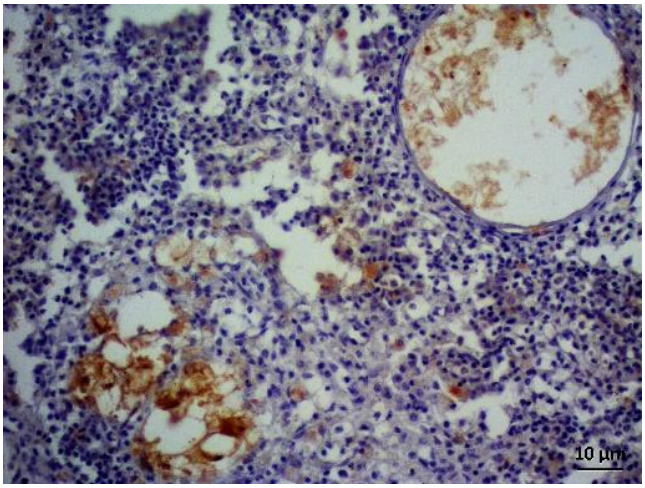


FIGURE 6. Photomicrograph of positive spleen for *Francisella spp* with the *in-situ* hybridization technique X 400

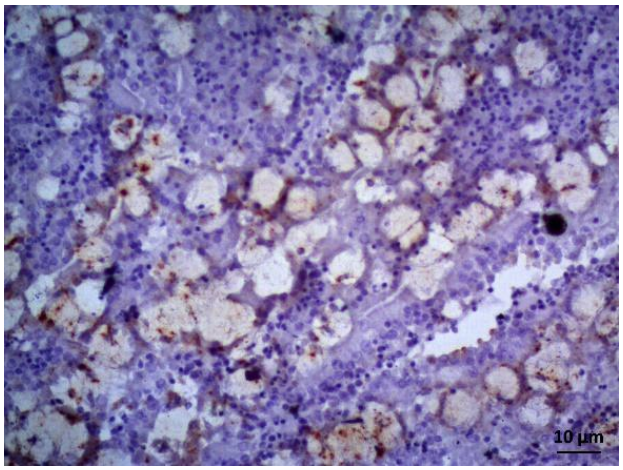


FIGURE 7. Photomicrograph of positive kidney for *Mycobacterium* spp with the *in-situ* hybridization technique X 400

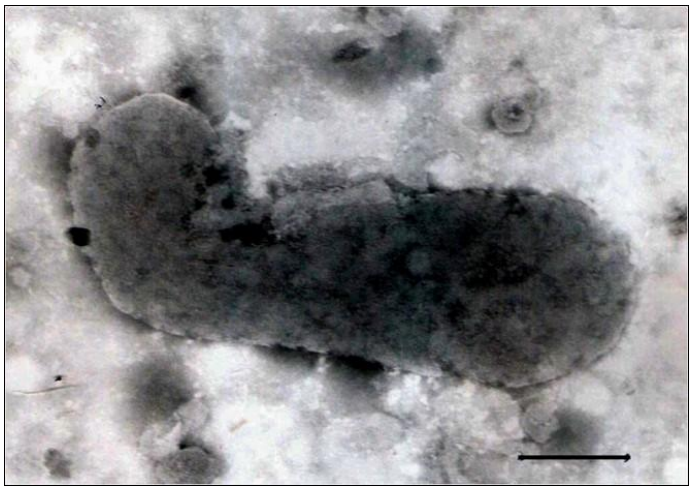


FIGURE 8. Electron micrography of *Mycobacterium spp.*, contrasted by ammonium molybdate in the negative staining technique. Bar: 100 nm

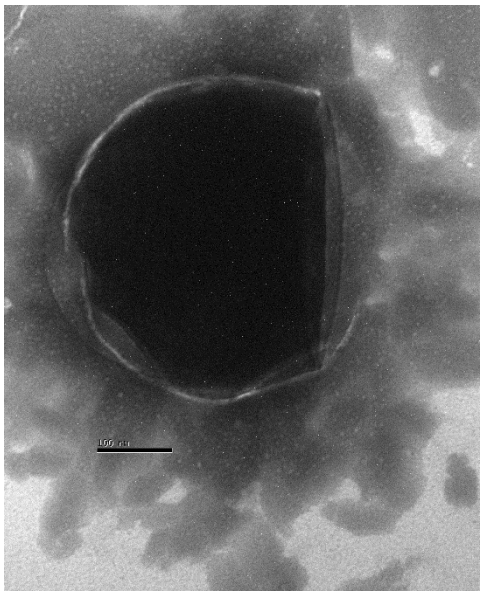


FIGURE 9. Electron micrography of *Francisella* spp., Contrasted by ammonium molybdate in the negative staining technique. Bar: 100 nm

IV. DISCUSSION

Brazil has enormous potential for animal and fish farming production, given its vast land, water sources, and favourable weather conditions. In Brazil, fish are typically bred in lakes, rivers, and the sea, to be used as food. Those used to carry on this study, though, were not intended for consumption.

High concentrations of fish can favour the onset of epizootic disease outbreaks, caused by *Mycobacteria spp* or *Francisella spp*, although in natural environmental conditions spontaneous disease outbreaks can also occur [19, 51]. Therefore, *Mycobacterium* spp and *Francisella* spp have serious implications for Animal Health, Public Health, and Agribusiness, and, still, in Brazil, there is little knowledge about the best diagnostic techniques to detect and characterize them.

This study was first focused at the diagnosis of bacterial diseases in fish, the behavioural changes of the affected animals, the clinical signs and the mortality rate and, if possible, the bacterial isolation through molecular and conventional microbiological methods, considering the characteristic disease and the peculiarity of each agent.

The culture and identification of *Mycobacteria* spp or *Francisella* spp, however, is hampered by its fastidious nature, co-infection with other fast-growing bacteria, low levels of microorganisms in tissues and/or submission of inadequate samples to the diagnostic centre [52, 53].

Several fish bacterial diseases can present in a similar way to francisellosis and mycobacteriosis, commonly characterized by granulomas in multiple organs, usually macroscopically visible.

In the present study, out of the 519 fish examined, 135 were found positive for granulomatous inflammatory disease, caused by unknown organisms, through routine histopathological analysis (H&E). Histopathological examinations are important for early diagnosis of infection in fish. Granulomas (fig 7.8) are suggestive of mycobacteriosis or francisellosis but are not pathognomonic of the diseases. The genera Nocardia, Edwardsiella, Photobacterium, Piscirickettsia, Renibacterium and streptococci are etiologic agents also inducing granulomatous diseases in wild and cultured fish species worldwide [42, 43].

Thus, histopathological diagnoses in tissue fragments, using routine hematoxylin-eosin staining, are particularly important because they allow a morphological evaluation. In 54 animals, a Fite-Faraco Z-N technique revealed the presence of Mycobacterium, eosinophilic small pleomorphic bacteria within vacuolated macrophages located in areas of moderate to severe chronic inflammatory cell infiltration or in the necrotic central area of granuloma. However, the use of the histochemistry, Fite-Faraco Ziehl Neelsen, was complicated. Some results generated doubts and, therefore, for the final diagnosis it was not considered the most conclusive. The same difficulty in this histochemical diagnosis was mentioned by Toenshoff et al. [53], who had observed that mycobacteria can be refractive to culture and are not always readily observable in histological preparations, even when stained by Ziehl-Neelson [54].

The difference in sensitivity between Z-N and IHC may be explained by the Z-N technique detecting only "perfectly preserved" organisms, whereas IHC detects mycobacterial antigens in fragments of living or dead organisms, even with "defective" cell walls [55].

Electron microscopy (TEM) corroborated the results obtained by the IHC and HS, by allowing the visualization of the microorganism and becoming more efficient when associated with antibodies linked to the colloidal gold technique. The main advantage of TEM is the ability to analyze the interior of the sample, the subcellular ultrastructure. There are several drawbacks to the TEM technique. Many materials require extensive sample preparation to produce a sample thin enough to be transparent to electrons, making TEM analysis a relatively time-consuming process, with a low volume of samples. The sample structure can also change during the preparation process and the field of view is relatively small, increasing the possibility that the analyzed region may not be characteristic of the entire sample.

***In situ* hybridization (IHS)** is a technique with which specific nucleotide sequences are identified in cells or histological sections. These can be DNA or RNA, endogenous, bacterial, or viral. This research technique is being translated into a diagnostic practice, mainly in the areas of genes expression, infection, cytogenetics of interphase, and rapid diagnosis [56].

The great advantage of the *in-situ* hybridization reaction, furthermore, is the possibility of precisely locating a specific gene or its transcripts in a paraffinized or frozen tissue. With PCR, mRNA or DNA can be detected in tissue extracts, however we cannot observe the distribution of transcripts or the DNA of a type of cell population, or in the areas of an adult and/or developing tissue [56].

Immunohistochemistry (IHC) is the technique that combines morphology (it has a histological style remarkably like the one of conventional histology) with antigen-antibody reactions marked with a chromogen. Between IHC and IHS, the latter still has the advantage of being ten times less expensive, that is, monoclonal or polyclonal biotinylated antibody is ten times more expensive than a biotinylated probe.

Another great advantage of these techniques is the possibility of retrospective studies because Hybridization and Immunohistochemistry are performed in histological sections, fixed in formalin and paraffinized, therefore, like those present in archival materials or museums [52], also reported that the current availability of highly specific and sensitive molecular diagnostic techniques facilitated the detection, description, and characterization of many previously unidentified or misdiagnosed pathogens from archived samples.

Therefore, the use of molecular techniques in the diagnosis of fish diseases by infectious agents, mainly bacteria and viruses, provided a significant improvement in the efficiency and quality of the diagnosis of these pathologies.

The benefits resulting from the association of these more refined technologies with conventional Clinic and Pathology are unquestionable, and, as a result, our knowledge about some diseases is admirably increasing. Consequently, it is always necessary to associate the findings by molecular techniques with the isolated clinical history and the macroscopic and microscopic findings [57, 58].

V. CONCLUSIONS

In general, molecular techniques are more accurate, sensitive, and specific, and may result in positive predictive values and negative predictive values remarkably high, depending on the prevalence of the diseases researched in the population studied. The results indicate that both assays, alone or in combination, constitute sensitive tools for initial, rapid diagnosis of mycobacteriosis or francisellosis in fish.

ACKNOWLEDGMENTS

Thanks to FAPESP for the aid to the Research grant 2016 / 12930.

REFERENCES

[1] Miller, J.D.&Neely,N. (2005). Large-Scale Screen Highlights the Importance of Capsule for Virulence in the Zoonotic Pathogen *Streptococcus iniae*.Infection and Immunity, 73(2):921–934.

[2] Wang, X., Wang, Q., Xiao, J., Liu, Q., Wu, H., Xu, L., Zhang, Y. (2009).*Edwardsiella tarda* T6SS component evpP is regulated by esrB and iron and plays essential roles in the invasion of fish. Fish Shellfish Immunology, 27:469–477.

[3] Martins, A.M.C.R.P.F.,Hipolito M., Cassiano, L.L., Stroebel, R.C.,Catroxo, M.H.B, Ruggiero-Couceiro, A.P., Campos, C.A., Caldas, P.C., Ramos, J., Dib, C. & Roxo, E. (2017). *Mycobacterium gordonae* infection in freshwater fish from lakes and ponds in a park at São Paulo city, Brazil. International Journal of Environmental & Agriculture Research (IJOEAR), 3(8):44-48.

[4] Davis, M. & Ramakrishnan,L. (2009).The role of the granuloma in expansion and dissemination of early tuberculous infection.Cell, 136(1):37-49.

[5] Clemens, D. L., Lee, B.Y. & Horwitz, M.A. (2004). Virulent and avirulent strains of *Francisella tularensis* prevent acidification and maturation of their phagosomes and escape into the cytoplasm in human macrophages. Infectand Immunity, 72:3204–3217.

[6] Morales-Covarrubias, M.S. Enfermedades del camarón. Detección mediante análisis en fresco e histopatología. Editorial Trillas, SA de CV., México, D.F. 2010. pp. 1-130.

[7] Barros, G.C., Langenegger, C.H., Langenegger, J. & Peixoto, P.V. (1988). Surto de micobacteriose em criação de rãs (*Rana catesbeiana*) causado por *Mycobacterium marinum*. Pesquisa Veterinária Brasileira, 8 (3/4): 75-80.

[8] Magalhães, H., Carneiro, G. M., Pires, A. R., Medeiros, M. I. M. Gonçalves, W. M., Silva, M. C., Fonseca, L. S., Santiago, S. S. F. & Mello, S.C.R.P. (1991). Micobacteriose em rãs-touro (*Rana catesbeiana*, Shaw, 1802). Revista de Microbiologia, 22 (3):204.

[9] Moraes, J.R.E., Torres, H.A., Martins, M.L, Souza, V.N.;& Moraes, F.R. Tuberculose em *Rana catesbeiana* Shaw, 1802, em ranários comerciais diagnosticados pelo Centro de Aqüicultura da UNESP. In: Encontro brasileirode patologistas de organismos aquáticos, 4, 1996. Pirassununga, SP.

[10] Souza, C.W.O. Ocorrência de Mycobacterium sp. em exemplares *de Rana catesbeiana*, Shaw, 1802, de alguns ranários comerciais do Estado de São Paulo, Brasil. Florianópolis, SC. 83 p. Dissertação (Mestrado) - Departamento de Aqüicultura, Universidade Federal de Santa Catarina. 1993.

[11] Ishikawa, C.M. Quantificação bacteriana e avaliação das lesões em peixes da espécie *Oreochromis niloticus* (tilápia do Nilo) inoculados experimentalmente com *Mycobacterium marinum* ATCC 927. São Paulo, SP. 59 p. Dissertação (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo,1998.

[12] Ishikawa C.M., Matushima E.R., Oliveira de Souza C.W., Timenetsky, J. & Ranzani-Paiva, M.T. (2001). Micobacteriose em peixes**.** Boletim Instituto de Pesca 27:231-242.

[13] Ippen R.Vergleichende pathologische Untersuchungen über die spontane und experimentelle Tuberkulose der Kaltblüter. Akademische Verlag GmbH, Berlin, 1964. 90 pp.

[14] Whipps, C.M; Watral, V.G. &Kent, M. L. (2003). Characterization of a *Mycobacterium* sp. in rockfish, *Sebastes alutus*(Gilbert) and *Sebastes reedi* (Westrheim & Tsuyuki), using rDNA sequencesJournal of Fish Diseases, 26(4):241–245.

[15] www.bacterio.cict.fr/m/mycobacterium.html

[16] Noga, E.J., Wright, J.F. & Pasarell, L. (1990). Some unusual features of mycobacteriosis in the cichlid fish *Oreochromis mossambicus*. Journal of Comparative Pathology, 102:335-344.

[17] Jacobs, J.M., Stine, C.B, Baya,A.M. &Kent, M. L.(2009). A review of mycobacteriosis in marine fish.Journal of Fish Diseases, 32(2): 119–130.

[18] Martins, A.M.C.R.P.F.,Hipolito, M., Stroebel, R.C.,Catroxo, M.H.B., Dib, C.C. &Roxo, E.Mycobacterial infection in freshwater fish from lakes and ponds parks in the city of São Paulo, Brazil. In: Aquaculture Europe 2011, Rhodes, Greece,2011.

[19] Gauthier, D.T. & Rhodes, M.W. (2009). Mycobacteriosis in fishes: A review. The Veterinary Journal 180(1): 33–47.

[20] Golovliov, I., Baranov, V., Krocova, Z., Kovarova, H. & Sjostedt, A. (2003). An attenuated strain of the facultative intracellular bacterium Francisella tularensis can escape the phagosome of monocytic cells. Infection and Immunity Journal, 71: 5940–5950.

[21] Collins, C.H., Grange, J.M., Noble, W.C.& Yates, M.D. (1985). *Mycobacterium marinum* infections in man. Journal of Hygiene, 94: 135 – 149.

[22] Decostere A., Hermans K. & Haesebrouck F. (2004). Piscine mycobacteriosis: A literature review covering the agent and the disease it causes in fish and humans. Veterinary Microbiolology, 99:59-166.

[23] Nigrelli, R. F. &Vogel, H. (1963). Spontaneous tuberculosis in fishes and in other cold-blooded vertebrates with special reference to *Mycobacterium fortuitumCruz* from fish and human lesions**.** Zoologica, 48:131-144.

[24] Bragg, R.R., Huchzermeyer, H.F.A.K. & Hanisch, M.A.M. (1990). *Mycobacterium fortuitum* isolated from three species of fish in South Africa. Onderstepoort Journal of Veterinary Research, 57(1):101-102.

[25] Hsieh, C.Y., Tung, M.C., Tu, C., Chang, C.D. & Tsai, S.S. (2006). Enzootics of visceral granulomas associated with *Francisella*like organism infection in tilapia (*Oreochromis*spp.). Aquaculture, 254:129–138.

[26] Hsieh, C.Y, Wu, Z.B., Tung, M.C. & Tsai. SS. (2007). PCR and *in situ* hybridization for the detection and localization of a new pathogen Francisella-like bacterium (FLB) in ornamental cichlids. Diseases and Aquatic Organisms, 75:29–36.

[27] Soto, E., Hawke, J.P., Fernandez, D. & Morales, J.A. (2009). *Francisella*sp., an emerging pathogen of tilapia, *Oreochromisniloticus*(L.), in Costa Rica. Journal of Fish Diseases, 32:713–722.

[28] Foley, J.E & Nieto, N.C. (2010). Tularemia.Veterinary Microbiology,140:332–338.

[29] Farlow, J., Wagner, D.M., Dukerich, M., Stanley, M., Chu, M., Kubota, K., Petersen, J. & Keim, P. (2005). *Francisella tularensis* in the United States.Emerging Infect Diseases Journal, 12:1835-41.

[30] Colquhoun, D.J. & Duodu,S. (2011). *Francisella* infections in farmed and wild aquatic organisms.Veterinary Research, 42(1):1-15.

[31] Ottem, K.F. *Francisella noatunensis*– Taxonomy and ecology. Thesis. University of Bergen, Norway,2011.

[32] Birkbeck, T.H., Bordevik, M., Froystad, M.K & Baklien, A. (2007). Identification of *Francisella* sp. from Atlantic salmon, Salmo salar L., in Chile. Journal of Fish Diseases, 30:505–507.

[33] Mikalsen, J., Olsen, A.B., Tengs, T. & Colquhoun, D.J. (2007). *Francisella philomiragia*subsp. noatunensis subsp. nov., isolated from farmed Atlantic cod (*Gadus morhua*L.). InternationalJournal of Systematic and Evolutionary Microbiology,57:1960– 1965.

[34] Sjösted, A. & Genus, I. (2005). *Francisella* Dorofe'ev 1947, 176al. Bergey's Manual of Systematic Bacteriology,The Proteobacteria, apud 2:200-210.

[35] Parker, R.R., Steinhaus, E.A., Kohls, G.M. & Jellison, W.L. (1951). Contamination of natural waters and mud with *Pasteurella tularensis* and tularemia in beavers and muskrats in the northwestern United States. Bulletin of National Instituteof HealthSciences, 193:1–161.

[36] Proctor, R.A., White, J.D., Ayala, E. & Canonico,P.G. (1975). Phagocytosis of *Francisella tularensis* by Rhesus Monkey Peripheral Leukocytes.Infection and Immunity,11(1):146-151.

[37] Hall, G.L., Sly, P.D., Fukushima, T., Kusel, M.M., Franklin, P.J., Horak, Jr. F. Patterson, H., Gangell, C. & Stick, S.M. (2007). Respiratory function in healthy young children using forced oscillations. Thorax,62:521–526.

[38] Kamaishi,T., Miwa, S., Goto, E., Matsuyama, T. & Oseko, N. (2010). Mass mortality of giant abalone Haliotis gigantea caused by a Francisella sp. bacterium. Diseases of Aquatic Organisms, 89:145–154.

[39] Santic, M., Molmeret, M., Klose, K.E., Jones, S. & Kwaik, Y. A. (2005). The *Francisella tularensis* pathogenicity island protein IglC and its regulator MglA are essential for modulating phagosome biogenesis and subsequent bacterial escape into the cytoplasm. Cellular Microbiology, 7:969-979.

[40] Birkbeck,T.H., Feist,S.W. & Verner-Jeffreys, D.W. (2011). *Francisella* infections in fish and shellfish. Journal of Fish Diseases, 34:173-187.

[41] Austin, B. & Austin, D.A. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, 5rd (revised) edn. Godalming, Springer-Praxis, United Kingdon, 1999.

[42] (https://www.sbcal.org.br/conteudo/view?ID_CONTEUDO=65;https://www.gov.br/mcti/pt-br; https://www.fc.unesp.br/Home/Pesquisa/diretriz-brasileira-para-o-cuidado-e-a-utilizacao-de-animais-para-fins-cientificos-e-didaticos.pdf); https://www.gov.br/mcti/pt-br).

[43] Fite, G.L., Cambre, F.J. & Turner, M.H. (1947). Procedures for demonstrating lepra bacilli in paraffin sections. Archives of Pathology, 43:624-625.

[44] Brenner, S. & Horne, R.W. (1959). A negative staining method for high resolution electron microscopy of viruses. Biochimica et Biophysica Acta, 34:103.

[45] Hayat, M.A. & Miller, S.E. Negative Staining. Mc. Graw-Hill Publ. Company, New York. 253 p.,1990.

[46] Shoup, D.I., Swayne, D.E., Jackwood, D.J. & Saif, L.J. (1996). Immunohistochemistry of transmissible gastroenteritis virus antigens in fixed paraffin-embedded tissues. Jornal ofVeterinary Diagnostic Investigation, 8:161-167.

[47] Hsieh, C.Y.,Wu, Z.B., Tung, M.C. & Tsai, S.S. (2007). PCR and *in situ* hybridization for the detection and localization of a new pathogen Francisella-like bacterium (FLB) in ornamental cichlids. Diseases of Aquatic Organisms, 75:29–36.

[48] Talaat, A.M., Reimschuessel, R. & Trucksis, M. (1997). Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis. Veterinary Microbiology, 58: 229-237.

[49] Braissant, O.,Wahli, W. (1998).Differential expression of peroxisome proliferator-activated receptor-alpha, - beta, and - gamma during rat embryonic development.Endocrinology,139(6):2748-54.

[50] Ramsay, J.M., Watral, V., Schreck, C.B. & Kent, M.L. (2009). Husbandry stress exacerbates mycobacterial infections in adult zebrafish, *Danio rerio* (Hamilton). Journal of Fish Diseases, 32(11):931–941.

[51] Maniatis, K., Morris. D.J., Adams, A. & Pearson, M. (2000). Detection of *Photobacterium damsela* subspecies piscicida in fixed tissue sections using immunohistochemistry and antigen retrievalimmunohistochemistry**.** Journal of Fish Diseases, 23:343–347.

[52] Toenshoff, E. R., Kvellestad, A., Mitchell, S. O., Steinum, T., Falk, K., Colquhoun, D. J. & Horn, M. (2012). A novel betaproteobacterial agent of gill epitheliocystis in seawater farmed Atlantic salmon (Salmo salar). PLoS ONE 7:1–7.

[53] Zerihun, M.A., Hjortaas, M.J., Falk, K. & Colquhoun, D.J. (2011). Immunohistochemical and Taqman real-time PCR detection of mycobacterial infections in fish Journal of Fish Diseases, 34(3):235–246.

[54] Sarli, G., Morandi, F.,Zanoni, R., Brunetti, B., Mandrioli, L., Florio, D. &Prearo, M. (2005). Comparison of immunohistochemistry and Ziehl-Neelsen for the detection of *Mycobacterium* infection in sea bass (*Dicentrarchus labrax).*BulletinEuropean Association of Fish Pathologists**,** 25(4):182.

[55] Jensen, E. (2014). Technical review: *in situ* hybridization.The Anatomical Record, 297(8):1349-1353.

[56] Tomaso, H., Scholz, H.C., Neubauer, H., Dahouk, S.A., Seibold, E., Landt, O., Forsman, M. &Splettstoesser, W.D. (2007). Real-time PCR using hybridization probes for the rapid and specific identification of *Francisella tularensis* subspecies tularensis. Mollecular and Cellular Probes 21:12–16.

[57] Abril, C., Nimmervoll, H., Pilo, P. & Brodard, I. Rapid diagnosis and quantification of *Francisella tularensis* in organs of naturally infected common squirrel monkeys (*Saimiri sciureus*). Veterinay Microbiology, 127:203–208 Austin B, Austin D: Isolation/detection. In: Bacterial fish pathogens diseases of farmed and wild fish, ed. Dobbins P, 4th ed., pp. 155–174, 2008.

Novel Ecofriendly Approaches for Controlling Soil Borne Fungal Pathogens: A Review

Mr. Prakash Vaghasiya[1*], Shekhar Sumit[2]

Vise Innovation Solution Enterprise Pvt. Ltd, Vadodra
*Corresponding Author

Received:- 01 August 2021/ Revised:- 08 August 2021/ Accepted:- 16 August 2021/ Published: 31-08-2021

Abstract— *The application of chemical fungicides for controlling soil borne plant pathogens is rapidly increasing due to their potential to deliver desirable results in a short span of time. However their rampant use has made many invasive plant pathogens resistant to any chemical control making them way harder to eradicate or eliminate as compared to the past days. The uncontrolled use of chemical fungicides is also causing soil toxicity and water pollution leading to several health hazards. The aim of the review article is to highlight the recent advancements in the field of eco-friendly disease management using the extracts obtained from natural resources and biologically active antagonistic organisms. The review article highlights the management of Black scurf disease (Rhizoctonia solani) in Potato using Bacillus subtilis V26 strain and by using a mixture of cattle manure and date palm compost. Biological control of Fusarium wilt of tomato (Fusarium oxysporum f.sp. lycopersici) by the application of endophytic bacterial isolates from Silver Leaf (Solanum elaeagnifolium) has been also mentioned. The review includes the management of Late blight of potato (Phytophthora infestans) using antagonistic Poplar (Populus nigra) bud extracts and peptide extracts obtained from Common Horsetail (Equisetum arvense). The review article also mentions the innovative method of management of Black shank disease of tobacco (Phytophthora parasitica var. nicotianae) by colonization of tobacco roots with Paenibacillus polymyxa C5 strain. As per the article, Foot rot of rice or rice bakanae (Fusarium moniliforme) can be effectively managed by the application of antifungal Surfactin-A extracted from Bacillus subtilis NH-100 and NH-217 strains. The article highlights the potential of the Bacillus subtilis RH5 strain as a bioformulation for controlling Sheath blight of rice (Rhizoctonia solani). The extracts and the antagonistic biocontrol agents can be used in the effective management of some economically important soil-borne plant diseases as a novel, innovative and environmentally safe approach.*

Keywords— Soil-borne fungus, Bio control agents, Late blight, Black scurf.

I. INTRODUCTION

Soil borne diseases are considered a major hurdle in crop production. Soil borne plant pathogens such as *Rhizoctonia spp., Fusarium spp., Sclerotinia spp., Verticillium spp., Pythium spp., and Phytophthora spp*. can cause around 50%–75% yield loss for many crops such as wheat, cotton, maize, vegetables, fruit and ornamentals as reported to date [1, 2, 3].

Soil-borne fungal pathogens like Rhizoctonia, Fusarium, Pythium, Verticillium, Phytophthora, Sclerotinia, Rosellinia, etc. exist in the form of dormant propagules or spores and start growing when the micro-environment becomes favorable [7]. Infected seeds act as a primary source of infection that travels from one place to another, crossing demographic boundaries [7].

The significant problems caused by soil borne pathogens in crop production include reduced crop performance, decreased yield, and higher production costs. The threats of soil borne disease epidemics in crop production, high cost of chemical fungicides and development of resistance towards fungicides, climate change, new disease outbreaks and increasing environmental concerns along with soil health are becoming increasingly evident. In organic farming most of the soil borne fungal diseases can be controlled by stimulating bio-diversity in and above the soil, by feeding soil life with organic soil amendments and good soil management [4]. The review article summarizes innovations for controlling soil borne pathogens using antagonistic microorganism and plant extracts.

II. MANAGEMENT OF BLACK SCURF IN POTATO

Rhizoctonia solani is the causal organism of Black Scurf disease in Potato. It causes reduction in the potato yields by 10 % in plains and 25 % in hilly areas of India [7]. Some of the following methods can be applied for controlling the disease effectively.

2.1 Application of *Bacillus Subtilis* V26 as A Biological Control Agent

The ability of the V26 strain of *Bacillus subtilis* to produce chitosanase and proteases makes it a promising biocontrol agent for controlling *R. solani* by antibiosis [5]. The mycelial growth of *R. solani* reduced by 80 % when a supernatant (3 % v/v) of V26 culture was applied [5]. An increased concentration of supernatant (9 % v/v) resulted in vacuolization, hyphal deformation and cell wall disintegration of the pathogen [5]. The activity of chitosanase hydrolysed chitosan, which is the main constituent of fungal cell wall. The activity of protease degraded the protein linkages of external cell wall [5].

2.2 Control of Black Scurf and Stem Canker using Cattle Manure and Date Palm Compost

Fungi associated with cattle manure and date palm compost (CMC) significantly inhibited the radial growth of *R. solani* [6]. The antagonistic fungi caused lyses of *Rhizoctonia* mycelium and formed mycelia cords between the mycelia filaments of *R. solani* exhibiting mycoparasitism and anastomosis [6]. The antagonistic fungal isolates of CMC caused vacuolization and lyses of hyphae [6]. The inhibitory effect of CMC on *R. solani* got enhanced after its amendment with peat-sand [6].

III. MANAGEMENT OF FUSARIUM WILT IN TOMATO

Fusarium wilt is one of the most devastating diseases that are prevalent in major tomato-growing regions of the world [8]. It is caused by *Fusarium oxysporum* f.sp. *lycopersici* (FOL) that causes wilting of plants, yellowing of leaves, browning of vascular tissues, stunting and eventually death of the plant [8]. Following method can be applied for effective management of Fusarium Wilt of Tomato.

3.1 Biocontrol of *Fusarium Oxysporum* F.Sp *Lycopersici* by the Application of Endophytic Bacterial Isolates Obtained from *Solanum Elaeagnifolium* Stems

Endophytic bacteria isolated from the internal stem tissues of an invasive weed, *Solanum elaeagnifolium* (Silverleaf Nightshade or Silver Leaf) caused reduction in the severity of Fusarium wilt and vascular browning by 77-83 % and 76 % respectively [8]. Phylogenetic analysis based on Neighbor-Joining methods and Blast analysis of sequenced 16S rDNA gene homology suggests that the anti-fungal isolates belonged to *Bacillus tequilensis* str. SV 101 and *Bacillus sp* [8]. Cell-free filterate of *B. tequilensis* caused alterations in the macro-morphological traits of the colonies of *Fusarium oxysporum* f.sp. *lycopersici* (FOL) such as change in mycelial texture affecting the mycelial density exhibiting antibiosis [8]. The isolate of *Bacillus tequilensis* caused antibiosis of FOL by producing cell wall-degrading lytic enzymes like pectinase, chitinase and protease [8].

IV. MANAGEMENT OF LATE BLIGHT OF POTATO

Late blight of Potato (*Solanum tuberosum*) caused by *Phytophthora infestans* (Oomycetes) is considered as one of the most devastating and economically important diseases in Potato [9]. It was responsible for the disastrous Irish Famine of the 1850s causing emigration, starvation and death of millions of people and children together [9]. The disease symptoms include rusty brown necrosis spreading from surface to the centre of the tuber and brownish black lesions on the leaves [10].

4.1 Utilization of Poplar Bud Extracts for Controlling *P. Infestans*

Bud extracts of Black Poplar (*Populus nigra*) have been found to be effective against *Phytophthora infestans* making it suitable for application under low cost management of Late Blight of Potato [9]. The compound 'Populin' is the potential compound in the Poplar bud extracts that reduces disease severity in the potato plants [9]. Application of Populin inhibited the germination of encysted zoospores and/or sporangia of *P. infestans* that resulted in the decline of hyphal growth in the infected plants [9]. Populin 4 % (v/v) proved to be the most effective concentration for controlling various strains of *P. infestans* [9].

4.2 Application of Peptide Extracts obtained from Common Horsetail (*Equisetum Arvense*) for Inhibiting *P. Infestans*

Peptide extracts obtained from *Equisetum arvense* have proven to be an effective inhibitory agent for *Phytophthora infestans* [11]. The peptide extracts inoculated on potato tuber discs inhibited the appearance of the symptoms of *P. infestans* like

soporiferous layers and necrotic spots [11]. The extracts of Common Horsetail possessed inhibitory capabilities towards the activity of zoosporangium germination, zoospore output and the development of *P. infestans* [11]. The inhibitory or anti-oomycete effects of the extracts are closely associated with the conversion of Rubisco (ribulose-1, 5-bisphosphate carboxylase/oxygenase) into a number of biologically active anionic peptides. Besides Rubisco, the active peptide extract also contained chitinases and aquaporins [11]. These proteins are responsible for some defense functions related to extracellular biotic stress (fungal infections caused by pest damage) [11].

V. MANAGEMENT OF BLACK SHANK OF TOBACCO

Phytophthora parasitica var. *nicotianae* is the causal organism of Black Shank disease of Tobacco (*Nicotiana tabacum*) [12]. It is one of the most devastating diseases that occur in the tobacco being cultivated across the globe [12]. *P. parasitica* var. *nicotiane* is a soil-borne fungus that severely infects the basal part of the tobacco stem along with the roots that markedly reduces the overall yield [12]. Prominent appearance of black necrotic patches on the stem leading to wilting of plant, black discoloration of stem and ultimately shriveling of the stem is the main symptom of the disease [13].

5.1 Colonization of Tobacco Roots with *Paenibacillus Polymyxa* C5 Strain for Controlling Black Shank Disease of Tobacco

The application of *Paenibacillus polymyxa* C5 amended with Bio-Organic Fertilizer (BIO) preparation exhibited a biocontrol efficiency of 80 % on *P. parasitica* var. *nicotiane* [14]. The antagonistic *P. polymyxa* C5 strain formed a protective biofilm around the elongation zone as well as the tip of the roots growing in the soil [14]. The mechanism of colonization of *P. polymyxa* C5 on the tobacco roots is mainly responsible for the suppression of *P. parasitica* var. *nicotiane* [12]. Moreover, the inhibitory and diffusible antifungal compounds produced and released by *P. polymyxa* C5 strain caused permanent inhibition of *P. parasitica* var. *nicotiane* [14].

VI. MANAGEMENT OF FOOT ROT OF RICE

Foot rot of Rice is also called as Rice Bakanae disease. It is caused by *Fusarium moniliforme (Gibrella fujikorai).* Typical symptoms of the disease include chlorotic, slender and elongated primary leaves and rotting of the basal part of the stem [15]. The disease causes lodging of the plant causing significant reduction in the final yield.

6.1 Application of Surfactin-A Extracted from Bacillus Subtilis Nh-100 and Nh-217 Strain

Application of 2000 ppm of purified Surfactin-A extracted from *Bacillus* (SPB) NH-100 and NH-217 strains on *Fusarium moniliforme* showed a reduction in disease incidence by 84 % [16]. Surfactin-A was found to exhibit anti-fungal activity in various pH levels ranging from 5 to 9 [16]. The anti-fungal extract was stable even under variable temperatures like 20◦C, 50◦C, and 121◦C making it suitable for its utilization as a commercial bio-control agent [16]. Surfactins are known to form persistent bio-films around the substrate which makes it a potent bio-surfactant [16]. Lipopeptides present in the surfactants are the main antagonistic agents against the plant pathogens [16].

VII. MANAGEMENT OF SHEATH BLIGHT OF RICE

Sheath Blight disease in rice is one of the major production constraints in rice crop causing an overall yield loss of 10-30 % [17]. *Rhizoctonia solani* is the causal organism of sheath blight of rice. Appearance of circular, ellipsoid or oblong, water soaked greenish grey spots on the stem near the water level are the initial symptoms [18].

7.1 Utilization of *Bacillus Subtilis* Rh5 Strain as A Potential Biocontrol Agent

The RH5 strain of *Bacillus subtilis* exhibited antagonistic activity by significantly reducing the incidence of fungal pathogen *R. solani* by 84.41 % [17]. Formation of protective biofilm is the main mechanism behind the biocontrol activity besides production of a diverse range of secondary metabolites with antimicrobial properties [17]. Strains of *Bacillus* producing endospore have the ability to directly inhibit and suppress plant pathogens by producing several hydrolytic enzymes (glucanases, chitinases and proteases), antimicrobial peptides and volatile substances [17]. *Bacillus* strains indirectly suppress the plant pathogens by competing with them for nutrition or niche [17]. These properties of the *B. subtilis* RH 5 strain makes it a potential candidate for a commercial bioformulation for the management of sheath blight of rice.

VIII. CONCLUSION / FUTURE PROSPECTS

The review article efficiently demonstrates the potential of non-chemically synthesized products (plant extracts and bioformulations) in the management of some of the most devastating diseases like Late blight, Fusarium wilt, Black shank

and Sheath blight occurring on economically important crops such as potato, tomato, tobacco and rice respectively. The exact mechanisms behind the management of Black scurf of potato using *Bacillus subtilis* V26 and date palm compost; biological control of Fusarium wilt using bacterial isolates from Silver Leaf stems and management of Late blight of potato using poplar bud extracts and peptide extracts of Common Horsetail have been clearly described in the research papers of some scientists. However, besides the mechanism of biofilm formation by the antagonists, the principal biocontrol mechanism behind the management of Black shank of tobacco by colonization of roots by *Paenibacillus polymyxa* C5 strain; biocontrol of foot rot in rice by Surfactin-A and management of Sheath blight of rice by RH5 strain of *Bacillus subtilis* have not been clearly elaborated. Therefore, comprehensive research works are needed on those areas for making them commercially successful. The innovative approaches mentioned in the review article can be emphasized upon for their commercialization so that the farmers across the globe can quickly switch over from chemically synthesized fungicides to the eco-friendly bioformulations for effective management of major soil-borne plant diseases.

REFERENCES

[1] Lewis, J. A. ; Papavizas, G. C. Biocontrol of cotton damping-off caused by *Rhizoctonia solani* in the field with formulations of *Trichoderma* spp. and *Gliocladium virens*. *Crop Prot.* **1991**, *10*, 396–402.

[2] Mihajlović, M. ; Rekanović, E. ; Hrustić, J. ; Tanović, B. Methods for management of soilborne plant pathogens. *Pestic. Fitomedicina* **2017**, *32*, 9–24

[3] Baysal-Gurel, F. ; Kabir, N. Comparative performance of fungicides and biocontrol products in suppression of Rhizoctonia root rot in viburnum. *J. Plant Pathol. Microbiol.* **2018**, *9*, 451.

[4] Reddy, SR. *Principles of Organic Farming*: Page-34

[5] Saoussen Ben Khedher. , Olfa Kilani-Feki. , Mouna Dammak. , Hayfa Jabnoun-Khiareddine. , Mejda Daami-Remadi. , Slim Tounsi. Efficacy of Bacillus subtilis V26 as a biological control agent against Rhizoctonia solani on potato. , Comptes Rendus Biologies. , Volume 338, Issue 12, 2015. , Pages 784-792. , ISSN 1631-0691

[6] El Khaldi, R. , Daami-Remadi, M. and Cherif, M. (2016), Biological Control of Stem Canker and Black Scurf on Potato by Date Palm Compost and its Associated Fungi. J Phytopathol, 164: 40-51. https://doi. org/10. 1111/jph.12423

[7] Hussain, T. , Khan, A. A. , *Bacillus subtilis HussainT-AMU* and its antifungal activity against potato black scurf caused by *Rhizoctonia solani* on tuber, *Biocatalysis and Agricultural Biotechnology* (2019), doi: https://doi. org/10. 1016/j. bcab. 2019.101443.

[8] Aydi Ben Abdallah, R. , Jabnoun-Khiareddine, H. , Nefzi, A. , Mokni-Tlili, S. and Daami-Remadi, M. (2016), Biocontrol of Fusarium Wilt and Growth Promotion of Tomato Plants Using Endophytic Bacteria Isolated from *Solanum elaeagnifolium* Stems. J Phytopathol, 164: 811-824. https://doi. org/10. 1111/jph.12501

[9] Turóczi B, Bakonyi J, Szabó K-A, Bálint J, Máthé I, Lányi S, Balog A. In Vitro and In Vivo Effect of Poplar Bud Extracts on *Phytophthora infestans*: A New Effective Biological Method in Potato Late Blight Control. *Plants*. 2020; 9(2):217. https://doi. org/10. 3390/plants9020217

[10] Henfling, Jan W. , *Late Blight of Potato.* , Technical information bulletin. , International Potato Centre. , 1987.

[11] Rogozhin EA, Vasilchenko AS, Barashkova AS, Smirnov AN, Zavriev SK, Demushkin VP. Peptide Extracts from Seven Medicinal Plants Discovered to Inhibit Oomycete *Phytophthora infestans*, a Causative Agent of Potato Late Blight Disease. *Plants*. 2020; 9(10):1294. https://doi. org/10. 3390/plants9101294

[12] Ma, L. , *Applied Soil Ecology* (2018), https://doi. org/10. 1016/j. apsoil. 2018. 05. 011

[13] Courtney A. , Gallup, Kestrel L. , McCorkle, Kelly L. Ivors. , David Shew. *Characterization of the Black Shank Pathogen, Phytophthora nicotianae, Across North Carolina Tobacco Production Areas*. *Plant Disease* (2018) 102:6, 1108-1114. https://doi. org/10. 1094/PDIS-02-17-0295-RE

[14] Ren, X. , Zhang, N. , Cao, M. *et al.* Biological control of tobacco black shank and colonization of tobacco roots by a *Paenibacillus polymyxa* strain C5. *Biol Fertil Soils* **48,** 613–620 (2012). https://doi. org/10. 1007/s00374-011-0651-4

[15] Wulff, E. G. , Sørensen, J. L. , Lübeck, M. , Nielsen, K. F. , Thrane, U. and Torp, J. (2010), *Fusarium* spp. associated with rice Bakanae: ecology, genetic diversity, pathogenicity and toxigenicity. Environmental Microbiology, 12: 649-657. https://doi. org/10. 1111/j. 1462-2920. 2009. 02105. x

[16] Ambrin Sarwar, Muhammad Nadeem Hassan, Muhammad Imran, Mazhar Iqbal, Saima Majeed, Günter Brader, Angela Sessitsch, Fauzia Yusuf Hafeez, *Biocontrol activity of surfactin A purified from Bacillus NH-100 and NH-217 against rice bakanae disease*, Microbiological Research, Volume 209, 2018, Pages 1-13, ISSN 0944-5013, https://doi. org/10. 1016/j. micres. 2018. 01. 006

[17] Jamali H, Sharma A, Roohi, Srivastava AK. *Biocontrol potential of Bacillus subtilis RH5 against sheath blight of rice caused by Rhizoctonia solani*. J Basic Microbiol. 2019;1–13. https://doi. org/10. 1002/jobm. 201900347

[18] Singh Shiv Kumar, Shukla Vivek, Singh Harpal, Sinha A. P. *Current status and impact of sheath blight in rice (Oryza sativa L.) – A review*. Agricultural Reviews. Year: 2004, Volume: 25, Issue: 4. First page: (289) Last page: (297). Print ISSN: 0253-1496.

Assessing the Value of Community-Based Tourism Approach in Community Development in the Surrounding area of the Volcanoes National Park in Rwanda

Nzabandora Dominique[1*], Kabera Callixte[2], Uwayo Pacifique[3]

[1,2]Department of Travel and Tourism Management, University of Tourism, Technology and Business Studies, Rwanda.
[3]Department of Environmental Economics and Natural Resources Management, Faculty of Environmental Studies, University of Lay Adventist of Kigali, Rwanda.
*Corresponding Author

Received:- 27 July 2021/ Revised:- 08 August 2021/ Accepted:- 14 August 2021/ Published: 31-08-2021

Abstract— *Community based-tourism (CBT) is both an integrated approach and a collaborative tool for socio-economic empowerment of communities through the development and marketing of natural and cultural community resources to add value to the experience of local and foreign visitors and simultaneously improve the level of the community. But there is lack of clear approaches to measure performances of CBTs, thus meaning that how they enhance socio-economic livelihoods of local communities and conserve protected areas is difficult to measure in both quantitative and qualitative terms. This study assessed the performance of Community Based Tourism on the socio-economic lives of local community around Volcanoes National Park, and it specifically 1) profiled and examined the performance of existing CBT ventures, 2) the factors affecting community-based tourism development around Volcanoes National Park and 3) the contribution of CBTs on social and economic lives of the local community. The methods used for data collection were sampling, key informant interviews, surveys, focus group discussions, observation and use of secondary data. Data was analysed using SPSS to generate descriptive information and further strata analysis was used. The study recommends that for Rwanda to achieve its goal of harnessing tourism for its vision 2050 the local communities around Volcanoes National Park should be empowered to embrace community-based tourism as an alternative to farming and fishing to improve their livelihood income.*

Keywords— CBT Approach, and Community Development.

I. INTRODUCTION

Community-Based Tourism (CBT) has been used to describe a broad range of different tourism models but usually refers to tourism that involves community participation and aims to generate benefits for local communities in the developing world by allowing tourists to visit these communities and learn about their culture and the local environment. Community participation in the tourism initiative is central to all the definitions, ranging from cooperative or individually owned and managed businesses to joint ventures between the community and the private sector (Zielinski et al., 2020). These ventures are characterized by high environmental consideration, increased control and involvement of the local residents, as well as significant benefits for the host community and coined the term community-based ecotourism (Hussin & Kunjuraman, 2014). This is used to describe any CBTs ventures that are characterized by high environmental and social considerations, increased control and involvement of the local residents, as well as significant benefits for the host community (Phuong et al., 2020).

Tourism Development Master Plan of 2010 noted that Rwanda has a growing community-based tourism sector providing visitors with an insight into how local Rwanda communities live and work. These are located mostly along the tourism routes and provide products and services such as home stays, village walks and interaction with village personalities (Safari, 2017). As described by Njenji (2020), these CBTs are managed and governed to pursue the economic and social goals of the communities in the country in a manner that yield sustainable individual and group benefits over the short- and long-term. However, the data on the economic activity of CBTs or indigenous entrepreneurs is still scarce (Gohori & van der Merwe, 2020). According to Mayaka et al., (2020), CBT was born as an alternative approach to the excesses of mainstream or mass tourism, such as repatriation of profits from developing economies by multinational companies and the negative impact on

destinations. It is consistent with alternative development and sustainable livelihood approaches, which focus on grassroots development and embrace participation, equity and empowerment ideas. Its interest resides in the fact that CBT projects are small or medium sized ventures that have the potential to generate a range of positive economic and social development impacts in rural areas, where other types of development may be inadequate (Pemayun & Maheswari, 2017).

Through local control of tourism businesses and activities, CBT is thought to contribute to cultural and environmental conservation and to the redistribution of economic benefits among the most vulnerable groups, such as indigenous communities. A range of studies about CBT initiatives have confirmed its potential benefits to communities, especially 'commercially grounded' initiatives (Kaur et al., 2016). In Rwanda, the government's policy framework prioritized Northern Province, which accommodates the Volcanoes National Park, a habitat of the rare endangered mountain gorillas as a great tourist destination where, Community Based Tourism (CBT) needs to be sustainably developed (Aboniyo & Mourad, 2017). A number of public and private sector investments have been encouraged by the Rwandan Government to provide tourist infrastructure and accommodation facilities. But, to engage the local communities in tourism in the province, SNV in the period of 2005-2012 initiated pro-poor tourism (PPT) projects in the country to encourage local participation and achieve local community economic diversification and alternative household income generation. Since these initiatives of CBT through PPT, the values to realizing socio-economic benefits and conserve wildlife have not been assessed in case of Volcanoes National Park in Rwanda. This creates a gap to understand whether CBT approach is effective or not (Njenji, 2020). Therefore, this study was conducted to fill this gap where, it assessed the value of community-based tourism approach in community development in the surrounding area of the Volcanoes National Park in Rwanda.

II. MATERIALS AND METHODS

2.1 Study Area Description

The study was carried out in Northern Province of Rwanda, Musanze District, precisely in both Kinigi and Nyange Sectors. The district is one of the four districts surrounding the Volcanoes National Park: Burera, Musanze, Rubavu and Nyabihu. According to the fourth Rwanda Population and Housing Census (PHC4) 2012, Northern Province had an estimated population of 1,726,370 residents representing 21.4% of the total country's population (Farmer et al., 2013). That population is predominantly female whose number represents 52.6% of the total population of the province. Gicumbi and Musanze, are the mostly populated districts with more than 360 thousand residents for each. Their populations represent 22.8 % and 21.4% of the total resident population of the Northern Province.

Kinigi and Nyange Sectors where the study was conducted, experience periodic temperature variations with the highest temperatures occurring in the dry season while the coldest occur in the rainy season. The average maximum temperature varies between 22-26^{o}C while the minimum ranges between 10-15^{o}C. The area has four main seasons: a short dry season occurring from January to March, a short rainy season from March to May, characterized by torrential rainfall, a long dry season from June to August and a long rainy season from September to December. The climate is typical tropical, characterized by high annual rainfall of up to 1, 500 mm per year.

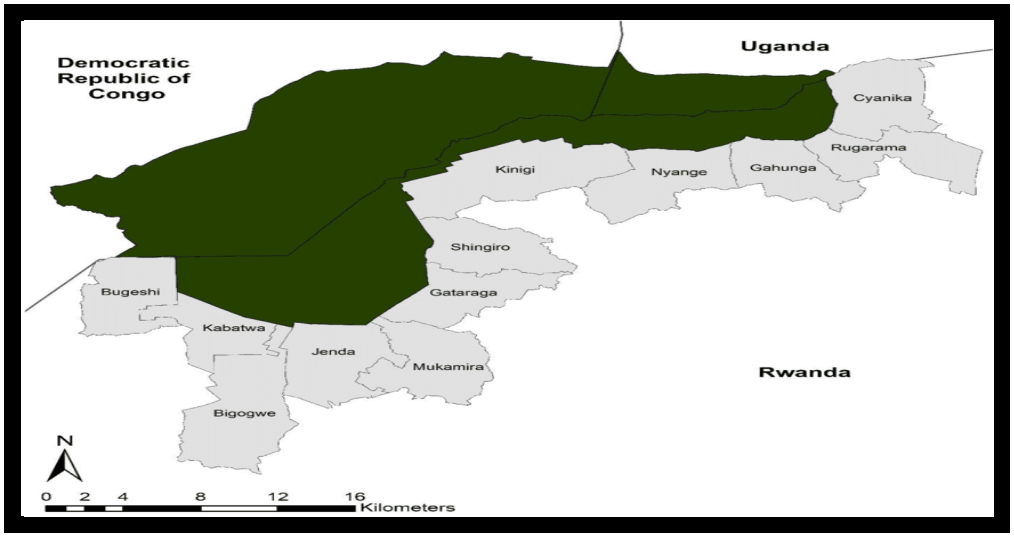


FIGURE 1: A map showing the different districts adjacent to VNP, Rwanda
Source: www.researchgate.net

2.2 Sampling Techniques and Data Collection

In this study, both purposive and random sampling techniques were used. Four CBT initiatives were purposively selected as case studies to profile structure of CBTs around VNP. Although, there were over 15 CBT ventures around VNP, the four initiatives which are the first to be started with technical and financial assistance from SNV and other external agencies and which have also grown to be large and renowned CBT venture examples around VNP area were chosen. Their deliberate selection aimed to extend existing studies on CBT approach in protected areas. The purpose was to collect random views on contributions of CBT approach to the community and household wellbeing. To determine sample size, Slovin formula was used.

$$n = \frac{N}{1+Ne^2} \quad (1)$$

Where: n = sample size (households that participate in CBT initiatives)

N = Total number of CBT members based on District CBO Register

e = margin of error

A sample size of 317 community respondents was selected from a study population of 54,687 residents (Kinigi 27,221 and Nyange 27,466). Cohort surveys were conducted to trace CBT venture group members who overtime shared the benefits to enhance livelihoods. The formula was applied to as: CBT venture Case 1 total members = 370: Case 2 total members = 300: Case 3 total members = 178 and Case 4 total members = 235. However, 10 CBT projects managers and 7 local leaders were deliberately included in the sample. Out of total sample size of 317, the actual respondents were 220 with 97 non-responses. Statistically response rate of 69.4% was considered significant for this study.

2.3 Data Analysis and presentation

The data collected on various aspects of the study (socio-economic or demographic variables of households, community tourism asset base and stakeholder views) were analysed using different methods. Resulting quantitative figures on demographic variables of households were entered into excel and transferred to the Statistical Package for Social Sciences (SPSS) for analysis. Descriptive information generated in as modes, means and standard deviations were further analysed using STRATA. To establish relationship between household variables and their ability to participate in CBTs Logit model was used. The model assumes nonlinear probability models with cumulative probability distribution function:

$$P = \frac{1}{1+e^{(-\beta X_i)}} \quad (2)$$

Where:

P - Probability that an individual participate in CBT

B - Coefficient of the covariate for every unit change in the covariate.

e - Exponential value.

Explanatory factor analysis (EFA) was run to identify critical factors that enabled growth of CBTs based on the existing asset base for tourism development.

III. RESULTS AND DISCUSSIONS

3.1 The main economic activities in Kinigi and Nyange Sectors

Based on the research results, excessive soils erosion and declining land size due to overpopulation and over farming on fragmented plots of land around the park boundary, limited formal employment opportunities and poor accessibility to the park undermined long term dependability of the community on current means of livelihoods. However, other alternative means of livelihood were provided by CBT ventures with assistance from SNV and other agencies. They encouraged recruitment of households and individuals as members of CBT ventures. Indigenous private or family investment in tourism sector were encouraged using collective CBT venture models like private small accommodation facilities, small restaurant and food establishments, tour guiding and porter services.

TABLE 1
COMMUNITY/HOUSEHOLD MAIN ECONOMIC ACTIVITIES AND THEIR PERSPECTIVE ON THEIR SUSTAINABILITY

Major economic activities	Community perspective on the status over long term prospects
Farming (Crop growing)	Excessive soil erosion and much more land taken up for crop growing.
Animal keeping	Disease outbreaks and shortage of grazing land.
Forestry	Illegal logging and excessive charcoal burning.
Commerce and trade	Increasing poverty due to declining productivity activities of fishing and farming leading to lowering household purchase power.
Formal employment	Limited employment opportunities due to limited growth around the park's major economic activities, civil service sector and rapid population growth (natural growth and high level of immigration).
Transport sector	Poor connection with other towns due to bad road infrastructure in the region and further transport sector development discouraged by declining economic opportunities around the park.

The reported encouragement was due to unique tourism resource base provided by the Volcanic Mountains, forests on the mountain slopes, wildlife and birdlife, as well as Rwandan cultural heritage attributes. The leaders of Musanze District informed that the district office had received applications for ecotourism projects by the private investors seeking concession for investment. The researcher also noted the growing accommodation and tourism development related activities, the way that the trend of tourism development offered the communities opportunities to invest in CBT ventures.

This perception based on the projected socio-economic benefits that could arise from CBTs to include sharing park entry fees, joint investment ventures with private sector, employment creation, improved household income, improved transport services and self-community social service provisioning like primary and secondary schools, health centres and safe water sources. It was noted that tourist expenditures on lodging, internal movement, food, guiding services and purchase of local crafts and souvenirs could offer tourism income to provide these socio-economic benefits. It was also observed that CBTs could play a major role as local collective entrepreneurs to provide opportunities for community-based ecotourism development to fill the gap of limited government or private sector investment interest in tourism sector around the park. Local community entrepreneurial gap would be crucial in developing and implementing productive investment opportunities offered by immense Mountain forest, Lakes, wildlife and birdlife potentials.

3.2 Resident Participation and its relationship to socio-economic determinants

Given the centrality of socio-economic determinants of the residents in influencing willingness to participate, Logit Model was used to establish the relationship between various socio-economic factors and willingness to participate (Table 2). The significances of coefficients provided the relationships explaining how socio-economic enable willingness to participate in CBTs by residents of Kinigi and Nyange sectors. These variables provide significant factors that influence willingness to participate. Land size (those with no land were reference category); Household Size ('1-3' people in the household was the reference category); Gender (Males were the reference category); Dwelling conditions (temporary was the reference category); Occupation (peasant farmers were the reference category); Education level attained (No education was the reference category); Age (15-29 category was the reference category); Residence Duration (was the period a house hold head had lived in the area); Income level (less than 50,000 RWF was the reference category; *** Significant at 10 percent level. The reference category is the category (in each explanatory variable) with which the comparisons are made. Results presented in Table 2 summarize the relationships between socio-economic factors and resident willingness to participate in CBT development. Socio-economic factors like gender, age (apart from group 45- 59), family size, dwelling and residence period generally had insignificant estimated coefficient and low marginal effect implying low probabilities of influencing and encouraging resident participation in CBT. Education, occupation, income and land size provided positive and statistically significant influences on participation in CBT venture formation, management and running. Particularly, education and income had shown increasing marginal effects of household participation. This means that the higher the education or income levels, the more residents around the park are willing to participate in CBT development. Income was the estimated monthly amount of money earned by a household from different economic activities including tourism activities.

TABLE 2
SOCIO-ECONOMIC VARIABLES INFLUENCING COMMUNITY WILLINGNESS TO PARTICIPATION

Variable	Explanatory variables	Coefficients	z-statistic	Marginal effects (r)
	Constant:	-1.900	-5.21	0.713
Gender	Female:	0.120	0.59	0.326
Age	30-44 years	-0.315	-1.03	0.505
	45 – 59 years	-1.199***	-2.70	0.510
	60+ years	0.037	0.11	0.639
Household size	4-6 people	-0.354	-1.09	0.390
	7-9 people	-0.383	-0.50	0.492
Land size	1 - 2 acres	0.861***	2.26	0.421
	3 -5 acres	1.822***	4.24	0.536
	6 - 8 acres	1.338	1.94	0.789
Income	50,000 – 100,000	2.196***	2.84	0.69
	100,001 – 150,000	1.823***	2.27	0.83
	150,001 – 200,000	2.626***	3.15	0.78
	200,001 – 250,000	2.314***	2.91	0.82
	Over 250,001+	3.376***	4.32	0.86
Education	Primary	1.201***	2.39	0.388
	Secondary	1.412***	2.67	0.473
	Tertiary	1.732***	1.95	0.836
Dwelling	Semi-Permanent	0.321	1.33	0.337
	Permanent	0.178	0.10	0.424
Occupation	Commercial fishing	2.012***	3.82	0.599
	Farming	-0.265	-0.27	0.973
	CBT members	2.764***	2.61	0.853
	Retail business	1.237***	2.03	0.685
	Bodaboda transportation	0.214	0.71	0.748
	Employed	1.561***	1.03	1.275
Residence	6 – 10 years	0.759	1.71	0.555
	11 – 15 years	0.865	1.68	0.499
	16 – 20 years	0.529	0.97	0.523
	21 – 25 years	0.885***	2.06	0.412
Log likelihood = -141.363		Pseudo R^2 = 0.3521		
LR chi2(24)=191.81		Prob> chi2 = 0.000	Sample size =330	

The results indicate that those residents engaged in commercial farming, employed in tourism enterprises, tourism entrepreneurs, government officials or collective self-help CBT ventures participated in CBT development. However, tourism entrepreneurs, government officials or residents who formed collective self-help CBT ventures had a higher marginal effect (0.91) than others collectively at (0.642). This was found to be linked to those residents who understand how CBTs could be used as self-help approaches collectively by residents to achieve park and their development. In this regard, it was found out that CBT acted as a strategy of community development through park tourism development.

When asked about their involvement in CBT projects and/or programmes, participation in meetings was identified as a popular activity undertaken by local residents. 76.6% of total respondents having participated in CBT meetings. However, majority (80.2%) participated few times (only once or twice). About their roles in decision-making, a total of 52.4% noted their engagement in deciding for CBT plans, activity project initiation or diversification. When comparing men and women in decision-making, the findings revealed a higher percentage of men in decision-making (42.5% versus 12.8%). Pearson chi-square tests were also run to show any significant differences between men and women in decision making about CBT plans, programmes and activities. CBT planning, programme and activity plans showed the greatest difference between men and women, corresponding with traditional roles between men and women in making decisions, there was significant difference in these activities between the two groups. However, in issues of craft making women made their own decisions on what type (s) of crafts when to make and at what prices to sell.

This was done through the park community socio-economic analysis and willingness to participate. Assessment was also done of existing strength and opportunities offered by their livelihoods in order to approximate their willingness to undertake alternative livelihood strategies in CBT. The assessment of socio-economic factors focused on: general socio-economic features of the residents as proxy approximation of undertaking opportunities in CBT ventures; - relating socio-economic variables with willingness to participate; level of knowledge and organizational skills. Awareness, knowledge and organizational skills are the basis for successful participation and management of CBTs. Long term prospects of their current sources of livelihood were investigated by applying the "resident development influence matrix" with the target group through focus group discussion.

With reference to the community participation, incorporating local communities' thoughts in tourism planning and development is a vital element of sustainable tourism. Notably, community participation is essential for tourism development, as tourism has a close relationship with the livelihood of the local community and the tourism destinations are communities with which local residents interact. This makes CBT as an important strategy to improve tourism development around Volcanoes National Park. Communities around parks' understanding of tourism potential for development can motivate their participation which can also improve their livelihoods. However (Mak et al., 2017), cautioned understanding potential of tourism development needs examining the extent to which local residents are thoroughly informed and invited to join in the tourism initiative process. A number of tourism scholars have adopted various frameworks to understand the level of participation and power distribution within communities and to determine whether the frameworks can be applied to both developed and developing countries. But, these frameworks were not applied in this study, because the study interest was to evaluate CBT tourism development initiatives only and understand how much tourism had penetrated the Kinigi and Nyange sectors of Volcanoes National Park development. It examined the relationships in terms of how rural communities with low economic activity and low tourism development residents have expectations about the future tourism development. The study found out more favorable perceptions towards tourism development. Similarly, the findings from this study also indicated that residents are willing to participate in CBT because its development relates closely with the level of economic activity within the park. However, a study by Nugroho & Numata (2020), in Fiji noted that residents of communities' dependent on tourism can clearly differentiate between its economic benefits and social costs.

3.3 Improvement of livelihoods and achieving conservation objectives

An assessment of CBT initiatives in Kinigi and Nyange sectors was done in order to provide a clear picture of how the four cases have played major roles in improving community/household livelihoods. This was done by means of Tourism Penetration Index (TPI) to relate venture development with livelihood improvement by understanding the degree of CBT venture development as alternative means of livelihoods. Its penetration as an alternative livelihood means relate directly with improvements of community/household wellbeing. The findings are guided by the questions: What are the CBT ventures existing in Kinigi and Nyange? What do they contribute to socio-economic lives band conservation? How has CBT approach in Kinigi and Nyange helped to enable tourism to penetrate the local economy in and around the Volcanoes National Park? These questions guide the findings about how CBT approach improves community livelihoods.

TABLE 3
TOURISM INVESTMENT IN KINIGI AND NYANGE AS A MEASURE OF TOURISM PENETRATION

Names	Ownership	Venture Type	Bed Capacity	Employment	Land occupied (in Acres)
Gorilla Guardian	Community	Cultural centre		43	10
SACOLA	Joint venture	Accommodation	45 Rooms	45	1
Volcano National Park	Government	National Park	162	90	12500
Buhanga Eco-Park	Government	Eco-Park		16	31
Nyange Community	Community	Cultural Village		25	40
Buhanga Sacred Forest	Government	Forest nature walks		24	8
Bulera and Ruhondo	Government	Canoeing on the twin Lakes		40	2800
Musanze Cave	Government	Cave visits			
Totals			**162**	**238**	**15389**
Percentage of private sector investment					**0 %**
Percentage of community group involvement and investment					**28.6 %**
Percentage of CBT initiatives					**42%**
Percentage of community members employed by external investors					**0 %**
Tourist Spending/annum					225.3Bn
Size of the Island (KM^2)					15389
Population (2014)					54687
Visitor arrivals					185000
Average stay					2.5

3.3.1 CBT ventures existing in Kinigi and Nyange Sectors

Different scenarios were used to categorize CBTs in Kinigi and Nyange sectors; thus individually owned community-based businesses, cooperatives, community associations, and concessions. The categorization was purely based on the elements of community-ownership or management. As it is shown by the data presented in Table 3 above, four main types of CBTs were identified and classified as:

a. Tourism project in which community members are employed by outsiders.

b. Tourism project that involves family ventures within the communities, based on community assets.

c. Group initiatives that are involved in collective ventures to run either as cooperatives or community associations within the park communities based on the park natural and cultural heritage assets.

d. Joint venture between a community or family and an outside business partner.

This categorization based on community ability to embrace individual or collective initiatives within the Kinigi and Nyange sectors. Table 3 summarizes the investment types by CBTs in Kinigi and Nyange sectors.

3.3.2 Contributions of CBT ventures to socio-economic lives and conservation

The contributions of CBT ventures in community development are vital to encourage participation and creating self-help initiatives in the local community economies for those living around national parks. The linkage between community assets, participation and benefits was determined by asking the following questions. How does CBT approach encourage community assets/capitals development in and around the park? What are the different ways in which tourism contributes to park communities' livelihoods in Kinigi and Nyange sectors? These questions were used in the study to assess the socio-economic benefits and CBT venture contributions to the local communities living in neighborhood with VNP. However, the ability of CBT approach to contribute to socio-economic lives of the communities was analyzed by how much tourism has penetrated in the socio-economic lives of the community as an alternative livelihood activity. These were determined by different agencies support by providing finances to supplement community or household livelihood strategies and by use of tourism penetration index (TPI).

3.3.3 Contributions of agencies to support CTB initiatives

Agencies support to CTB approaches was determined by different supports to community-based groups who are engaged in tourism. The findings noted various financial and technical supports provided to community-based groups. Table 4 shows the various contributions by different agencies in supplementing community-based initiatives around the park. As noted in the table, agencies' supports focused on supplementing community-based initiatives of commercial ventures like accommodation provisions, capacity-building and social service supports in form of school fees. However, the monetary contributions in these various support could not be got because the agencies regarded the information confidential.

TABLE 4
CONTRIBUTIONS OF AGENCIES TO SUPPORT CTB INITIATIVES

Agencies	Projects supported	Types of contributions
Rwanda Development Board	Revenue-sharing projects	Revenue-sharing fund of 10%
SNV	Financing community-based project under pro poor tourism initiatives	Funds to start community enterprises
SNV	Community capacity building as technical support	Training
International Gorilla Conservation Programme (IGCP) jointly with Flora & Fauna International (FFI)	Community capacity building	Training
IGCP	Community accommodation project support	Co-funding of SACOLA lodge jointly with a private investor
Africa Wildlife Foundation	Support to community-private joint ventures	Co-funding of SACOLA lodge jointly with IGCP and a private investor
Dian Fossey Fund	Building gorilla-community relationships for mutual co-existence	Paying school for vulnerable children, Amani yaJuu Artisan Sewing Project for women groups and the Kigali Public Library Project

3.3.4 Tourism Penetration Index in the Local Economy of Kinigi and Nyange sectors

As provided in Table 5 below, average per capita visitor spending was Rwf. 91,447.58 per total population in the Year 2014 and average visitor density is calculated at 0.0021 tourists per total population per 1000 residents. This implies that visitors represented 0.021 percent increase in most of the year round population of the park. This is critical in determining demand increase for resources and utilities. The current land use percentage occupied by tourism related investment is only 0.101 (about 10.1%) per total land size of the park implying tourism is still at infant stage of development. Tourism employment only represents an indicative figure of 0.11. The overall TPI score for the park is 0.457 combining five variables of spending, density, bed capacity and land use. Therefore, since the purpose of this study was to assess the performance of CBT ventures on the socio-economic lives of local community around Volcanoes National Park, the TPI provides the degree to which tourism has become part of the community economic activity. It assists the community, Musanze District Local

Administration, Rwanda Development Board (RDB) and other stakeholders to know the role of tourism in socio-economic development of the communities.

TABLE 5
CONSTRUCTION OF TOURISM PENETRATION INDEX AND PARK TPI SCORE

Selected Tourism Indicators	
Spend/Population	91,447.58
Visitor Density per 1000	0.0021
Rooms Km^2	0.47
Land use Acres	0.56
Employment Ratio/Population	0.003
Impact Indices	
Spending	0.084
Density	0.0021
Bed capacity	0.16
Employment	0.11
Land use	0.101
TPI score	**0.457**

However, TPI of Kinigi and Nyange sectors showed very low tourism development and limited community participation. This means that currently tourism is less visible as a major development strategy. This requires a major community-wide planning effort to achieve three objectives: (1) to identity the unique assets/attractions; (2) to encourage tourism investment and; (3) to determine the sectors identity compatible with the "genius of the place". This is a formidable task necessitating development of a special policy by the district of Musanze to use tourism as a local economic development tool. The three impact scores provided by the TPI also mean that tourism development should be socially acceptable, environmentally compatible and economically viable.

3.4 Contributions

The CBT initiatives resulted in the provision of community-based accommodation (in form of *bandas*, tented camps, camping grounds, cottages and hostels), craft making and selling, food services, guiding services, economic -museums, retail shops, car hires and motorcycle transport services. Income realized from these initiatives formed a critical alternative finances for household/community livelihood improvement. Participant responses put these as alternative means of survival apart from agriculture, retail businesses and motorcycle riding as community major livelihood activities. Overall, it was estimated in focus group discussion that households participating in these activities generate over 12,778.83$/month which was 28.2% more than that realized from other activities. The estimated total flow of funds into local households' budget from tourist hotels in the area is 13,093.9$/month. Generally, at Kinigi and Nyange Sectors the shares of benefits generated were noted to be high. This was expected mainly because two sectors are the most active in terms of having a high number of business ventures (both private and by communities). Field data further reveals that the CBT ventures had over 500 people employed directly or indirectly in tourism activities forming over 60% of local people employed in an alternative sector apart from agricultural sector.

This means that CBT approach have a significant contribution in local communities' livelihood. Lo & Janta(2020), argue that the contribution of livelihood in terms of employment and income are critical in asset building. The contributions of this study mostly focused on community asset building which was the focus of SNV. Asset building was noted by SNV to increase community ability to adapt and transform. Community adaptive capacity response to change. It involves informed decision-making, forward-looking planning and flexibility created through CBT approach. While transformative capacity implied structural and systemic changes that enable the communities to improve other capacities.

IV. IMPLICATIONS OF THE RESEARCH AND CONCLUSION

4.1 Implications

Rwanda's tourism industry has witnessed a faster growth and is now a major source of foreign exchange income, creating much-needed employment and development opportunities (Mora et al., 2019).With a steady increase in non-resident visitor arrivals and domestic movements, it is important to understand how tourism development in Volcanoes National Park (especially CBT) may be a major sector for the park and community economic development. As evidenced by a number of scholars including Pasanchay(2019), many communities are actively pursuing tourism growth, yet the substantial and growing CBT remains relatively unnoticed and poorly documented in tourism development particularly in Kinigi and Nyange sectors in Musanze District. Understanding the role of CBTs more thoroughly through planned research is a logical step in better evaluating partly park tourism development in Kinigi and Nyange sectors, and this can help to ensure that both the national tourism objectives and the demands of tourists can be met as well as allowing sound marketing decisions to be made by the Rwanda Development Board (RDB) to promote tourism in Musanze District.Le et al.,(2016), observed that CBT referred to in the early 1980s as the sine qua non of alternative tourism, aided rural communities in the global south through grassroots development, resident participation, empowerment and capacity building. Community Based Tourism plays a great role in community development, capacity building, local control and local enterprise development, sustainable livelihoods and poverty alleviation (Kaur et al., 2016). The criteria identified by various scholars like (Haque et al., 2016), were tested in this study to evaluate the success of tourism development in Kinigi and Nyange sectors, Volcanoes National Park in relation to community participation, benefit-sharing, tourism resources conservation, partnership and support from within and outside the community, local ownership, and scale of tourism development. Based on a wide range of case studies in various countries, this study concurred with Hatton(1999), conclusion that while CBTs present an opportunity for economic gain, leadership, empowerment and employment in Kinigi and Nyange sectors. This was done in this study by assessing CBT initiatives and their benefit streams to the communities around the park. The study also concurred with Bittar Rodrigues & Prideaux (2018), assertion of the four dimensions of community empowerment: economic (income and employment related); psychological (considers community pride and self-esteem); social (community cohesion and well-being); and political (shift balance between the powerful and powerless, between the dominant and dependent, for greater political equity) through CBT initiatives in Volcanoes National Park. But, it was observed that CBT initiatives in the park lacked full dedication to projects, as most residents were engaged in farming activities, retail trade and other traditional activities they have been performing over generations.

4.2 Conclusion

The study noted the following as key conclusions.

1. Four types of CBT initiatives have emerged in Volcanoes National Park area: businesses that employed local residents, family-ran enterprises, joint venture with private sector and community-based social enterprises.
2. Majority of local residents who participated in tourism are in the age bracket 30 – 40 and completed either secondary or tertiary education showing that CBT only attracted educated people.
3. Operational and marketing factors influenced by policy and technical supports acted as critical success factors to facilitate CBT approach.
4. Tourism development for communities living around protected areas is still low determined by TPI. This supports previous findings on low tourism development in a number of small communities due to limited local initiatives.

ACKNOWLEDGEMENTS

We are pleased to thank all respondents who voluntarily accepted to provide the necessary information that were used in this study.

REFERENCES

[1] Aboniyo, J., & Mourad, K. A. (2017). Role of community based organizations in transferring climate change information in Rwanda.

[2] Bittar Rodrigues, C., & Prideaux, B. (2018). A management model to assist local communities developing community-based tourism ventures: a case study from the Brazilian Amazon. *Journal of Ecotourism, 17*(1), 1-19.

[3] Farmer, P. E., Nutt, C. T., Wagner, C. M., Sekabaraga, C., Nuthulaganti, T., Weigel, J. L., . . Karasi, J.-C. (2013). Reduced premature mortality in Rwanda: lessons from success. *Bmj*, *346*.

[4] Gohori, O., & van der Merwe, P. (2020). Towards a tourism and community-development framework: An African perspective. *Sustainability*, *12*(13), 5305.

[5] Haque, M., Reza, M., Alam, M., Ahmed, Z. U., & Islam, M. (2016). Discovery of a potential site for community-based sustainable ecotourism in the Sundarbans Reserve forests, Bangladesh. *Wasiul, Discovery of a Potential Site for Community-Based Sustainable Ecotourism in the Sundarbans Reserve Forests, Bangladesh*, 553-566.

[6] Hatton, M. J. (1999). *Community-based tourism in the Asia-Pacific* (Vol. 99): School of Media Studies.

[7] Hussin, R., & Kunjuraman, V. (2014). Sustainable community based tourism (CBT) through homestay programme in Sabah, East Malaysia. *Proceeding of the Social Sciences Research ICSSR, 2014*, 41-61.

[8] Kaur, P., Jawaid, A., & Othman, N. B. A. (2016). The impact of community-based tourism on community development in Sarawak. *Journal of Borneo Kalimantan*, *2*(1), 15-26.

[9] Le, T.-A., Weaver, D., & Lawton, L. (2016). Community-based tourism and development in the periphery/semi-periphery interface: A case study from Viet Nam *Reframing sustainable tourism* (pp. 161-181): Springer.

[10] Lo, Y.-C., & Janta, P. (2020). Resident's perspective on developing community-based tourism–a qualitative study of Muen Ngoen Kong Community, Chiang Mai, Thailand. *Frontiers in Psychology*, *11*.

[11] Mak, B. K., Cheung, L. T., & Hui, D. L. (2017). Community participation in the decision-making process for sustainable tourism development in rural areas of Hong Kong, China. *Sustainability*, *9*(10), 1695.

[12] Mayaka, M. A., Lacey, G., & Rogerson, C. M. (2020). Empowerment process in community-based tourism: Friend relationship perspective. *Development Southern Africa*, *37*(5), 791-808.

[13] Mora, J. A., Yamova, O., & Murtuzalieva, T. (2019). Community-Based Tourism as the Leading Approach to the Rural Development. *Sustainable Leadership for Entrepreneurs and Academics*, 503-510.

[14] Njenji, J. (2020). Community-based Tourism in Rwanda.

[15] Nugroho, P., & Numata, S. (2020). Resident support of community-based tourism development: Evidence from Gunung Ciremai National Park, Indonesia. *Journal of Sustainable Tourism*, 1-16.

[16] Pasanchay, K. (2019). An Examination of Rural Homestay Operations through the Lens of the Sustainable Livelihood Framework: A Case Study of Phou Khao Khouay National Park Community-Based Tourism (PKK NP CBT), Lao PDR.

[17] Pemayun, A., & Maheswari, A. (2017). Economic impacts of craftsman statue on community based tourism development. *International Journal of Social Sciences and Humanities (IJSSH)*, *1*(3), 59-73.

[18] Phuong, N. T. M., Van Song, N., & Quang, T. X. (2020). Factors Affecting Community-Based Tourism Development and Environmental Protection: Practical Study in Vietnam. *Journal of Environmental Protection*, *11*(2), 124-151.

[19] Safari, E. (2017). *A model of the contribution of information communication technology to the tourism value chain for pro-poor benefits in Rwanda*. Cape Peninsula University of Technology.

[20] Zielinski, S., Kim, S.-i., Botero, C., & Yanes, A. (2020). Factors that facilitate and inhibit community-based tourism initiatives in developing countries. *Current Issues in Tourism*, *23*(6), 723-739.

Detection of Coronavirus in Giant Anteater (*Myrmecophaga tridactyla*) by Transmission Electron Microscopy in São Paulo, SP, Brazil.

Catroxo, M.H.B.[1*]; Martins, A.M.C.R.P.F.[2]; Santos, E.M.[3]

Electron Microscopy Laboratory, Research and Development Center in Animal Health, Biological Institute, São Paulo, SP, Brazil
Biological Institute, Avenida Conselheiro Rodrigues Alves, 1252, Vila Mariana, São Paulo, SP, Brazil
*Corresponding Author: Marcia H.B. Catroxo

Received:- 20 July 2021/ Revised:- 05 August 2021/ Accepted:- 16 August 2021/ Published: 31-08-2021

Abstract**—** *Coronaviruses belong to the order Nidovirales, family Coronaviridae and have four genera, Alphacoronavirus, Betaconavirus, Gammacoronavirus and Deltacoronavirus. They infect humans and several animal species, causing various diseases. Coronavirus constitute zoonotic risk to global public health because of their ability to adapt to new species and establish sppilover events. In this study, we evaluated the presence of coronavirus particles in the feces of giant anteaters (Myrmecophaga tridactyla). Under the transmission electron microscope, particles with coronavirus-like morphology, pleomorphic, rounded or elongated with radial projections forming a corona and measuring 80-140 nm in diameter, were visualized in all examined samples. The technique used was extremely useful for rapid viral diagnosis in affected animals. This report is the first occurrence of coronavirus in Giant anteater (Myrmecophaga tridactyla).*

Keywords— Coronaviruses, Giant Anteater (Myrmecophaga tridactyla), Transmission electron microscopy.

I. INTRODUCTION

Coronaviruses infect humans and a wide diversity of animal and bird species causing respiratory, enteric, neurologic and hepatic disorders [1].

They constitute a zoonotic risk to global public health because their ability to adapt to new species and establish sppilover events [2].

Due to their zoonotic potential, they have a strong tendency to cause catastrophic impacts, such as the recent human viral pandemics, originating from bat, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and Covid 19 [3].

In animals, the coronavirus has also caused devastating diseases, such as porcine epidemic diarrhea (PEDV) that eliminated 10% of the US pig population in less than a year [4, 5, 6] and the infectious bronchitis virus that decimated flocks of chicken and turkey in different parts of the world, causing economic losses to the poultry industry [7].

Coronaviruses are positive-stranded RNA viruses, belong to the order *Nidovirales*, family *Coronaviridae* and have four genera, *Alphacoronavirus* (human coronavírus NL63 (HCoV-NL63), porcine transmissible gastroenteritis coronavírus (TGEV), PEDV, and porcine respiratory coronavírus - PRCV), *Betacoronavirus* (SARS-CoV, MERS-Cov, bat coronavírus HKU4, mouse hepatites coronavírus (MHV), bovine coronavírus (BCoV), and human coronavírus OC43, *Gammacoronavirus* (avian (infectious bronchitis coronavírus - IBV) e *Deltacoronavirus* (porcine deltacoronavirus (PdCV) [8].

Companion animals, such as cats, dogs, ferrets, horses and alpacas can also be infected [9].

The electron micrographs of coronavirus revealed a diverging spherical outline with some degree of pleomorphism, virion diameters varying from 60 to 140 nm, and distinct spikes of 9 to 12 nm, giving the virus the appearance of a solar corona [10].

Coronavirus genome measures 27 to 32 kb and encodes three tipos of proteins, structural, accessory, and non-structural proteins. The structural proteins comprise the nucleocapsid (N), spike (S), membrane (M), and envelope (E) proteins [11].

The S protein mediates attachment of the virus to the host cell surface receptors resulting in fusion and subsequent viral entry.
The M protein is the most abundant protein and defines the shape of the viral envelope and the pleomorphic variability in different species. The E protein is the smallest of the major structural proteins and participates in viral assembly and budding. The N protein is the only one that binds to the RNA genome and is also involved in viral assembly and budding [12]. The roles of most of the accessory proteins remain poorly understood, but, the SARS coronavirus encodes accessory proteins that antagonize the development of type I interferon (IFN) responses [13]. The nonstructural proteins reassemble to form a viral replicase-transcriptase complex, consisting of the RNA-dependent RNA polymerase (RdRp, nsp12), helicase (nsp13), nsps with accessory functions [14].

Viral replication occurs quickly and mainly in the villous epithelial cells of the small intestine, resulting in marked villous atrophy due to necrosis [15, 16].

Animals become infected through the faecal-oral route, respiratory or inhalation of aerossol [17, 18].

The incubation period is 2–8 days [19] and the morbidity is very high, up to 100% [20].

The giant anteater (*Myrmecophaga tridactyla*) is the largest known species of anteater and the only species in Myrmecopahaga [21], listed as "vulnerable" by IUCN. It has become extinct in some parts of its geographic distribution, such as Uruguay. In Brazil it is considered extinct in the states of Vitoria, ES and Rio de Janeiro, RJ, and in condition of vulnerability in other states at great risk of disappearance in Central America [22].

The main threats to the survival of the species are hunting and habitat destruction, being an animal susceptible to being fatally hit by fires and traffic-accidents [21].

The incidence of studies on the presence of coronavirus in animals with potential reservoir has been little discussed, especially in animals at risk of vulnerability [23].

Electron microscopy techniques (rapid preparation) allow an accurate detection of viral particles, especially those of the coronavirus, enabling a rapid diagnosis in samples of different specimens [24].

To the detriment of the efficiency of detection by means of electron microscopy, this study sought to evaluate the presence of coronavirus particles in fecal samples of giant anteaters from the Ecological Park of the State of São Paulo, SP, Brazil.

II. MATERIAL AND METHODS

Description of the case: In 2016, there were cases of diarrhea among giant anteaters (*Myrmecophaga tridactyla*), from Parque Ecológico of State São Paulo, SP, Brazil. Fecal samples from the five individuals were sent to the Electron Microscopy Laboratory of the Biological Institute, São Paulo, SP, Brazil, for research on viral agents. The feces were watery to pasty and yellowish to greenish in color.

Negative staining technique (rapid preparation): The 5 fecal samples received were processed using the negative staining technique (rapid preparation) and subjected to examination using a Philips EM 208 transmission electron microscope.

In the negative staining technique, stool samples were suspended in phosphate buffer 0.1 M, pH 7.0. Drops of the obtained suspensions were placed in contact with metallic copper grids with carbono stabilized supporting film of 0.5% collodion in amyl acetate. Next, the grids were drained with filter paper and negatively stained at 2% ammonium molybdate, pH 5.0 [25].

III. RESULTS

Through the negative staining technique (rapid preparation) and under the transmission electron microscope Philips EM 208, it was possible to identify coronavírus like-particles, rounded or elongated, enveloped, with an average diameter of 140 nm, containing radial projections typical in the form of a solar corona, measuring 10 nm. (Fig.1 and Fig. 2).

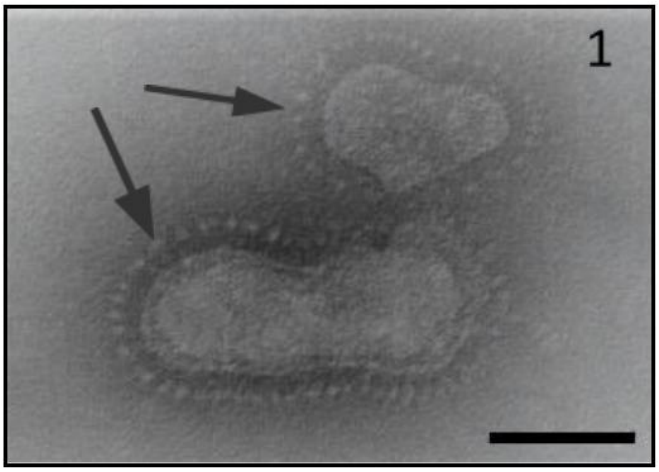


FIGURE 1: Negative staining of coronavirus particles in a suspension of giant anteater feces, rounded or elongated, pleomorphic, containing a characteristic envelope in the shape of a solar corona. Observe thin, wispy, and widely spaced spikes forming the envelope. Bar: 120 nm.

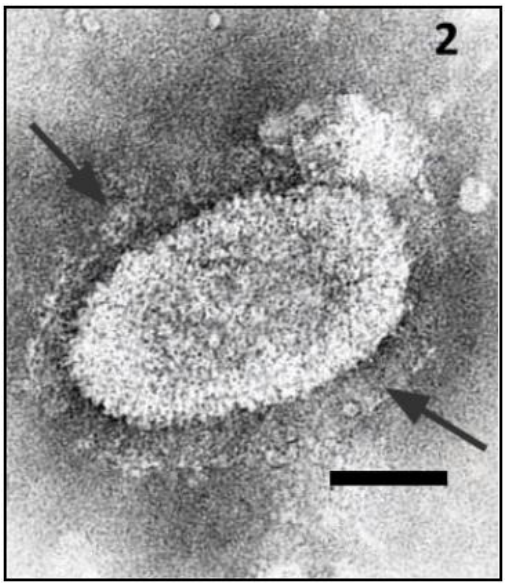


FIGURE 2: Coronavirus particle showing a characteristic envelope in the form of a solar corona or goblet (arrows), negatively contrasted with 2% ammonium molybdate. Bar: 50 nm.

IV. DISCUSSION

Observation under the transmission electron microscope, using the negative staining technique, clearly showed typical particles of coronavirus in the 5 fecal samples, confirming a small outbreak of infection among specimens of giant anteaters from Parque Ecológico of State São Paulo SP, Brazil.

The particles were pleomorphic, rounded to elongated, containing a characteristic envelope with typical radial projections in the shape of a solar corona and measuring an average of 140 nm in diameter.

Similar cases of outbreaks in wild animals with the presence of coronavirus particles have already been reported in Collared peccary [26]; Brocket deer [27], Greater rhea[28], White-lipped peccary[29], Golden-faced lion tamarin [30], Coatis [31], Montain lion [32], capybaras [33], pigeons [34], wild boar [35], peregrine falcon[36], ferrets [37], buffaloes [38].

The coronavirus has also been reported in other species of wild animals, such as dromedaries [39], rodents ([40]; bats [41], gorillas [42] and wild birds [43].

We observed that the spikes of the coronavirus particles in our study measured 10 nm long, as also described by [10] in human coronaviruses.

The negative staining technique is commonly used to detect coronaviruses in other animal species, such as swines[44], dogs, [45] bovines[46, 47] and equines[48], and in humans [10, 49].

The presence of yellowish or greenish diarrhea with watery to pasty consistency in the animals in our study was also reported in other coronavirus research in Brocket deer, Collared peccary, White-lipped peccary, Greater rhea, Golden-faced lion tamarin, dogs, bovines, wild boar, coati, Montain lion, capybaras, equines, ferrets, pigeon, buffaloes, swines, dogs, bovines and equines [26, 27, 28,29, 30, 45, 46,31,32, 47, 33, 34, 35, 36, 48, 37, 38,44].

New studies on the animal ability to be a reservoir for the coronavirus must be conducted to develop a more accurate viral origin [50].

Ecological assessments regarding the human-nature interrelationships should be studied, also addressing the interactions within breeding centers and efforts that allow a direct and indirect management with animals, since new infectious diseases can develop and lead to new worldwide damages, taking as an example the new coronavirus pandemic started in 2019 [51].

Integrative management together with surveillance planning programs for giant anteaters would reduce the occurrence of new outbreaks, avoiding economic and faunal losses [52].

Giant anteaters, like other mammals, have a sociable behavior with humans [21], therefore, it is extremely important for public health to better understand the aspects involved in possible contagions.

Considering that spillovers are a consequence of environmental degradation by human activity, studies must be instituted in order to understand how they arise and avoid new occurrences. Considering that bats and other wild animals act as host of several viral types, genetic and computational research must be developed for the previous knowledge of the viruses they carry, as well as the affinity of such viruses for the proteins of human cells, constituting an alert to avoid contagion and the prior establishment of prevention and treatment measures [53].

A better understanding of the animal's coronaviruses, their capacity for cross-species transmission, and the sharing of genetic information may facilitate improved prevention and control strategies for future emerging zoonotic coronaviruses [3].

The correct application of transmission electron microscopy techniques allows a quick clinical diagnosis, and, therefore, the elaboration of prophylactic and disease control measures, avoiding the rampant dissemination of viruses [54].

The use of the negative staining technique (rapid preparation), allowed to capture photographic images with greater focus, allowing to accurately and smoothly visualize the club-shaped surface projections, and can be widely used in the research of coronavirus particles in samples from different specimens.

Coronavirus has already been reported in different animals of the mammal class; however, this is the first occurrence in giant anteater (*Myrmecophaga tridactyla*).

REFERENCES

[1] Woo, P.C., Lau, S.K., Huang, Y.& Yuen, K.Y. (2009). Coronavirus diversity, phylogeny and, jumping. Experimental Biology and Medicine, 234:1117–1127.

[2] Dhama, K., Khan S., Tiwari, R., Sircar, S., Bhat, S., Malik, Y.S., Singh, K.P. Chaimcupa, W., Aldana, D.K.B., Morales, A.J.R. (2020). Coronavirus disease 2019-COVID-19. Clinical Microbiology Reviews, 33, 1-48.

[3] Haake, C., Cook, S., Pusterla, N., & Murphy, B. (2020). Coronavirus infections in companion animals: Virology, epidemiology, clinical and pathologic features. Viruses 12:1023.

[4] Mole, B. (2013). Deadly coronavirus found in bats. Nature News & Comments.

[5] Stevenson, G.W., Hoang, H., Schwartz, K.J., Burrough, E.R., Sun, D., Madson, D., Cooper, V.L., Pillatzki, A., Gauger, P., Schmitt, B.J., Koster, L.G., Killian, M.L.,& Yoon, K.J. (2013). Emergence of porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences. Jornal Veterinary Diagnostic Investigation, 25:649–654.

[6] Chen, Q., Li, G., Stasko, J., Thomas, J.T., Stensland, W.R, Pillatzki, A.E., Gauger, P.C., Schwartz, K.J., Madson, D., Yoon, K-J., Stevenson, G.W., Burrough, E.R., Harmon, K.M., Main, R.G. & Zhang, J. (2014). Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. Journal of Clinical Microbiology, 52:234–43.

[7] Bande, F., Arshad, S.S., Omar, A.R., Hair-Bejo, M., Mahmuda, A. & Venugopal Nair, V.(2017). Global distributions and strain diversity of avianinfectious bronchitis virus: a review. Animal Health Research Reviews,18(1): 70–83.

[8] Li, F. (2016). Structure, function, and evolution of coronavirus spike proteins.Annual of Review Virology, 3(1): 237–261.

[9] Decaro, N.& Lorusso, A. (2020). Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses.Veterinary. Microbiology,244,108693.

[10] Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G.F. & Tan, W. (2019). A novel coronavirus from patients with pneumonia in China. New England Journal of Medicine, 382:727–733.

[11] Fenner's, F. *Coronaviridae*. Veterinary Virology. MacLachlan, N.J. & Dubovi, E.J. (Eds). Elsevier, Amsterdam, The Netherlands, 2017. pp. 435–461.

[12] Malik, Y.A. (2020). Properties of coronavirus and SARS-CoV-2. Malaysian Journal of Pathology, 42(1): 3–11.

[13] Freundt, E.C., Yu, L., Park, E., Lenardo, M.J., Xu, X.-N. (2009). Molecular Determinants for subcellular localization of the severe acute Respiratory syndrome coronavirus open reading frame 3b protein. Journal of Virology,83: 6631–6640.

[14] Imbert, I., Snijder, E.J., Dimitrova, M., Guillemot, J.-C., Lécine, P.& Canard, B. (2008). The SARS-Coronavirus PLncdomain of nsp3 as a replication/transcription sca_olding protein. Virus Research, 133:136–148.

[15] Zhou, P., Fan, H., Lan, T., Yang, X.L., Shi, W.F., Zhang, W., Zhu, Y., Zhang, Y.W., Xie, Q.M., Mani, S.; et al. (2018). Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. Nature, 556:255–259.

[16] Wang, Q., Vlasova, A.N., Kenney, S.P. & Saif, L.J. (2019). Emerging and re-emerging coronaviruses in pigs. Current Opinion Virology, 34:39–49.

[17] Oma V.S., Travén M., Alenius S., Myrmel M. & Stokstad M.(2016).Bovinecoronavirus in naturally and experimentally exposed calves;viral shedding and the potential for transmission, Virology Journal, 13(100):1-11.

[18] Guo, M., Tao, W., Flavell, R.A., & Zhu, S. (2021). Potential intestinal infection and faecal–oral transmission of SARS-CoV-2. Nature Reviews Gastroenterology & Hepatology, 18:269–283.

[19] Boileau, M.J. & Kapil, S. (2010). Bovine coronavirus associated syndromes. Veterinary clinics: Food Animal Practice. 26:123–146.

[20] Takiuchi, E., Barry, A.F., Alfieri, A.F., Filippsen, P. & Alfieri, A.A. (2009). An outbreak of winter dysentery caused by bovine coronavirus in a high production dairy cattle herd from a tropical country. Brazilian Archives of Biology and Technology, 52:57-61.

[21] Braga, F.G., Santos, R.E.F., & Batista, A.C. (2010). Marking behavior of the giant anteater *Myrmecophaga tridactyla* (Mammalia: Myrmecophagidae) in Southern Brazil. Zoologia, 27 (1): 7–12.

[22] Miranda, F., Bertassoni, A. & Abba,A.M. (2014). *Myrmecophaga tridactyla*. The IUCN Red List ofThreatened Species, 2014. Available in:<https://www.iucnredlist.org/species/14224/47441961>. Accessed in: January 7, 2021.

[23] Wu, D., Wu, T., Liu, Q. & Yang, Z. (2020). The SARS-CoV-2outbreak: what we know.International Journal of InfectiousDiseases, 94:44-48.

[24] Zhang, Y., Hung, T., Song, J.D. & He, J.S. (2013). Electron microscopy: Essentials for viral structure, morphogenesis and rapid diagnosis. Science China Life Science Journal, 56:421-430.

[25] Brenner, S. & Horne, R. W. (1959). A negative staining method for high resolution electron microscopy of viruses. Biochemical Biophysical Acta, 34:103.

[26] Catroxo, M.H.B., Miranda, L.B., Lavorenti, A., Cappellaro, C.E.M.P.D.M., Mueller, S.B. Detection under transmission electron microscopy of coronavirus-like particles in feces of Collared peccary (*Tayassu tajacu*). In: 4 th Congresso of the Zoological Society of São Paulo (SPZ), 1995a. Santos, SP, Brazil.

[27] Catroxo, M.H.B., Castro, M.S.; Silva, J.C.R., Cappellaro, C.E.M.P.D.M. Visualization of coronavírus-like particles in faeces of Brocket deer (*Mazama gouazoubira*). In: 5 th Virology, 1995b. Ribeirão Preto, SP, Brazil, p.B-05.

[28] Catroxo, M.H.B., Ito, N.M.K., Miranda, L.B., Miyaje, C.I., Cappellaro, C.E.M.P.D.M., Andrade, P.C.M., Sanchez, M.E. Presence of coronavirus-like particles in feces of Greater rhea (*Rhea americana*) with diarrhea. In: 9th Annual Meeting of the Biological Institute, 1996a. São Paulo, SP, Brazil. Arquivos do Instituto Biológico, 63 (Supl.):24.

[29] Catroxo, M.H.B., Miranda, L.B.,Lavorenti, A., Cappellaro,C.E.M.P.D., Mueller, S.B.K.Determination under the transmission electron microscopy of coronavirus-like particles in feces of White-lipped peccary (*Tayassu pecari*). In: XV Pan American Congresso of Veterinay Sciences (PANVET), 1996b, Campo Grande, MS, Brazil, Summary in the proceedings of the event, Campo Grande, MS, Brazil, 1996, p.73.

[30] Catroxo, M.H.B, Patricio, F.D.,Schmich, H. Detection of coronavirus-like particles in faeces of golden-faced lion tamarin (*Leontopithecus chrysomelas*)., 1997.São Paulo, SP, Brazil. In: 10 th Annual Meeting of the Biological Institute, 1997. Arquivos do Instituto Biológico, 64(Supl.):27.

[31] Catroxo, M.H.B.; Godoy, S.N.;Milanelo, L.; Curi, N. A. Detection of coronavirus-like particles in the small intestine of coati (*Nosua nosua*) by transmission electron microscopy. In: 16 th Annual Meeting of the Biological Institute, 2003, São Paulo, SP., Brazil. Arquivos do Instituto Biológico, 70(Supl.3):544-548.

[32] Catroxo, M.H.B., Pongiluppi, T., Godoy, S.N., Milanelo, L., Ferreira, J.P.N., Curi, N.A. Morphological detection of the association of coronavirus and astrovirus in feces feline (Montain lion-*Felis concolor*) with diarrhea. In: 17th Annual Meeting of the Biological Institute, 2004. São Paulo, SP, Brazil. Arquivos do Instituto Biológico, 71(Supl.):238-241.

[33] Catroxo, M. H. B., Miranda,L.B., Lavorenti, A., Petrella,S., Melo, N. A., Martins, A. M. C. P.R. F. (2010). Detection of coronavirus in capybaras (*Hydrochoeris hydrochaeris*) by transmission electron microscopy in São Paulo, Brazil. International Journal of Morphology, 28(2):549-555.

[34] Catroxo, M.H.B., Martins, A.M.C.R.P.F., Petrella, S. Curi, N.A., Melo, N.A. (2011). Research of viral agent in free-living pigeon feces (*Columba livia*) in the city of São Paulo, SP, Brazil, for transmission electron microscopy. International Journal of Morphology, 29(2):628-635.

[35] Bersano, J.G., Catroxo, M.H.B., Villalobos, E.M.C. & Curi, N.A. Detection of coronavirus-like particles in an outbreak of wild boar enteritis in the State of São Paulo, SP, Brazil. In: 14 th Annual Meeting of the Biological Institute, 2001. São Paulo, SP, Brazil. O Biológico, 68(Supl.). p.132.

[36] Pongiluppi, T., Catroxo, M.H.B., Godoy, S.N., Milanelo, L., Ferreira, J.P.N. & Curi, N.A. Detection of coronavirus-like particles in feces of Peregrine falcon (*Falcon peregrinus*) by transmission electron microscopy. In: 2 th Congress of Scientific Initiation Agrarian, Biological and Environmental Sciences, 2004. São Paulo, SP, Brazil. O Biológico, 69(2):73.

[37] Gregori, F., Catroxo, M.H.B., Lopes, V.S., Ruiz, V.L.A., Brandão, P.E. (2010). Occurrence of ferret enteric coronavirus in Brazil (preliminary report). Brazilian Journal Veterinary Research Animal Science, 47(2):156-158.

[38] Martins, A.M.C.R.P.F., Catroxo, M. H.B. & Petrella, S. Detection ofcoronavirus in buffaloes (*Bubalus bubalis*) by transmission electronmicroscopy. XXVII World BuiatricsCongress, 2012. Lisboa, Portugal, p.249.

[39] Corman, V.M., Ecklerle, I., Memish,Z.A., Lijander, A.M., Dijkman, R., Jonsdottir, H., Ngeiywa, K.J.Z.J., Kamau, E. Younan, M.; Masri, M.A., Assiri, A., Gluecks, I., Musa, B.E., Meyer, B., Müller, M.A., Hilali, M., Bornstein, S., Werery, U., Thiel, V., Jores, J., Drexler, J.F., Drosten, C. (2016). Link of a ubiquitous human coronavirus to dromedary camels. Proceedings of the National Academy of Sciences, 113(35):1-6.

[40] Corman, V.M., Muth, D., Niemeyer, D., Drosten, C. (2018). Hosts and sources of endemic human coronaviruses. Advances in virus research, 100:163-188.

[41] Zi-Wei, Y., Yuan, S., Yuen, K.-S., Fung, S.-Y., Chan, C.-P. & Jin, D.-Y. (2020). Zoonotic origins of human coronaviruses. International Journal of Biological Sciences, 16(10):1686-1697.

[42] San Diego Zoo. Gorilla troop at the SanDiego Zoo Safari Park test positivefor COVID-19. 2021. Disponible in:https://www.reuters.com/business/healthcare-pharmaceuticals/two-gorillas-san-diego-zoo-test-positive-covid-19-2021-01-Accessed in: Jan 15, 2021.

[43] Montassier, M.F.S., Teixeira, F.C., Barboza, K.B., Werther, K., Ávila, F.A. &Montassier, H.J. (2013). Detection of coronavirus in wild birds in the northeastern region of the State of São Paulo, Brazil, by molecular techniques of RT-PCR and Nested PCR. ARS Veterinaria, 29(4):13.

[44] Martins, A.M.C.R.P.F., Bersano, J.G., Ogata, R., Amante, G., Nastari, B.D.B. & Catroxo, M.H.B. (2013). Diagnosis to detect porcine transmissible gastroenteritis virus (TGEV) by optical and transmission electron microscopy techniques.International Journal of Morphology, 31(2):706-715.

[45] Catroxo, M.H.B., Lima, J.L.L., Schmich, H., Curi, N.A. Detection of coronavirus-like particles by transmission electron microscopy, in feces of dogs during gastroenteritis outbreak. In: 11 th Annual Meeting of the Biological Institute, 1998. São Paulo, SP, Brazil. Arquivos do Instituto Biológico, 65(Supl.):98.

[46] Catroxo, M.H.B. & Curi, N.A. Detection of coronavirus-like particles by transmission electron microscopy in bovine feces during an outbreak of enteritis. In: 12 th Annual Meeting of the Biological Institute, 1999. São Paulo, SP, Brazil, Brazil. Arquivos do Instituto Biológico, 66(Supl.):45.

[47] Catroxo, M.H.B., Gregori, F., Brandão, P.E., Melo, N.A. Detection of rota and coronavirus in calves during an outbreak of diarrhea in the city of Dourados, Mato Grosso do Sul, Brazil. In: 20 th Annual Meeting of the Biological Institute, 2007. São Paulo, SP, Brazil. O Biológico, 69(2):114.

[48] Lopes, V.S., Brandão, P.E., Catroxo, M.H.B., Ruiz, V.L.A., Gregori, F. Occurrence of coronavírus in equines in the city of São Paulo, SP, Brazil. In: VI th Congresso of Scientific Initiation in Agrarian, Biological and Environmental Sciences, 2008. São Paulo, SP, Brazil. O Biológico, 70(1):18.

[49] Prasad, S., Potdar, V.,Cherian, S., Abraham, P. &Basu, A. (2020). Transmission electron microscopy imaging of SARS-CoV-2**.** Indian Journal Medical Research, 151(2-3):241-243.

[50] WSAVA. World Small AnimalVeterinary Association. The New Coronavirus and Companion Animals. (2020). Disponible in: <https://www.crmvpr.org.br/uploads/noticia/arquivos/COVID-19_WSAVA-AdvisoryDocument-Feb-29-2020-Portuguese.pdf> Acessed in:january 10, 2021.

[51] Acosta, A.L., Xavier, F., Chaves, L.S.M., Sabino, E.C., Saraiva, A.M. & Sallum,M.A.M. (2020). Interfaces to coronavirus transmission and spillover between forests and cities.Estudos Avançados, 34(99):191-207.

[52] WHO. World Health Organization. The control of neglected zoonotic diseases. From advocacy to action. In: Report of the fourth international meeting held at WHO, 2014. Headquarters, Geneva, Switzerland, p.1-48.

[53] Arruda, E. (2021). Eurico Arruda: An admirer of viruses. Revista Fapesp, ed. 301, março 2021. Available in: https://revistapesquisa.fapesp.br/eurico-arruda-um-admirador-dos-virus/.

[54] Popov, V.L., Tesh, R.B., Weaver, S.C. & Vasilakis, N. (2019). Electron microscopy in discovery and emerging viruses from collection of the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA). Viruses, 11(477):1-17

Impact of Ginger Enrishment on Biochemical Characteristics of Tisane from *Aloysia Citrodora* Leaves, Cultivated at a small scale in the Area of Man (West Region of Côte d'Ivoire)

Mohamed Anderson YEO[1*], Lêniféré Chantal SORO[1], Ernest Kouadio KOFFI[2], Lacina COULIBALY[3]

[1]Departement of Agronomic, Forestry and Environmental Engineering, University of Man (BP 20 Man), Côte d'Ivoire
[2]Laboratory of Biochemistry and Food Science, Department of Bioscience, University of Félix Houphouët Boigny of Cocody, Abidjan, Côte d'Ivoire.
[3]Departement of Environmental Science and Management, University of Nangui Abrogoua 02 B.P. 802 Abidjan, Côte d'Ivoire
*Corresponding Author: Mohamed Anderson YEO, phone: (+225) 07 09 12 42 08

Received:- 28 July 2021/ Revised:- 06 August 2021/ Accepted:- 12 August 2021/ Published: 31-08-2021

Abstract— *This study confirm one of the official missions attributed to the University of Man, those to enhance natural's resources of the region of Man and its properties. The assessment of the biochemical characterization of a tisane from Aloysia citrodora's leaves enriched at ginger has been conducted.*

To reach this goal, tisane has been prepared with Aloysia Citrodora. An aqueous extract of Zingiber rhizomes has been also produced. Ginger extract incorporated in tisane of Aloysia citrodora at 2.5 % level. The results have shown that many studied parameters increased highly. The dry matter increased from 88.92±3.92% to 90.07±2.91%. Incorporated ginger in tisane of Aloysia citrodora improved total phenolic content, antioxidant capacity, insoluble solids. Total amount of phenolic compounds was 11.68±4.05mg GAE/g and it's increased to 15.90±0.42 mg GAE /g. Antioxidant activity of this enriched tisane was also 3.96±1.58 µM Trolox Eq / Kg for the ABTS method. The analysis concerning mineral content of obtained tisane has noted a high content particularly those of Calcium. Its content were three time (535 130.4 ppm) important in enriched ginger tisane at 2.5% than Aloysia Citrodora's tisane (192888.9 ppm).

Keywords— Aloysiacitriodora, tisane, Zingiber officinale, Man, Côte d'Ivoire.

I. INTRODUCTION

Nowadays, several aspects of research data point out that specific dietary compound act like preventive agent against some diseases like cardiovascular disorders, some types of cancer, inflammatory circumstances, and obesity. Functional foods, which have specific physiological benefits, contain bioactive ingredients recognize as prebiotics, probiotics, flavonoids, phenolic compounds, phytosterols, bioactive peptides (Arvanitoyannis, 2005).

Aloysia citrodora belongs to the family Verbenaceae and has several other synonyms for the scientific name including *A. triphylla* (L'Hér.) Britton, *A. citridora* Paláu, *A. citriodora* Paláu, *Lippia citriodora* Kunth, *L. citrodora* Kunth, and *L. triphylla* (L'Hér.) (Somanchi *et al*. 2017).

The plant is a perennial shrub with up to 3 m height, striate and scabrous branches, and lanceolate 7-10 cm margined leaves with short petioles. The tiny flowers have white or light blue color which appears on a hairy calyx with four tips in the panicle-like spikes. The petals form 4-5 mm funnel at the base which covers 2 long and 2 short stamens (Vitali *et al*. 2009).

This plant has a wide geographical distribution from South America to North Africa and South of Europe. It should be mentioned that due to the pleasant lemony fragrance and its application in food industries and cosmetics, as well as its use as a home remedy for several health problems, the plant is currently available in other parts of the world, as well (Carnat *et al*. 1999). One of these species is Erica arborea.

Aloysia Citrodora leaves contains several phytochemicals, which show high antioxidant activity, and may be the cause of several health benefits such as a hypotensor, diuretic. Several parts of this herb are preferred in various regions as a urinary antiseptic, and against constipation (Pascual *et al.* 2001).

Also, the processed leaves are used in tea manufacturing and as an ingredient of alcoholic or non-alcoholic herbal drinks. The plant "cedrón" is also included in the Código Alimentario Argentino (CAA) as a corrective and coadjutant, in the section referred to vegetal condiments (Código Alimentario Argentino) (Ghédira, 2017). Tisane is not only a source of water (Goetz, 2004). According to all kind properties of *Aloysia Citrodora* and the goal to preserve the health of the people of « tonpki »'s region, the polytechnic University of Man has planned to describe different tisanes from local plant or plant from another area.

The present investigation is undertaken to evaluate biochemical properties, antioxidant activity and sensory evaluation of *Zingiber officinale* enriched tisane from *Aloysia citrodora* leaves, cultivated at a small scale in the west region (Man) of Côte d'Ivoire. To reach the objectives, we must:

- Prepare the leaves of *Aloysia citrodora.*
- Produce the tisane from *Aloysia citrodora'*s leaves.
- Extract the juice of Zingiber officinale.
- Assess biochemical, microbiological load and of the resulting tisane.
- Evaluate sensory evaluation.

II. MATERIALS AND METHODS

2.1 Materials

Dried leaves of *Aloysia Citrodora* and fresh ginger rhizomes were the materials of this study. The fresh leaves were harvested from those which were cultivated on 600 m^2 space in the village called Kouitongouiné in the department of Gbangbégouiné Yati, the region of Man (Côte d'Ivoire). The camp is 4 km from Man's University. Fresh ginger rhizomes were obtained from local « Ermankono » market (Côte d'Ivoire).

2.2 Methods

2.2.1 Preparation of *Aloysia citrodora's* leaves

Fresh leaves were harvested and quickly send to the laboratory. The leaves were manually cleaned to remove stones and other unwanted debris, washed under tap water to remove dust, dirt and adhered material. They were also washing thoroughly in sterile de-ionized water and 1% salted water during 1 to 5 minutes to remove germs. They are rinsed again after the previous step (De Saint Sauveur, 2010). The sample was placed in a nettle for 15 minutes. The fleshed leaves were dried at room temperature without ligth (Bidima, 2016).

2.2.2 Production of Herbal Tea Beverage

The herbal tea beverage was prepared according to Felgines *et al.* (2014). 40 g of dried leaves of *A. citrodora* were soaked in 1L boiling water for 30 min. the infusions leaves were liquated and keep at -20°C before different analysis.

2.2.3 Production of aqueous extract of ginger

Clean fresh ginger rhizomes were chopped to small pieces using a knife by cutting cross with a thickness. Furthermore, the rhizomes were put into 50 mL of de-ionized water and the overall were grounded by using laboratory scale blender (Kenwood blender) until to get a fine extract. 100 mL of water was added to the sample of ginger, furthermore, filtration using filter paper was conducted to separate filtrate / extract from the solid waste. Ginger extracts were stored in sterile dark amber bottles at 5°C (Yeo *et al.* 2014).

2.3 Biochemical characterization

- pH was determined with a pH meter (Testo 230 type 4, France) (Guerra and Mujica 2010).
- Dry matters were determined following the methods of Official AOAC (AOAC, 1990).

- Ash was determined following the methods of Official BIPEA (BIPEA, 1976).
- Insoluble Solids quantified by Weende's method (Multon, 1991).
- Fat acid was determined by extraction with soxhlet (AOAC, 1975).
- Total sugar and Reducing sugar, content of was measured following the methods described by the official AOAC (AOAC, 1995).
- The total phenol content of the solution was determined spectrophotometrically using Folin-Ciocalteu's method described by Singleton et al. Rossi (1965) modified by Wood *et al.* (2002).
- Antioxidant Activity was evaluated as ABTS according the method described by par Choong *et al.* (2013).
- Micronutrients such as : Phosphorus (P), Potassium (K), Zinc (Zn), Calcium (Ca), Iron (Fe) and Iod (I) by fluorescence X, following the method described by Sawadogo *et al.* (2014).

2.4 Microbiological analysis

Microbial analysis was carried out on the tisane control and tisane of Ginger (*Zingiber officinale)* and *Aloysia Citrodora*. Different dilutions have been conducted according to NF ISO 7218 (AFNOR. 1996).

2.4.1 Total viable count

Microbial count was carried out using plate count agar (PCA) for total viable (TVC), incubated at 30 °C for 72h. The method used was described by AFNOR rules NF V 08-051 (AFNOR, 1999).

2.4.2 Fungal count

Potato dextrose agar (PDA) was those used for fungal count and incubated at 37°C overnight and enumerated while PDA was incubated for 48 h according to NF-V08-022 rules.

2.4.3 Enumeration of indicator bacteria, contamination

2.4.3.1 Staphylococcus aureus.

Mannitol salt agar (MSA) for staphylococci count was used by modified AFNOR V-08-014 method.

2.4.3.2 Thermotolerant Coliform and Escherichia coli

The count of thermotolerant coliforms was done by AFNOR (1974), by AFNOR V-08-013 and sulfite-reducing anaerobes by NF EN 15213.

2.5 Sensory Attributes

Characterizations evaluated for sensory attributes were color, texture, hardness, chewiness, sweetness, saltiness, pleasantness, spicy and overall acceptance by unstructured scaling method or quantitative descriptive analysis (QDA) (Stone *et al*. 1974). The panel consisted of 17 people comprising both male and female, often participated in sensory evaluation. The descriptors of sensory attributes explained orally to the panelists. The scorecard consisted of a scale from 0 to 10. Panelists asked to record each evaluation, by marking it (0 to 10) according to their intensity or perception of the magnitude of each attribute.

2.6 Statistical Analysis

All the experiments were carried out in triplicates (n = 3) and the results expressed as mean ± standard deviation and percentage (SD) using Microsoft Excel software. Post hoc comparisons made by least significant difference (LSD). IBM SPSS Statistical software version 20.0 used to analyze the results. The comparisons of mean were made by the analysis of variances (ANOVA) at 0.05 significance level.

III. RESULTS

3.1 Biochemical properties of Ginger (*Zingiber officinale*), control tisane of *Aloysia citrodora* leaves (T0) and enriched tisane of *Aloysia citrodora* at 2.5% *Zingiber officinale* (T2.5)

The results of physical properties like pH, color, dry matter, turbidity, filterability, insoluble solids, water activity and ash content of Z. officinale enriched jaggery are represented in Table 1.

It was noted an improvement of the studied parameters of tisane enriched by ginger. This improvement concerned dry matter content which was 88.92±3.92% with control tisane (T0) increased to reach 90.07±2.91% with tisane enriched at 2.5% ginger.

Results have also shown increased phenolic content, crude fiber and antioxidant capacity in enriched tisane compared to it respective control. Its where respectively 11.68±4.05mg gallic Acid Eq /g, 5.12±2.7% et 3.96±1.58 µM Trolox Eq / Kg versus 15.90±0.42 mg Gallic Acid Eq /g, 3.07±0.40% et 6.06±0.51 µM Eq Trolox/ Kg.

TABLE 1:
BIOCHEMICAL PROPERTIES OF GINGER (*ZINGIBER OFFICINALE*), CONTROL TISANE OF ALOYSIA CITRODORA LEAVES (T0) AND ENRICHED TISANE OF *ALOYSIA CITRODORA* AT 2.5% *OFFICINALE* (T2.5) *ZINGIBER*

Parameters	Solution of *Zingiber officinale*	Control Tisane of *Aloysia citrodora* (T0)	Enriched tisane of *Aloysia citrodora* at 2,5% *Zingiber officinale* (T2.5)
pH	5.94±0.07a	6.9±2.17b	6.04±0.17c
Dry matter (%)	19.07±6.22a	88.92±3.92b	90.07±2.91c
Total phenol content (mg GAE* /g)	5.91±0.32a	11.68±4.05b	15.90±0.42c
Total sugar (mg equivalent glucose/g)	1.20±0.12a	47.78±3.06b	48.50±4.22c
Reducing sugar (mg equivalent glucose/g)	0.08±0.00a	0.10±0.03a	0.11±0.00a
Fat (%)	10.63±2.58a	13.13±3.72b	12.67±3.68c
Ash (%)	3.57±0.05a	1.64±1.29c	3.07±0.40b
Insoluble solids (%)	2.02±0,02a	5.12±2.70b	6.07±0.02c
Antioxidant Activity (µM Eq Trolox/ Kg)	5.01±0,01a	3.96±1.58b	6.06±0.51c

**** GAE: Gallic acid equivalent***

3.2 Minerals assessment

Significant results were noticed for the mineral analysis of ginger enriched tisane as depicted in table 2.

The controlled sample exhibited lower values for all the minerals as presented in table 2. The level of Ca particularly increased three times in enriched ginger tisane compared to the control tisane (192888.9 ppm vs 535 130.4 ppm). The observation was the same concerning Zn, the content was the twice of those of the control tisane (991.2 ppm vs 1920.6 ppm). The level of phosphorus was also increased 9 times in the enriched ginger tisane.

TABLE 2
MINERALS COMPOSITION OF CONTROL TISANE OF *ALOYSIA CITRODORA* LEAVES (T0) AND ENRICHED TISANE OF *ALOYSIA CITRODORA* AT 2.5% *ZINGIBER OFFICINALE* (T2.5)

Parameters	Composition (ppm)	
	T0	**T2.5**
P	10.7	98,58
K	15977.0	247730.7
Zn	991.2	1920.6
Ca	192888.9	535130.4
Fe	50062.5	6641.7
I	9.18	9,8

3.3 Microbial quality of different tisane samples

Results of the microbial load of tisane of Aloysia citrodora with or without ginger were presented in Table 3. A kind of reduction in microbial load was also found for each sample for yeast, fungus and coliform (Table 3). Coliform load was not found in all samples. Fecal coliform is very harmful to human health and in this study fecal confirmation test was done and fecal coliform was not observed. The same results were found for staphyloccocus aureus, Sulfite-reducing Anaerobes, Salmonella spp, Yeasts and moulds. So, it can postulate that properly boiled tea is safe to drink for human health.

TABLE 3
MICROBIALS LOAD OF CONTROL TISANE OF *ALOYSIA CITRODORA* LEAVES (T0) AND ENRICHED TISANE OF *ALOYSIA CITRODORA* AT 2.5% *ZINGIBER OFFICINALE* (T2.5)

Microbial load (CFU)	Samples		
	T0	T2.5	E U criteria 2005
Total viable count 30°C	< 10cfu/ml	< 10cfu/ml	
Total Coliforms /g	No growth	No growth	10 ufc/ml
Thermotolerant coliforms	No growth	No growth	10 ufc/ml
Staphylococcus aureus /g	No growth	No growth	No growth
Sulfite-reducing Anaerobes	No growth	No growth	No growth
Salmonella spp	No growth	No growth	No growth
Yeasts and moulds	No growth	No growth	No growth

CFU* = Colony forming unit

3.4 Sensory Attributes of control tisane of *Aloysia citrodora* leaves (T0) and enriched tisane of *Aloysia citrodora* at 2.5% *Zingiber officinale* (T2.5)

Sensory attributes such as color, texture, appearane, taste, Aroma and overall acceptance of control tisane and tisane of *Aloysia citrodora* at 2.5% *Zingiber officinale* (T2.5) were evaluated by a quantitative descriptive analysis method (Figure 1).

Concerning the color, the score obtained was respectively 6.8 and 6.7 for the T2.5 and T0. There was no difference in taste among T2.5 and T0 (5 vs 5.1). But it has been noted another trend about texture and appearance. Texture's score of T0 was 3.6 but 4.4 for T2.5. Those of appearance has increased from 3.4 (T0) to reach 4.5 (T2.5: tisane with incorporate ginger), it was an improvement of appearance.

The result has showed that aroma has changed positively. The score was 4 for control tisane and 6 for tisane T2.5

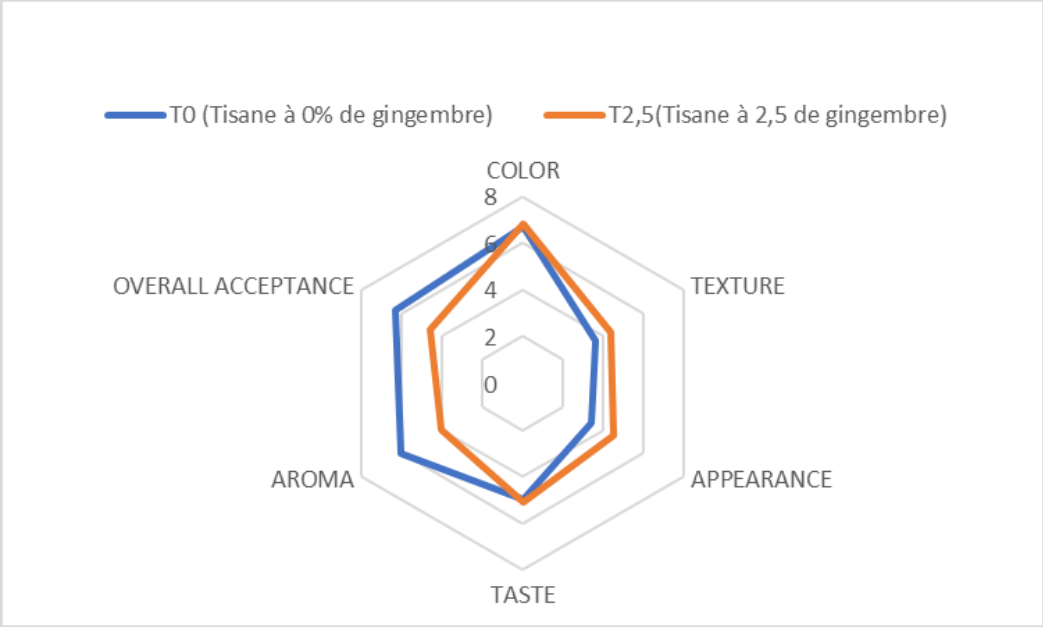


FIGURE 1: Star diagram of sensory attributes of control tisane and enriched tisane at 2.5 % Ginger (*Z. officinale*) Enriched

IV. DISCUSSION

Since a long time, Aloysia citrodora is a medicinal plant which is used by native people for several indications such as asthma, flu, diarrhea, flatulence, insomnia, and rheumatism (Abuhamdah *et al.* Mohammed, 2013). Lemon verbena is also used in the treatment of nervous condition, tachycardia, migraine headache (Pascual *et al.* 2001). According to Yousef zadeh *et al.* Meshkatalsadat, (2013) and Abdi and co-workers (2020), it's also used to treat hyperglycemia and certain cancers.

The high antioxidant activity of *Aloysia citriodoara* tisane would be due to the trapping activity of the superoxide radical and a moderate activity vis à vis to the hydroxyl radical and hypochlorous acid (Valentao *et al.*, 2002).

Add aqueous extract of *Zingiber officinale* at the tisane from *Aloysia citriodora* improved the phenol content of the obtained tisane. Lenoir (2011) showed that Polyphenols enhance total oxidant-scavenging. Indeed, during the inflammation, hydroxyl radical increased in tissues and plasma. The study done on mice, have indicated that, increasing of hydroxyl radical was reduced among them by using luteonin (Ashok kumar *et al.*, 2008). Phenolic compounds have been widely studies not only for its antioxydants properties but also for its ability to improve Cellular Antioxidant Defense. Furthermore, the study conducted among human has shown that tisane at 2.5% of ginger did not presented an anxiety and sedative effect mechanistically (Marty 2017).

V. CONCLUSION

This study confirms one of the official missions attributed to the University of Man. One of the goals is to generate an innovative beverage by local products from the region or not. The study consist to formulate and analyse the herbal infusions prepared from formulations of indigenous herbs (*Aloysia Citrodora*) and spices (*Zingiber officinale*) and to analyse for its biochemical, nutrient content and evaluate sensoials properties.

At the end of this study, the results have showed that the formulate beverage which have high amounts of bioavailable bioactive compounds and mineral contents. The elemental content of tea beverage was found to be high for Calcium and Zinc. Microbial load were found in acceptable limit. The sensory qualities were better with incorporate aqueous extract of ginger.

REFERENCES

[1] Abdi, I., Lahouel, N., Lebiod, B., and Hireche, S. E. (2020). Activité anti-inflammatoire *d'Aloysiacitriodora* (Doctoral dissertation, Université de Jijel).

[2] Aggarwal BB et Shishodia S, 2006. Molecular tragets of dietary agents for prevention and therapy of cancer. *Biochemical Pharmacology*, 71: 1397-1421.

[3] Abuhamdah R et Mohammed A, 2013. Chemical, molecular pharmacology and neuroprotective properties of the essential oil derived from *Aloysiacitrodora* Palau: Durham University.

[4] Arvanitoyannis I. S., 2005.The Nutritional Trace Metals. International Journal of Food Science Technology: 1, 3–12.

[5] Ashokkumar, P. and G. Sudhandiran (2008). "Protective role of luteolin on the status of lipid peroxidation and antioxidant defense against azoxymethane-induced experimental colon carcinogenesis." *BiomedPharmacother*62: 590-597.

[6] Bayala, B. (2014). Etude des propriétés anti-oxydantes, anti-inflammatoires, anti-prolifératives et anti-migratoires des huiles essentielles de quelques plantes médicinales du Burkina Faso sur des lignées cellulaires du cancer de la prostate et de glioblastomes (Doctoral dissertation, Université Blaise Pascal-Clermont-Ferrand II; Université de Ouagadougou (Burkina-Faso)).

[7] Bordjihane, Y., Brahmi, F. E., and Bouaziz, S. (2018). Enrichissement d'un produit laitier par des antioxydants d'origine naturelle.Cuisine africaine. Jus de gingembre et ses bienfaits. (En ligne) disponible sur : http://www.recettesafricaine.com/jus-de-gingembre.html (page consultée le 20/04/2021)

[8] CarnatA., Carnat A.P, Fraisse D, LLamaison J., 1999.The aromatic and polyphenolic composition of lemon verbena tea. Fitoterapia, 70, 1, 44-49.

[9] Felgines, C., Fraisse, D., Besson, C., Vasson, M. P., and Texier, O. (2014). Bioavailability of lemon verbena (*Aloysiatriphylla*) polyphenols in rats: Impact of colonic inflammation. *British Journal of Nutrition*, 111(10), 1773-1781.

[10] Goetz, P. (2004). Plaidoyer pour la tisane médicinale. *Phytothérapie*, *2*(1), 8-15.

[11] Konan, J. L. K., Turquin, L., Attah, H., Yao-Kouame, A., Allou, K., and Ake, S. (2010). *Lippiamultiflora* (verbenaceae) en Côte d'Ivoire: point des premiers résultats de recherche et Enjeu cultural. *Agronomie Africaine*, *22*(2).

[12] Lenoir, L. (2011). Effet protecteur des polyphénols de la verveine odorante dans un modèle d'inflammation colique chez le rat (Doctoral dissertation, Université d'Auvergne-Clermont-Ferrand I).

[13] Marty, M. (2017). Les MICI: enquête auprès des patients sur l'utilisation des thérapies complémentaires (Doctoral dissertation, Université Toulouse III-Paul Sabatier).

[14] Pascual M, Slowing K, Carretero E, Mata DS et Villar A, 2001. Lippia: traditional uses, chemistry and pharmacology: a review. *Journal of ethnopharmacology*, 76(3): 201-214.

[15] Saidi, S. Etude de l'effet antioxydant des huiles essentielles de*Lippiaciiriodora*de la région de Tlemcen (Doctoral dissertation).

[16] Sawadogo J. Nisol B. Bougouma M. Ramde T. Boubie G., Buess-Hermann C. and Legma JB. 2014. Caractérisations chimiques des ustensiles de cuisine artisanale en aluminium fabriqués au Burkina Faso: cas de Ouagadougou. *International Journal of Biological and Chemical Sciences*, *8*(6), 2813-2827

[17] SomanchiMeena, Katherine Phillips, Ermias Haile, Pamela Pehrsson, 2017. *Aloysiacitrodora* Paláu (Lemon verbena): A review of phytochemistry and pharmacology. Journal of Ethnopharmacology, 222, 10, 34-51

[18] Soro, L. C., Munier, S., Pelissier, Y., Grosmaire, L., Yada, R., Kitts, D., ... and Poucheret, P. (2016). Influence of geography, seasons and pedology on chemical composition and anti-inflammatory activities of essential oils from *Lippia multiflora* Mold leaves. *Journal of ethnopharmacology*, *194*, 587-594.

[19] Valentao P, Fernandes E, Carvaiho F, Andrade PB, Seabra RM and De Lourdes Basto M, 2002. Studies on the antioxidant activity of *Lippia citriodora* infusion scavenging effect on superoxide radical, hydroxyl radical and hypochiorous acid. *Biological and Pharmaceutical Bulletin*, 25(10): 1324-1327.

[20] Vitali D, Dragojević IV, Šebečić B,2009.Effects of incorporation of integral raw materials and dietary fibre on the selected nutritional and functional properties of biscuits. Food Chemistry, 114, 4: 1462-1469.

[21] Yéo Mohamed Anderson*, Chatigre O., Elleingand E., Koni Pascal and Koffi Ernest. 2014 Analysis of quality attributes of banana drinks blended with aqueous extracts of ginger. *African Journal of Food Science Volume* 8 (5), pp. 260 -263

[22] Yousefzadeh N and MeshkatalsadatMh, 2013. Quantitative and qualitative study of bioactive compounds of essential oils of plant Lippia citriodora by use of GC-MS technique. Journal of Novel Applied Sciences, 2(2S): 964-968.

Economic Analysis of Costs and Returns of Vitamin A Cassava Production in Anambra State, Nigeria, West Africa

Umebali, E.E[1]; Nkamigbo, D.C[2*]; Ekpunobi, C.E[3]; Isibor, A.C.[4]

Department of Agricultural Economics and Extention, Faculty og Agriculture, Nnamdi Azikiwe University, Awka, Nigeria, West Africa
*Corresponding Author

Received:- 05 August 2021/ Revised:- 17 August 2021/ Accepted:- 24 August 2021/ Published: 31-08-2021

Abstract— *The study investigated the costs and returns of vitamin A cassava production in Anambra State, Nigeria. Multi-stage and simple random techniques were adopted in selecting one hundred and thirty eight respondents for the study. Data were collected using well structured questionnaire and analysed using descriptive statistics, multiple regression, budgetary technique and benefit cost ratio. The specific objectives were to ascertain costs and returns on vitamin A cassava based production; ascertain influence production costs have on the financial value of the crop's output and to identify the constraints to production of the crop. Findings on costs and returns showed that gross margin, net farm income and net return on investment were ₦41,128.00, ₦41,097.00 and 1.6 respectively. This implies that for every 100 kobo invested in the production, 160% was gained. The result of Benefit Cost Ratio is an indicator that the venture is a profitable business. The findings also revealed that out of the five predictors included in the model, three namely cost of planting material, cost of labour and cost of renting land statistically and significantly influenced production returns earned by the farmers. High cost of labour, poor access to yellow stem, poor access to capital, poor pricing of yellow cassava tubers and poor transportation infrastructure were perceived as the most serious constraints encountered by vitamin A cassava production. Farmers should be encourage to form cooperative in order to enable them access or purchase tractors which should be made available and affordable to farmers to ease the cost of labour, government and other stakeholders should be encourage to multiply vitamin A cassava stems and investors should be encouraged to set up industries that would enter into contracts with vitamin A cassava farmers in the State in order to buy off their produce and process them into value added products were recommended.*

Keywords— Cassava, Vitamin A, Production, Profitability.

I. INTRODUCTION

Cassava (*Manihot esculenta*) is a major staple food, a major source of energy in the diet of many Nigerians and can easily adapt to wide range of climatic and soil conditions (Ekpunobi, Nwigwe and Nkamigbo,2020). Nigeria is currently the largest producer of cassava in the world and the largest cassava market in Africa. (Ijigbade, Fatuase and Omisope, 2014 and Epkpunobi *et al*, 2020).

Nigeria produces approximately 45 million tonnes of cassava annually and the system is highly dominated by small-scale farmers' holding, cultivating average of 0.5 hectares. Cassava which is the major food consumed by Nigerians, though inexpensive and good source of carbohydrates has low protein content and lacks vitamin A. As a result, Nigerians who are restricted to the consumption of cassava based diet could be at risk of being exposed to having diseases associated with vitamin A deficiency (VAD) (Eguonu, 2014, Eze, Nwibo, 2014 and Ekpunobi, 2020). According to (Bouis, Hoty, McClafferty, Meenakshi and Pfeiffer (2011), the deficiency of this all important micronutrient – vitamin A, is a leading cause of morbidity and mortality, especially in young children, pregnant and lactating mothers. Vitamin A deficiency has continued to be a significant public health problem in Nigeria despite improving diets as a result of rising incomes and administration

of vitamin A capsules over the years (Ilona, Bouis, Moursi, Palenberg and Oparinde, 2017). To address the challenges associated with vitamin A deficiency and its severity, Nigerian government embarked on supplementation program with vitamin A for children within the ages of 6 months to 5 years during immunization and went ahead to mandate the fortification of some food item like wheat flour, sugar, vegetable oil with Vitamin A since year 2000 (Adeola, Ogunleye and Bolarinwa, 2017). However, the recurring transportation and administration cost associated with these efforts seems unsustainable especially for those in rural areas due to prevalence of poverty[5]. It is estimated that Nigeria losses over 1.5 billion dollars to vitamin and mineral deficiencies [8]. This is quite enormous for a developing country like Nigeria that is still grappling with poverty and unemployment. Animal foods that are good sources of Vitamin A are not affordable by the poor communities thus leaving food of plant origin as important source of pro-vitamin A in developing countries (World Bank, 2018). Vitamin A cassava has also been reported to be high yielding, resistant to major pests and diseases, and have shown delay in the onset of post-harvest deterioration (Tumuhimbise, Namtebi, Turyashemerwa and Muyong, 2013). However, variance in the colour of vitamin A cassava to that of other cassava varieties, which are traditionally white, raises question on whether the crop would generally be accepted by both the farmers and consumers in the State. Cases have been reported in the past where farmers and consumers rejected improved variety of a crop like maize, due to variance in colour (Ekpa and Linneman, 2018). This is one major issue that would determine the prospects of vitamin A production of the crop and its sustainability, in the State. Even though study carried out by (Nzeh and Ugwu,2014) on the cost and return on cassava production showed that cassava production and marketing is profitable, the researcher however observed that there is paucity of research study on the cost and return on vitamin A cassava production in the State. Hence, with the changing social characteristics of cassava farmers; changes in economic decisions they make; changes in economic realities; the costs and returns on vitamin A cassava production in Anambra State is called to question. As vitamin A cassava is increasingly being pushed to get to more smallholder farmers in Nigeria, it became important to ascertain the costs and returns of its production, identify the influence of production cost on financial value of the output and identify constraints to production of the crop.

II. MATERIAL AND METHODS

The study was carried out in Anambra State. The predominant occupations in these areas include farming, fishing, trading, craft, etc. It is situated on a generally low elevation on the eastern side of the River Niger sharing boundaries with Delta State to the west, Imo, Abia and Rivers State to the south, Enugu state to the East and Kogi state to the North. The state occupies an area of about 4,844 Km^2, lies within longitude $5^0 55^1$ and $6^0 42^1$N. The annual rainfall ranges from 1400 mm in the North to 2500 mm in the south with temperature of 25^0C- 35^0C. The population of the State is 4,182,232 with 863 Sqkm density (NPC, 2006). It consists of twenty-one (21) Local government areas (LGAs) and four agricultural zones.

The Population of the study was vitamin A cassava producers in Anambra State. According to the Anambra State Agricultural Development Programme, the state has eight thousand five hundred (8500) registered cassava producers. However, the list is not categorised into the different varieties of cassava produced. Pre- survey test carried out showed that about 5% (425) of the farmers produced vitamin A cassava. Multi-stage sampling technique was adopted in selecting sample for the study.

Stage one, simple random sampling technique was used to select three agricultural zones from the four agricultural zones of the state.

Stage two, simple random selection technique was used to select four Local Government Areas from each of three agricultural zones totaling twelve LGAs.

Stage three, simple random technique was used to select three communities from each of the twelve LGAs making a total of thirty six communities.

Stage Four, respondents were selected from each of the thirty six communities except in Anambra zone where only two respondents each were selected in three communities due to paucity of yellow cassava producers, while others were four. This made it a total of one hundred and thirty eight respondents (138) which formed the sample size.

TABLE 1
SAMPLE SIZE ALLOCATION

One plot (100 x 50Ft)			
Agricultural Zones	**LGAs**	**communities**	**Sample size allocation**
Anambra Zone	Anambra West (6.4902^{o}N, 6.7922^{o}E)	Miata	2
		Umuewelum	2
		UmuezeAnam	2
	Anambra East (6.3093^{o}N, 6.8673^{o}E	Umueri	4
		Aguleri	4
		Igbariam	4
	Oyi (6.2246^{o}N, 6.8887^{o}E)	Nteje	4
		Awkuzu	4
		Ogbunike	4
	Ayamelum (6.553553^{o}N, 6.986939^{o}E)	Omor	4
		IfiteOgwari	4
		Igbakwu	4
Awka Zone	Awka North (6^{o}12^{1}45.68N, 7^{o}0419”E)	Mgbakwu	4
		Isuaniocha	4
		Achala	4
	Awka South (6^{o}09^{1}60.00”N, 7^{o}0360.00”E)	Awka	4
		Amawbia	4
		Nibo	4
	Dunukofia (6^{o}16’20”N, 6^{o}5738”E)	Ukpo	4
		Nawgu	4
		Ukwulu	4
	Njikoka (6^{o}11’3.12”N, 6^{o}58’35.58”E)	Abagana	4
		Abba	4
		Enugwu-ukwu	4
Aguata Zone	Orumba North (6”02’46N, 7”12’36E)	Ajali	4
		Ufuma	4
		Awa	4
	Orumba South (5^{o}58’0”N, 7^{o}13’0”E)	Umunze	4
		Ihite	4
		Ibughubu	4
	Aguata (6^{o}01’0”N, 7^{o}05’0”E)	Umuchu	4
		Uga	4
		Umuona	4
	Nnewi South (6^{o}0’37.8684”N, 6^{o}54’37.2420”E)	Otolo	4
		Uruagu	4
		Umudim	4
Total			138

Values in parenthesis represent the Global position of the site

2.1 Method of Data Collection

Primary data used for the study were derived from set of structured questionnaire and also subjected to descriptive and inferential analysis- mean, standard deviation percentages, frequency, multiple regression, budgetary technique and benefit cost ratio.

2.2 Model Specification

The regression function analysis was used in four functional forms from which the lead equation was chosen on the basis of the values of the coefficient of Multiple Determination (R^2) as well as the signs and significance of the regression parameters. This is stated explicitly as;

$Y = a + b_1X_1 + b_2X_2 + e$; as described by (Akinbile, 2015).

Y= Output

The regression function postulated for cassava production in the study area is shown in the explicit form using four functional forms; the linear, semi log, double log and exponential. The four functional forms were evaluated using ordinary least square method. The explicit forms of the functional forms are as follows:

Linear function

log Y = b0+b1COLAB+b2COFERT+b3CPM+b4COSTPEST+b5COSTLAND+e

Exponential function

LogQ= b0+b1COLAB+b2COFERT+b3CPM+b4COSTPEST+b5COSTLAND+e

Semi-log function

Q= b0+b1logCOLAB+b2logCOFERT+b3logCPM+b4logCOSTPEST+b5logCOSTLAND+e

Double log

LogQ= b0+b1logCOLAB+b2logCOFERT+b3logCPM+b4logCOSTPEST+b5logCOSTLAND+e

Where Y is the total output in kg

Where

Q	=	Total output (in kg)
Py Q	=	Value of total output (in naira)
COLAB	=	Cost of Labour (in naira)
COFERT	=	Cost of fertilizer (in naira)
CPM	=	Cost/amount spent on planting material (in naira)
COSTPEST	=	Cost of pesticides and herbicides (in naira)
COSTLAND	=	Cost/amount spent on renting/leasing land (in naira)
E	=	Stochastic error term (error term assume to have a zero mean and constant variance).

Profitability was estimated using budgetary tool, which measured the difference between total revenue (TR) and the total cost (TC). Net return is given as *TR-TC*

Where; TR = Total Revenue =P.Q (P=Price, Q=Quantity); TC= Total cost.

III. RESULTS AND DISCUSSION

3.1 Cost and Returns of Vitamin A Cassava Production in Anambra State

The analysis of profitability of vitamin A cassava production using enterprise budgeting and benefit cost ratio is shown in Table 2. Total revenue (TR) from yellow cassava production was ₦ 66,726.00 while total variable cost (TVC) and total cost of production were ₦ 25,598.00 and ₦ 25, 629.00 respectively. The cost of labour (35.5%) and fertilizer (28.1%) constitute

the major cost in the production of vitamin A cassava in the study area while hoe and cutlass (35.5%) has the least annual depreciation value. The analysis further revealed that gross margin, net farm income and net return on investment were ₦41,128.00, ₦41,097.00 and 1.6 respectively. This implies that for every 100 kobo invested in the production, 160% was gained. From the result, investors are encouraged to go into the production of the crop as they are sure of making profit. This agrees with (Nkamigbo, Atiri, Gbughemobi and Obiekwe, 2015) who reported a mean rate of return of 153% of hybrid maize in their study area. The implication of this is that vitamin A cassava production in the study area is a better investment.

TABLE 2
COST AND RETURNS ON VITAMIN A CASSAVA FARMERS IN ANAMBRA STATE

Variables	Amount (₦)	Percentage (%)
Total Revenue	***66, 726.00***	
Cost of planting material	4, 160.00	16.3
Cost of labour	9,526.00	37.2
Cost of fertilizer	7,190.00	28.1
Agro chemicals	2,814.00	10.1
Cost of land renting	1,908.00	7.5
Total Variable Cost (TVC)	**25,598.00**	**100**
Fixed Cost		
Depreciation on wheelbarrow	10.00	32,3
Depreciation on hoe	5.00	16.1
Depreciation on cutlass	5.00	16.1
Depreciation on sprayers	11.00	35.5
TOTAL FIXED COST	31.00	100
Total Cost (TVC+TFC)	25,629.00	
Gross Margin (TR-TC)	41.128.00	
NFU (TR-TC)	41,097.00	
NROI (NFI/TC)	1.6	

Also the analysis of profitability of vitamin A cassava production using Benefit Cost Ratio is shown below.

$$\text{Benefit Cost Ratio (BCR)} = \frac{\text{Total Revenue}}{\text{Total market Cost}} = \frac{₦66,726.00}{₦25,629.00} = 2.6$$

BCR > 1 = Profitable.

From the result of the analysis, yellow cassava production in the study area with BCR > 1 indicator that the venture is a profitable business.

3.2 Influence Production Costs have on Financial Value of Vitamin A Cassava Output in Anambra State

Table 3 shows outputs of the four functional forms of the regression model for predictors of the influence production cost have on the financial value of yellow cassava output in the study area. The result indicated that output of the Exponential function gave the best result in terms of number of significant predictors, signs and sizes of the predictors as well as the value of F- statistic, R^2 adjusted and was chosen as the lead equation. The coefficient of multiple determination (R^2) 54.6% meant that 54% of the variation in the profit was explained by the variations in the independent variables while the remaining 46%

was due to error. The F-statistic value of 31.70 was significant and confirms to overall significance of the regression analysis. The equation is given as:

log Y = b0+b1COLAB+b2COFERT+b3CPM+b4COSTPEST+b5COSTLAND+e

log Y = 4.549+2.873COLAB+0.115COFERT+1.965CPM+0.68COSTPEST+1.989COSTLAND+e .

Out of the five predictors included in the model, three namely cost of planting material, cost of labour and cost of renting land statistically and significantly influenced production returns earned by the farmers. The cost of planting material was positive and highly statistically significant at 1% probability level. This implies that unit increase in the cost of procurement of planting material will result in increase in the financial value of vitamin A cassava output in the study area. This is in consonance with (Ekpunobi, *et al*, 2020) who stated that increase in planting material is bound to increase output and producers could procure additional planting material for available land space. The effect of cost of labour was also positive and highly statistically significant suggesting that it is a crucial input in vitamin A cassava production. The positive sign of the co-efficient of cost of renting/leasing land suggests a direct relationship between farm size and output level. The implication of this is that the more farm acquired will generate a higher output (income) to the farmer. This agrees with the report of (Okeke, Nkamigbo and Chukwuji, 2013) that farm size has a direct relationship with the output and larger farm size generate higher income to the farmer.

TABLE 3
INFLUENCE PRODUCTION COSTS HAVE ON THE MONETARY VALUE OF VITAMIN A CASSAVA OUTPUT IN ANAMBRA STATE

Influence Production Costs have on Monetary Value of Vitamin A Cassava Output in Anambra State					
Variable	**Linear**	**Semi log**	**Exponential[1]**	**Double log**	**Decision**
Constant	-92811.457 (-1.477)	200732.312 (0.302)	4.549 (104.915)***	3.086 (6.276)***	
Cost of plant. Material (CPM)	-0.200 (-0.215)	-89068.393 (-0.597)	1.965 (3.062)***	0.332 (3.011)***	Reject
Cost of fert. (COFERT)	0.097 (0.57)	33386.058 (1.112)	0.115 (1.761)	0.046 (2.082)**	Accept
Cost of pest and herb (COPEST)	9.715 (0.632)	41873.519 (1.048)	0.068 (0.921)	0.032 (1.072)	Accept
Cost of Lab. (COSTLAB)	2.300 (3.040)***	1.2052.147 (0.195)	2.873 (5.501)***	0.004 (0.0087)	Reject
Cost of renting/ Leasing land (COSTLAND)	20.265 (5.570)***	63547.053 (2.395)**	1.989 (7.919)***	0.060 (0.003)*	Reject
R^2 F statistics Sample size	0.261 9.310*** 138	0.085 1.931** 138	0.546 31.701*** 138	0.297 8.795*** 138	
Figures in parenthesis are t- ratios. *** Significant at 1%; ** Significant at 5%; * Significant at 10%.					

Source: Computed from the Field Survey Data, 2020

3.3 Constraints to Vitamin A Cassava Production in Anambra State

The constraints associated to vitamin A cassava production were shown in Table 4. High cost of labour, poor access to vitamin A stem, poor access to capital, poor pricing of vitamin A cassava tubers and poor transportation infrastructure were perceived as the most serious constraints encountered by vitamin A cassava production with high percentages of 62.3, 54.3, 50.7, 43.5 and 43.5 respectively. High cost of fertilizer (39.1%) and insecurity challenge/fear of herdsmen attack (36.2%) also affect its production. Yakassai (2010) and Emmanuel (2013) highlighted finance as a factor impeding emergence of modern cassava production system. Other constraints of less importance were high cost of transportation (16.7%), difficulty in accessing large expanse of land (15.9%), pest and diseases (8.7%) and poor access to extension services (4.3%). This is

contrary to (Nkamigbo, Nwoye, Makwudo and Gbughemobi, 2018) who reported inadequate extension and pest and disease infestation as the major constraints to maize production.

TABLE 4
CONSTRAINTS TO VITAMIN A CASSAVA PRODUCTION

Constraints	Frequency	Percentage
Poor access to yellow cassava stem	75	54.3
High cost of fertilizer	54	39.1
Poor pricing of yellow cassava tubers	60	43.5
Pest and diseases	12	8.7
Poor access to capital	70	50.7
High cost of labour	86	62.3
Insecurity challenge (fear of herdsmen attack)	50	36.2
Difficulty in accessing large expanse of land	22	15.9
Poor transportation infrastructure	60	43.5
High cost of transportation	23	16.7
Poor access to extension services	6	4.3

Multiple responses **Source: Field survey, 2020**

IV. CONCLUSION

The result of costs and returns of vitamin A cassava production from one plot (100 x 50Ft) revealed that gross margin, net farm income and net return on investment were ₦41,128.00, ₦41,097.00 and 1.6 respectively. This implies that for every 100 kobo invested in the production, 160% was gained. The cost of labour (35.5%) and fertilizer (28.1%) were the major cost in the production of vitamin A cassava. Multiple regression analysis revealed that cost of planting material, cost of labour and cost of renting land statistically and significantly determined net farm income earned by the farmers while cost of fertilizer and cost of pest were not significant. . High cost of labour (62.3%), poor access to vitamin A cassava stem (54.3%), poor access to capital (50.7%), poor pricing of vitamin A cassava tubers (43.5%) and poor transportation infrastructure 43.5%) were perceived as the most serious constraints to vitamin A cassava production in the study area. It was recommended that farmers should be encouraged to form cooperatives in order to enable them access or purchase tractors which should be made available and affordable to farmers to ease the cost of labour, government and other stakeholders should be encouraged to multiply yellow cassava stems and investors should be encouraged to set up industries that would enter into contracts with vitamin A cassava farmers in the State in order to buy off their produce and process them into value added products.

REFERENCES

[1] Ekpunobi C.E, Nwigwe A.C, Nkamigbo D.C. (2020). Socio economic determinants of yellow cassava production in Anambra State, Nigeria. Intl Journal of Applied Science and Research 3(2), 90-100.

[2] Ijigbade J.O, Fatuase A.I and Omisope E.T. (2014). Conduct and profitability of gari production for increased food security in Ondo State, Nigeria. *IOSR Journal of Humanities and Social Science*. 19(7), 89-95.

[3] Eguono I.(2014). A look at cassava production in Nigeria. Intl Journal of Agricultural Sciences. 5 (5), 818-819.

[4] Eze A.V, Nwibo, S.U.(2014). Economics and technical efficiency of cassava production in Ika North East Local Government Area Delta State, Nigeria. Journal of Development and Agricultural Economics. 6(12), 429-436.

[5] Bouis, H.E, Hoty, C, McClafferty, B., Meenakshi, J.V., Pfeiffer, W.H. (2011). Bio-fortification: A New Tool To Reduce Micronutrient. 31-40.

[6] Ilona. P., Bouis, H.E., Moursi, M, Palenberg, M., Oparinde, A. (2017). Vitamin A cassava in Nigeria: crop development and delivery. African Journal of Food, Agriculture, Nutrition and Development. 17(2):12000-12025.

[7] Adeola, R.G., Ogunleye, K.Y, Bolarinwa, I.F. (2017). Yellow cassava attributes influencing Its utilization among cassava processors in Oyo State. International Journal of Environment Agriculture and Biotechnology. 2(5): 2650-2658.

[8] World Bank.(2018). Nigerias - *Accelerating Nutrition Results in Nigeria Project (English).*Washington, D.C: World Bank Group. 1-120.

[9] Tumuhimbise, G.A, Namtebi, A., Turyashemerewa, F., Muyong, J.(2013). Provitamin A Crops: Acceptability, Bioviability, Efficacy and Effectiveness. Food and Nutrition Science. 4(4), 430-435.

[10] Ekpa, O., Linneman, A.R. (2018). Sub-saharan African maize-based foods: Technological perspectives to increase the food and nutrition security impacts of maize breeding programmes. *Global Food Security*. 17, 48-56.

[11] Nzeh, E.C,Ugwu, J.N.(2014). Economic analysis of production and marketing of cassava in Akoko North-West Local Government Area of Ondo State, Nigeria. International Policy and Research. 2 (6), 234-237.

[12] NPC (2006). Report of Naigeria's National Population Commission on 2006 census. Population and Development Review. 2006; 33(1): 206-210.

[13] Akinbile, A. (2015). Application of linear regression analysis in agricultural extension research. In: M.C. Madukwe (ed.) A Guide to Research in Agricultural Extension Agricultural Society of Nigeria (AESON), Agricultural and Rural Management Training Institute, Ilorin, Nigeria.

[14] Nkamigbo, D.C, Atiri, O.A, Gbughemobi, B.O., Obiekwe, U.C. (2015). Comparative analysis of costs and returns in hybrid and non hybrid maize production in Osun State, Nigeria. Journal of Agric and Vet Sci. 7(2), 55-69.

[15] Okeke, D.C, Nkamigbo, D.C, Chukwuji, C.O. (2013). Economics of legume based intercropping systems in Anambra State. Journal of Vocational and Technical Education. 8 (1), 125-131.

[16] Yakasai, M. (2010). Economic contribution of cassava production (a case study of Kuje area council federal capital territory, Abuja, Nigeria). Journal of Pure and Applied Sciences. 3 (1): 87-96.

[17] Emmanuel, T. (2013). Enhancing Cassava Marketing and Processing in Cameroon: Drivers, Constraints, and Prospects of the Value Chain, In: Rebuilding West Africa's Food Potential, A. Elbehri (ed.). FAO/IFAD. 505-535.

[18] Nkamigbo, D.C, Nwoye, I.I, Makwudo, E.O, Gbughemobi, B.O.(2018). Economics of maize production in Oyi LGA, Anambra State, Nigeria. Intl Journal of Agriculture and Bioseiences.8 (2), 112-116.

Effect of Socio-economic Characteristic on Maize Farmers in Zing Local Government Area, Taraba State, Nigeria

Tukura Ejati Danladi[1], Oruonye, E.D.[2*], Menwo Ukechi Wilson-Osigwe[3], Kesunga Bala[4]

Department of Geography, Taraba State University, Jalingo, Nigeria
*Corresponding Author

Received:- 05 August 2021/ Revised:- 13 August 2021/ Accepted:- 19 August 2021/ Published: 31-08-2021

Abstract— *Smallholder farmers are one of the most important stakeholders in Nigeria's agrarian economy. This study examined the effect of socio-economic characteristic on maize farmers in Zing Local Government Area, Taraba State, Nigeria. This study adopted descriptive survey design and used primary and secondary data. Questionnaires were used to elicit information from the respondents. Five (5) wards were purposively selected out of ten (10) wards in the study area. Data was analyzed using descriptive statistics. The findings of the study reveal that men are more involved in farming activities in the study area than women because of their ability to handle complex farming operations such as land preparation (clearing bushes and creating mounds and ridges). The study findings reveal that 33.73% of the respondents were in their prime age, between 20-30 years. As much as 63.86% of the respondents have small farm holdings between 3 to 5 hectares and 42.77% of the respondents acquire their farmlands through inheritance, 28.31% bought their farmlands, 19.28% rent their farmlands while 9.64% obtain their farmlands by lease. The study reveals that 34.64% of the respondent's income ranges between ₦10,000 to ₦20,000. This income is very low, thereby forcing the local people to take to other alternative sources of livelihood such as commercial cyclist riding, carpentry, welding and petty trading. In terms of labour, 43.97% of the respondents use family labour exclusively in their farming operation, 27.11% used hired labour, 21.69 used mechanical power in form of tractor and 7.23% use animal draught in their farming operations. The poor socio-economic characteristics of the farmers contribute greatly to increasing decline in maize production in the study area which also translate to low income of the rural farmers. Based on the findings, the study recommended the need to assist the rural farmers to organize themselves into cooperatives and increase provision of up-to-date information and technology by extension workers to improve the skills of the rural farmers in modern agronomic practices.*

Keywords— Maize farmers, Smallholder farmers, Socio-economic characteristics and Zing.

I. INTRODUCTION

Smallholder farmers are one of the most important stakeholders in Nigeria's agrarian economy. Even though the contribution of agriculture to Nigeria's GDP continues to decline, about half of the populations are still employed in the sector (Food and Agriculture Organisation [FAO], 2015). Maize is one of the staple foods for some one billion people in 105 countries of the world over, where a third of the caloric needs of the people are met (OECD-FAO, 2015). The importance of maize to Africa's age-old problem of food insecurity cannot be overemphasized. It is believed that maize could help protect the food and energy security needs of poor countries, especially in Sub Saharan Africa now threatened by volatile food prices (FAO, 2008). Maize in Nigeria is largely produced by smallholder farmers on marginal and often degraded lands of the humid tropics. The crop is cultivated on fragmented land holdings where the farmers produce to feed their family and sell surplus produce for income. Nonetheless, the smallholder sector plays crucial role as far as livelihoods for the vast rural population is concerned.

Its production is influenced by several factors ranging from geographical to socio-economic. The production levels of the crop have been increasing on a yearly basis and constitute a significant percentage of Nigeria's agricultural Gross Domestic Product [GDP] (FAO, 2013). For sub-Saharan Africa (SSA), maize is regarded as one of the most important crops due to its general acceptance and less input demand. However, socio-economic factors continue to play crucial role in determining the levels of crop production and the sort of crops planted accros Nigeria. The production levels are not the only areas affected

but also the way agricultural businesses are managed which put the socio-economic characteristics of the farmers into consideration (von Braun & Mirzabaev, 2015). Previous studies have showed that if support is to be extended to crop farmers in production locations, their basic characteristics are worth studying to fully understand their needs for need-driven intervention. For instance, Mwaniki (2006) stressed that, boosting agricultural production capacity of farmers requires that adequate information about the demographic and socio-economic characteristics of the farmers become part of the wider strategy to improve production. Many rural farmers often missed out from supports due to their geographic and socio-economic characteristics and this influence their production output levels. The well to do farmers are easily identified because they have voices to be heard while the poor farmers remain voiceless. Primary areas of interest identified in earlier studies consist of a mixture of some socio-economic and demographic factors.

On demographic characteristics, empirical studies have shown that educational level of farmers tend to increase their output levels through increased knowledge of the production processes and the ability to easily understand recent research findings on new agronomic practices (Seyoum, Battese & Fleming, 1998; Hassan & Ahmad, 2005). Further, the magnitude of time and efforts needed to convince producers to undertake innovative and improved farming practices are reduced with literate farmers. Illiterate farmers on the other hand, are sometimes trivial and unnecessarily focused on the personality of the extension personnel rather than the message (Onubogu, Esiobu, Nwosu and Okereke, 2014). Of late, there is burgeoning concerns for farm size and output level relationship.

Conventionally, age and experience are directly proportional in the smallholder farmer operations. The relationship between age of farmers and their potential output levels has engaged and continue to engage the attention of scholars at least for some time. The argument surrounding age as far as efficiency, productivity and output potentials are concern gathered momentum and show no sign of ending anytime soon. Depending on the effects of other demographic and socio-economic factors on age, it can either enhance or reduce the output levels of farmers in production process. According to some studies, age influences output levels positively because farming is an activity that requires prolonged practice before attaining perfection. (Abdul-kareem & Isgin, 2016; Oren & Alemdar, 2006; Erhabor & Emokaro, 2007). Other studies argued otherwise as young farmers being more positioned to realise higher outputs than older farmers (Latruffe, 2010; Samuel, Debrah & Abubakari, 2014). They hold the view that older farmers may be reluctant to change and sometimes their unwillingness or inability to adopt new innovations could affect their production abilities leading to low level of outputs realised.

The gender of farmers according to studies has some production implications. Many studies have concluded that male farmers are likely to obtain higher outputs than their female counterparts from the employment of the same factors of production (Abdulai, Nkegbe & Donkoh, 2013; Asante, Osei, Dankyi, Berchie, Mochia, Lamptey, Haleegoah, Osei & Bolfrey-Arku, 2013; Onumah, Onumah, Al-Hassan & Brummer, 2013). They contend that in some geographical localities, the culture of the people will likely exclude women in extension information dissemination because they are not considered as farmers like their male counterparts. Also, due to gender alignment issues, extension information content may not address the needs and conditions of women producers. Few studies have shown that the women off-farm time could be used to gain more knowledge and information thereby increasing their knowledge of the production process (Latruffe, 2010; Onumah, Al-Hassan & Onumah, 2013).

On the socio-economic characteristics, empirical literature is flooded with arguments for and against farm sizes in productions. Many studies have concluded that the larger farm size is preferable to smaller farm size in terms of outputs obtainable from the production process (Hassan & Ahmad, 2005). However, findings of other studies in the same area have argued otherwise (Badunenko, Fritsch & Stephen, 2006; Masterson, 2007). Their conclusive assertions lend credence that farms with smaller land sizes produce higher output than their larger size counterparts. There has not been a consensus on this matter, but quite strangely, the approach adopted by scholars from both sides of the block raises more questions than answers. Importantly, one thing that is driving the debate in a subtle manner is the productivity level of the land or the fertility level of the land under cultivation. That is to say how much is obtained from a parcel of land is a function of several factors rather than just the number of acreages engaged. Additionally, in making a case for either of them, there is always some unintended neglect of the influences of other factors of production in the production process which may lead to erroneous conclusions of one being preferable to the other (Masterson, 2007).

Despite the popularity of maize as staple food in Zing Local Government Area of Taraba State, there is low productivity of this crop. This may be due to factors such as sowing of poor quality variety, late sowing, inaccurate spacing, inappropriate fertilizer application, (at the right time and quantity), non-use of chemicals to control pest and diseases, poor knowledge of appropriate moisture content of maize to be harvested and stored. Other factors include non-adoption of some farming

practices such as dig and cover method of fertilizer placement, measurement of seed quantity to be planted and measurement of spacing between hills were considered too labour intensive and unnecessary. The major constraints to adoption were found to be lack of capital, high cost of fertilizer and lack of market for produce. Moreover, non-adoption of improved maize production practices negatively affects maize production in the study area.

Despite the economic importance and popularity of Maize to the teeming populace in Nigeria, it has not been produced to meet food and industrial needs of the country and this could be attributed to low productivity from Maize farms or that farmers have not adopted improved technologies for Maize production. Additionally, other factors like price fluctuation, diseases and pests, poor storage facilities have been associated with low Maize production in the country thereby causing a fluctuation trend in maize production.

This fluctuating trend in maize production threatens household food security and income sources. To reverse this trend and ensure increase maize in the study area and the country at large there is need to examine demographic and socio-economic characteristics that influences the poverty status of farmers and how their productivity can be improved upon. This is very important in order to ensure agricultural growth and development in Nigeria mostly in the rural areas where we have 70% of her over 140 million involved in agriculture production is addressed.

No doubt maize is in high demand both for local human consumption and preparation of animal feed. Nigeria is still importing maize to supplement local production. The major challenge is the dominance of smallholder farmers which leads to low output and insufficiency to meet the country's food need. There is therefore need to study how these demographic and socio-economic characteristics influences maize farmers in the study area. This will not only contribute to existing knowledge on maize farmer's productivity and their poverty status but will also help in formulating future strategies to improve maize farmer's productivity, increase productivity for effective economic development, achieve long run economic growth per person through increases in capital.

Although, there are studies on socio-economic characteristics of farmers in the Savannah zone, not many studies have been done on the effects of demographic and socioeconomic characteristics on maize farmers in the study area. Moreso, considering the importance of maize as local staple food to the country, basic demographic and socio-economic information of maize farmers would interest policy makers and provide a basis for other studies involving the staple crops in the country. It is against this background that this study examines the effects of the demographic and socio-economic characteristics on maize farmers in Zing Local Government Area, Taraba State, Nigeria.

Description of the Study Area

Zing Local Government Area is situated to the North-eastern part of Taraba State. It is bounded to the East and North by Adamawa state and to the West and South by Yorro Local Government Area. Zing Local Government Area lies between latitudes 8^{o} 45′N and 9^{o} 10′N and longitudes 11^{o} 35′E to 11^{o} 50′E. Zing LGA has a total land mass of 1,030km². The study area consists of 6 districts with 75 villages. Each village has approximately between 255-783 farm families (TADP, 2005). It also has a total population of approximately 127,362 according to the 2006 National census with annual growth rate of 3.0% (NPC, 2006). Ethnic groups found in the study area are Mumuye, Yendang and Fulani.

The study area has tropical climate marked by dry and wet seasons. The annual rainfall ranges between $819mm^2$ to $1250mm^2$ spreading over seven months, usually between April to October. Average rainfall is about $850mm^3$ annually and mean temperature of 30^{o}C. Temperature and evapo-transpiration are high for a greater part of the year (Oruonye, 2014). The study area falls within the Sudan Savannah woodland characterized by scattered deciduous tall trees with broad leaves and tall grasses. The major soil types are the hydromorphic and ferruginous tropical soils. The soil consist of mixture of loamy and sandy soils on hilly terrain and deep loamy soil in between the rocks.

The relief of the study area can be categorized into two zones, highland mountain ranges and lowlands. The highlands occupy the southern regions stretching from West to South in chains of mountain elevation, ranging from an average of 1,800 – 2,400 metres high, forming the Atlantica, Shebshi and Adamawa Massifs ranges. The lowlands which occupies about 60% of the region hosts most of the settlements in area.

Zing LGA is underlain by crystalline rocks of basement complex origin dominated by low rising hills and rock outcrops. The underground aquifer is poorly developed. The existence of basement complex rocks near the surface in the valleys results in poor aquifer since the rocks are mainly igneous and metamorphic and so impermeable.

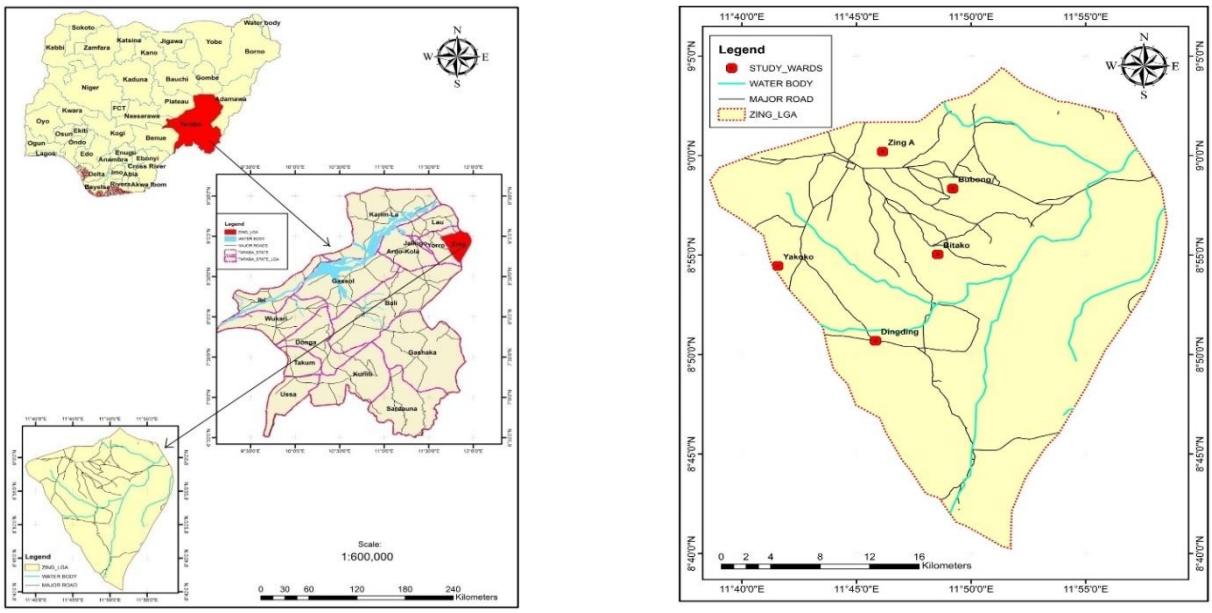


FIGURE 1: Map of the study area.
Source: Ministry of Land and Survey Jalingo (2018)

Agriculture is the dominant economic activity practiced by the vast majority of the people in the area. The major food crop cultivated in the area include, maize, yam, sorghum, bambara nut, groundnut, millet and rice. The recent relocation of the Taraba State College of Education from Jalingo to Zing town and its upgrade to a degree awarding tertiary educational institution has also increased socioeconomic activities in the area.

II. MATERIALS AND METHODS

This study adopted descriptive survey design. This study design was considered appropriate because it enabled data collection from the sample on the demographic and socio-economic factors influencing maize production among rural household in the study area. The type of data used in the study includes both primary and secondary data. The primary data was generated from questionnaires. The secondary data was generated from existing published materials which include journals, seminar papers, proceeding papers, newspapers etc.

The questionnaire used was closed-ended. The respondents were informed ahead about the purpose of the study. The questionnaire was subjected to validation by expert in the field to ascertain the content of the questionnaire, clarity of wordings and capacity of the instruments to generate the required information. The heads of the sampled farmer's household were given the questionnaires to complete. The questionnaires were then retrieved after some days.

Multi-stage sampling technique was adopted in this study. This involves the purposive selection of five (5) wards that are known for high maize production out of ten (10) wards in the LGA. In the five (5) wards selected, random selection was adopted in the selection of the actual respondents for this study. Proportionate number of farmers was selected from each ward because the number of maize farmers varies from one ward to another. The data collected was analyzed using descriptive statistics such as frequency tables and percentages.

III. RESULT OF THE FINDINGS

3.1 Demographic Characteristics of Maize Farmers

The results of the demographic characteristics of the respondents are presented in Table 1. The result shows that 55.12% of the respondents were male while 44.88% were female. This indicates that men are more involved in farming activities in the study area than women. This result is because of the fact that men carry out more complex farming operations such as land preparation (clearing bushes and creating mounds and ridges), while women and children perform lighter operations, such as planting, fertilizer application and weeding. The result in Table 1 shows that 33.73% of the respondents were between 20-30 years, while 25.0% are between 31 to 40years. This indicates that the bulk of the farmers were still in their prime age. The result in Table 1 further reveal that majority of the farmers (47.89%) were single, 37.05% are married, 9.64% divorced and 5.42% are widowed. The result shows that 8.73% had primary education, 25.9% had secondary education, 40.96% had National Certificate of Education and 17.47% had University education. This suggests that most of the respondents are

educated, which is good for information gathering. FAO (1993) reports show that agricultural knowledge transfer, procurement and use flourish in areas where farmers are well trained.

TABLE 1
SOCIO-DEMOGRAPHIC CHARACTERISTICS OF MAIZE FARMERS

Characterstics	No of Respondents	Percentage (100%)
Gender		
Male	183	55.12
Female	149	44.88
Age (years)		
Less than 20 years	57	17.16
21– 30	112	33.73
31– 40	83	25
41– 50	46	13.9
51– 60	22	6.6
61– 70	11	3.31
Above 71	1	0.3
Level of Education		
Primary	29	8.73
Secondary	86	25.9
Nat. Cert. of Education	136	40.96
University	58	17.47
Post graduate	20	6.02
Marital Status		
Single	159	47.89
Married	123	37.05
Divorce	32	9.64
Widowed	18	5.42
Gender that Engage the Most in Maize Production		
Male	246	74.1
Female	86	25.9

Source: Fieldwork, 2020.

3.2 Socio-Economic Characteristics of Maize Farmers

Table 2 shows that 36.14% of the farmers had farm sizes between 3-5 hectares. This means that 63.86% of the maize farmers in the study area have small farm holdings. The Table 2 reveals that 38.55% of the farmers have farming experience between 3-6 years and 38.55% have farming experience of 7-10 years. This indicates that the majority of the farmers in the study area have extensive farming experience in their locality.

TABLE 2
FARM SIZE AND FARMING EXPERIENCE

Variable	No of Respondents	Percentage (100%)
Size of Farmland		
3 – 5ha	120	36.14
5 – 7ha	86	25.9
8 – 10ha	56	16.87
1 – 2ha	50	15.06
10 – 15ha	18	5.42
20ha and above	2	0.6
Level of Experience in Farming		
3 – 6 years	128	38.55
7 – 10 years	122	36.75
16 – 20 years	40	12.05
20 years and above	22	6.63
11 – 15 years	20	6.02

Source: Fieldwork, 2020

Table 3 reveals that 42.77% of the respondents acquire their farmlands through inheritance, 28.31% bought their farmlands, 19.28% rent their farmlands while 9.64% obtain their farmlands by lease. The result in Table 3 also reveals that 34.64% of the respondent's income ranges between ₦10,000 to ₦20,000. This income is very low, thereby forcing the local people to take to other alternative sources of livelihood such as commercial cyclist riding, carpentry, welding and petty trading. These activities are also important for reducing poverty since they provide some level of livelihood diversification. These practices, which are a part of their daily lives, were discovered to provide some income for maize farming.

TABLE 3
SOCIO-ECONOMIC CHARACTERISTICS OF RESPONDENTS

Land Acquisition Method	No. of Respondents	Percentage (100%)
Inheritance	142	42.77
Purchase	94	28.31
Rent	64	19.28
Lease	32	9.64
Income level (₦)		
10,000 – 20,000	115	34.64
30,000 – 50,000	94	28.31
60,000 – 100,000	67	20.18
100,000 – 200,000	35	10.54
300,000 – 500,000	10	3.01
500,000 and above	11	3.31
Income Level From Non – Agricultural Source (₦)		
10,000 – 20,000	150	45.18
30,000 – 50,000	100	30.12
60,000 – 100,000	44	13.25
500,000 and above	13	3.92
100,000 – 200,000	12	3.61
300,000 – 500,000	1	0.30

Source: Fieldwork, 2020

The result of the findings in Table 4 reveal that 43.97% of the respondents use family labour extensively in their farming operation, 27.11% used hired labour, 21.69 used mechanical power in form of tractor and 7.23% use animal draught in their farming operations. The result of the findings reveals that majority of the farmers in the study area depend so much on their personal labour and that of their family members, friends and relatives for farm labor to help them in their farming activities.

TABLE 4
SOURCE OF LABOUR IN FARM OPERATION

S/No.	Value	No of Respondents	Percentage (100%)
1	Unpaid family labour	146	43.97
2	Hired manual labour	90	27.11
3	Mechanical	72	21.69
4	Animal draught power	24	7.23

Source: Fieldwork, 2020.

Table 5 shows the age distribution of respondents that easily adopt new technology. This indicates that majority 54.53% of maize farmers between ages 20-30years easily adopt new technology. It also shows that 44.58% of maize farmers who have completed their college of education easily adopt new farming techniques. FAO (1993) and Zijp (1994) reports show that agricultural knowledge transfer, procurement, and use flourish in areas where farmers are well trained.

TABLE 5
ADOPTION OF NEW TECHNOLOGY

S/No	Value	No of Respondents	Percentage
1	**Age Bracket that Easily Adopts New Technology**		
2	20 – 30	181	54.52
3	30 – 40	90	27.11
4	40 – 50	43	12.95
5	50 – 60	11	3.31
6	Above 60	7	2.11
7	**Education Level that Easily Adopt to New Farming Technique**		
8	Primary	31	9.33
9	Secondary	77	23.19
10	College	148	44.58
11	University	56	16.87
12	Post graduate	20	6.02

Source: Fieldwork, 2020.

Credit is a valuable institutional service that helps poor farmers funds the purchase of farming inputs and eventually, the adoption of new technology. Some farmers, on the other hand, may not have taken credit because of issues with repayment and down payment in order to obtain input from formal sources. As a result, some farmers stop using farm credit. The result of the findings reveals that 58.43% of the farmers had ever access credit facility in carrying out farming operation as shown in Table 6. The result also shows that about 60.54% of the farmers does not belong to an organization or cooperative. This means that farmers do not have direct access to information that can aid their development. According to Adebayo and Adekunle (2016), farmers who belong to a party reap a lot of benefits and can achieve a lot more collectively than they can individually.

TABLE 6
ACCESS TO FINANCIAL AND CORPORATIVE MEMBERSHIP OF FARMERS

S/No.	Ever received loan	No. of Respondents	Frequency (100%)
1	No	194	58.43
2	Yes	138	41.57
3	**Last Time You Took Loan**		
4	Within the last one year	109	32.83
5	Within the last two years	21	6.33
6	Within the last three years	6	1.81
7	Four years and above	2	0.6
8	**Do you Belong to an Active Farmer Group or Cooperative**		
9	No	201	60.54
10	Yes	131	39.46
11	Do you think the Government can Assist Farmers with Affordable and Easily Accessible Credit?		
12	Yes	209	62.95
13	No	123	37.05

Source: Fieldwork, 2020.

IV. CONCLUSION

This study has examined the effect of socio-economic characteristic of rural farmers on maize production in Zing Local Government Area, Taraba State, Nigeria. The findings of the study reveal that men are more involved in farming activities in the study area than women because of their ability to handle complex farming operations such as land preparation (clearing bushes and creating mounds and ridges). The study findings reveal that 33.73% of the respondents were in their prime age, between 20-30 years. As much as 63.86% of the respondents have small farm holdings between 3 to 5 hectares and 42.77% of the respondents acquire their farmlands through inheritance, 28.31% bought their farmlands, 19.28% rent their farmlands while 9.64% obtain their farmlands by lease. The study reveals that 34.64% of the respondent's income ranges between ₦10,000 to ₦20,000. This income is very low, thereby forcing the local people to take to other alternative sources of livelihood such as commercial cyclist riding, carpentry, welding and petty trading. In terms of labour, 43.97% of the

respondents use family labour exclusively in their farming operation, 27.11% used hired labour, 21.69 used mechanical power in form of tractor and 7.23% use animal draught in their farming operations. The poor socio-economic characteristics of the farmers contribute greatly to increasing decline in maize production in the study area which also translates to low income of the rural farmers.

RECOMMENDATIONS

Based on the findings of the study, the following recommendations were made;

i. Farmers should be assisted in forming viable and functional cooperatives by extension workers. This will allow them to combine their resources and generate opportunities for them to obtain loans to improve their production capacity.

ii. Farmers should be provided with up-to-date information and technology by extension workers in order to improve their skills in modern agronomic practices especially in areas where they are lacking in relevant information.

REFERENCES

[1] Abdulai, S., Nkegbe, P.K. and Donkoh, S.A. (2013). Technical Efficiency of Maize Production in Northern Ghana. *Afr. Journal of Agric. Research.* 8(43):5251-5259.

[2] Abdul-kareem, M.M. & Isgin, T. (2016). Technical Efficiency of Cassava Production in the Savannah Zone of Northern Ghana: Stochastic Frontier Analysis. *J. Biol. Agric. Health*. 6(20):62-72.

[3] Adebayo, S.A. and Adekunle, O.A. (2006). Socio-Economic Status of Women in Group Membership in Selected Areas of Kwara State, Nigeria. *Agrosearch,* 16(1); 59-67.

[4] Asante, B.O., Osei, M.K., Dankyi, A.A., Berchie, J.N., Mochiah, M.B., Lamptey, J.N.L., Haleegoah, J., Osei, K. and Bolfrey-Arku, G. (2013). Producer Characteristics and Determinants of Technical Efficiency of Tomato Based Production Systems in Ghana. *J. Dev. Agric. Econ.* 5(3):92-103.

[5] Badunenko, O., Fritsch, M. and Stephan, A. (2006). What Determines the Technical Efficiency of a Firm? The Importance of Industry, Location, and Size. Jenaer Schriften zur Wirtschaftswissenschaft, 33/2006.

[6] Erhabor, P.O. and Emokaro, C.O. (2007). Relative Technical Efficiency of Cassava Farmers in the Three Agro-Ecological Zones of Edo State, Nigeria. *J. Appl. Sci*. 7(19):2818-2823.

[7] Food and Agriculture Organization (FAO) (2008). Food Security and Agricultural Development in Sub Saharan Africa; Building a case for more public support. Policy Brief No 1.

[8] Food and Agriculture Organization (FAO) (2013). Building a common vision for sustainable food and agriculture. Principles and Approaches.

[9] Food and Agriculture Organization (FAO) (2015). The State of Food and Agriculture. Social Protection and Agriculture: Breaking the Cycle of Rural Poverty. Food and Agriculture Organization of the United Nations, Rome, 2015.

[10] Hassan, S. and Ahmad, B. (2005). Technical Efficiency of Wheat Farmers in Mixed Farming System of the Punjab, Pakistan. *Int. J. Agric. Biol.* 7(3):431-435.

[11] International Labour Organization (ILO) (2016). World Employment and Social Outlook: Trends 2016. International Labour Office – Geneva.

[12] Latruffe, L. (2010). "Competitiveness, Productivity and Efficiency in the Agricultural and Agr-Food sectors" OECD Food. *Agric. Fish.* Papers P 30.

[13] Masterson, T. (2007). Productivity, Technical Efficiency and Farm Size in Paraguayan Agriculture. The Levy Economics Institute of Bard College. Working Paper No. 490.

[14] Mwaniki, A. (2006). Achieving Food Security in Africa: Challenges and Issues. Rep. N.p.: United Nations.

[15] National Population Commission (NPC)(2006). 2006 National Population Census Result.

[16] Organisation for Economic Co-operation and Development/Food and Agriculture Organization (OECD/FAO) (2015). Overview of the OECD-FAO Agricultural Outlook 2015-2024", in OECD-FAO Agricultural Outlook 2015. OECD Publishing, Paris.

[17] Onubuogu, G.C., Esiobu, N.S., Nwosu, C.S. and Okereke, C.N. (2014). Resource Use Efficiency of Smallholder Cassava Farmer in Owerri Agricultural Zone, Imo State Nigeria. *Scholarly J. Agric. Sci.* 4(6):306-318.

[18] Onumah, J.A., Al-Hassan, R.M. and Onumah, E.E. (2013a). Productivity and Technical Efficiency of Cocoa Production in Eastern Ghana. *Jour. Econ. Sustain. Dev*. 4(4):109-118.

[19] Onumah, J.A., Onumah, E.E., Al-Hassan, R.M. and Brümmer, B. (2013b). Meta-frontier Analysis of Organic and Conventional Cocoa Production in Ghana. *Agric. Econ. Czech.* 6:271-280.

[20] Oren, M.N. and Alemda, T. (2006). Technical Efficiency Analysis of Tobacco Farming in the South Eastern Anatolia. *Turk. J. Agric. For.* 30(2):165-172.

[21] Oruonye, E.D. (2014). Geographical Aspects of Jalingo Region. Phimela Concept Printing Press, Jalingo.

[22] Samuel, T.A., Debrah, I.A. and Abubakari, R. (2014). Technical Efficiency of Vegetable Farmers in Peri-Urban Ghana Influence and Effects of Resource Inequalities. *Am. J. Agric. For.* 2(3):79-87.

[23] Seyoum, E.T., Battese, G.E. & Fleming, E.M. (1998). Technical Efficiency and Productivity of Maize Producers in Eastern Ethiopia: A Study of Farmers within and outside the Sasakawa-Global 2000 Project. *Agric. Econ.* 19:341-348.

[24] Taraba Agricultural Development Programme (TADP)(2005). Taraba State Village Listing Form.

[25] Von Braun, J. and Mirzabaev, A. (2015). Small Farms: Changing Structures and Roles in Economic Development, ZEF- Discussion Papers on Development Policy No.204, Centre for Development Research, Bonn.

[26] Zijp W. (1994). Improving the transfer and use of agricultural information – a guide to Information Technology. Washington DC: World Bank.

Production Techniques and Quality Evaluation of Distilled Alcoholic Beverages (Rum Spirits) in Onitsha Metropolis of Anambra State, Nigeria

Sylvia Chinwe ONWUCHURUBA[1*], Ernest Chukwusoro IGWE[2]

Department of Food Science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
*Corresponding Author

Received:- 06 August 2021/ Revised:- 13 August 2021/ Accepted:- 21 August 2021/ Published: 31-08-2021

Abstract— *Evaluation of production techniques and quality of rum distilled alcoholic beverages (Rum spirits) sold in Onitsha metropolis, Anambra State, Nigeria was carried out using survey and laboratory studies. Rum distilled alcoholic beverage brands investigated included Nigerian and foreign makes. Nigerian made rums evaluated included DCL, SBD, S5BD, PBC, BWR, CBD and foreign made rums included KSCR, ELR, IVGR and GMCR. Field studies involved administration of questionnaires to the sellers of spirit alcoholic beverages in only three markets in Onitsha metropolis namely Ose-Okwodu, Relief and bridge Head markets that were purposely sampled for this study. Questions asked the sellers of distilled alcoholic beverages included names, background, and status of respondents and physicochemical characteristics of the products. There were also laboratory production of rum spirit beverage (LBC) based on survey studies and findings from producers. The laboratory produced and market samples were analyzed for physicochemical and organoleptic attributes. The mean and standard deviation of the data obtained were presented in tables whereas the statistical differences of the obtained data were determined by ANOVA ($P < 0.05$) using SPSS 22. The significant means were compared using Fisher's Least Significant Difference (LSD). The alcohol content, pH, titratable acidity, specific gravity, total solids, suspended solids and dissolved solids contents of the eleven rum spirit brands ranged from 42% to 50% and average of 47%, pH3.3- 5.1 (average pH value of 3.7), 011 to 4.5 (average value of 0.74), 0.87-0.99 and average value of 0.94, 3.03 to 32.18 (average of 8.45), 1.19 and 12.31(average of 6.08) and 1.53to 18.05 (average of 6.38) respectively. The consumer acceptance and preference evaluation of the rum spirit brands using 10 panelists and 9-point Hedonic scale revealed sensory scores of 3.70-6.40, 2.50-6.80, 3.20- 6.70, 2.60-6.70 and 3.30-6.60on colour, taste, aroma, mouth feel and general acceptance respectively.*

Keywords— Distilled Alcoholic Beverages, Rum Spirits, Anambra State.

I. INTRODUCTION

Alcoholic beverages have been prepared and consumed by mankind since ancient times in most of the world's culture (1; 2; 3; 4; 5).The forms of alcoholic beverages consumed in various regions of the world vary considerably in accordance to location and ingredients. But in many developing countries, various types of homemade or locally produced alcoholic beverages such as sorghum beer, palm wine or sugarcane sprits continue to be the main available beverage types (6).The global alcoholic drink industry exceeded USA $1 trillion in 2014 (7). Alcoholic drinks are most widely used recreational drugs in the world with about 33% of people being current drinkers (8). Furthermore, it was reported that alcoholic beverage production serves some nutritional, psychological, religious and social functions (9).

Alcoholic beverages are drinks that include wine of different sources; spirits such as brandy, whisky, gin, rum; and beer that contain ethanol, a type of alcohol produced by fermentation of grains, fruits or other sources of sugar (10). Depending on their alcoholic contents, Alexis, (11) reported that unsweetened, distilled alcoholic drinks that have alcoholic content of at least 20%Alcohol By Volume (ABV) are called spirits and that they include large variety of liquor produced with distillation process such as whiskey, Brandy, Gin, Vodka, Tequila, Rum, etc.

In general, spirits are liquids of high alcoholic contents which are obtained by distillation from such fermentable materials distilled to a point where they are purified yet still retain sufficient by-products to impart the particular characteristics of the original base material (12; 13;14). (15) Portrays distilled alcoholic beverages as being produced by distilling ethanol through fermentation of grains, fruits, vegetables, sugarcane juice, molasses, fermented mash of cereals and potatoes as well as fermented malt of barley and rye with alcohol content ranging between 40 and 60%. They further reported that the main ingredients for these beverages are water and ethanol and they account for all of 99% of the total content of the spirit beverages which range between 40 and 60% (9).

Rum is a distilled alcoholic beverage made from fermented sugar cane juice, sugar cane syrup or molasses (16). Most rums are produced from sugar cane molasses although as allied product cachaca is produced in Brazil from sugar cane juices (17;18). In Australia, rum is defined as a portable alcohol distillate from fermentation which unless otherwise required by this standard, contains at least 37% alcohol by volume, produced by distillation of fermented liqueur derived from food source so as to have the taste, aroma, and other characteristics generally attributable to rum (19).

In the U.S.A., rum is an alcoholic distillate from the fermented juice of sugar cane, sugar can syrup, sugar cane molasses, or other sugar cane by-products, produced at less than 190^{o} Proof in such manner that the distillate possesses the taste, aroma and characteristics generally attributed to rum and bottled at not less than 80^{0} Proof and also includes mixtures solely of such distillates (20). Elsewhere, European definition of rum portrays rum as a spirit drink produced exclusively by alcohol fermentation and distillation, either from molasses or syrup produced in the manufacture of sugar cane juice itself and distilled at less than 96% v/v, so that the distillate has discernible specific organoleptic attributes of rum; has the aromatic characteristic specific to rum and a content of volatile substances equal to or exceeding 225ghl-1 of alcohol of 100% v/v (2250 parts per million). It is bottled at minimum alcohol strength of 37.5% v/v (21).

The distinguishing features of rum from other spirits included its composition of higher quantity of caramel (0.6mls/litre), sweetener up to 9.6mls/litre sugar syrup and rum / coffee flavor that give the product discernible specific organoleptic characteristic of rum. The minimum alcoholic content of rum is 37.5% (22). Rum has different grades and colours such as gold rum, white rum, dark/ black rum and premium rum. Gin distilled spirit drink is colourless and flavoured with juniper berries. London dry gin may not contain added sweetening not exceeding 0.1g of sugar per litre of the final product nor colourants, nor any added ingredients other than water. The term 'London gin' is used interchangeably with dry gin. Gin has alcoholic strength of 37.0%ABV (22). Gin is termed "London dry gin" if it does not contain any sweetening agents (23).Whisky is bottled at not less than 40% ABV (80Proof). It contains caramel for colour enhancement and whisky flavor. Brandy contains caramel colouring, brandy flavor and bottled at not less than 36.0.% ABV(22).Brandy is a liquour produced by distilling wine which generally contains minimum of 36.0% alcohol by volume (22) and typically consumed as an after dinner digestive. Some brandies are coloured with caramel colouring to imitate the effect of aging while some are produced using combination of both aging and colouring. Vodka is a distilled beverage composed primarily of water and ethanol but sometimes with traces of impurities and flavourings. Flavoured Vodka is vodka with minimum alcoholic strength of 37.5%which has been sweetened, blended, flavoured with a predominant flavor other than that of the raw materials and matured or coloured (22).

NAFDAC Standard for rum include; 37.5%v/v minimum alcohol content, methyl alcohol(2ppm maximum), aldehydes (as acetaldehyde) 40ppm maximum, furfural (20ppm maximum), higher alcohol (50ppm maximum), total acidity (100ppm maximum), ethyl carbamate (0.015ppm maximum), ester (50ppm maximum), total ash (0.2ppm maximum), additives; food colours, flavourings, sweetening agents(As permitted by the agency), sulphur (10mg/kg maximum), appearance (free and clear), colour and flavor; characteristics of rumlead (0.2ppm minimum), arsenic (0.2ppm minimum), copper (2.0ppm minimum), iron (1.0ppm minimum). The product shall be free of pathogenic micro- organisms or their metabolic products or other toxic substances when analyzed according to the method prescribed in the standard (22)

The quality and identity of distilled alcoholic spirits is of paramount necessity especially as it pertains to their social, health and psychological implications. Therefore, this study was aimed at evaluating the production techniques and quality of rum spirit drinks, both produced and not produced in Onitsha metropolis, Anambra State.

II. MATERIALS AND METHODS

2.1 Sources of materials

This study covered Onitsha metropolis which included Onitsha and parts of adjoining towns in Ogbaru, Obosi, Ogidi, Ogbunike and Nkwelle Ezunaka. Onitsha is a seaport and market town in Anambra state, Nigeria with an estimated urban

population of over 7,425,000 (Demographia, 2016). The raw materials for laboratory produced rum spirit and market samples of rums were procured from this study location.

2.2 Research design

The design is such that the research consists of three phases namely field studies, production of rum based on findings of survey studies, laboratory and statistical analyses. Administration of questionnaires and oral interviews to the sellers and producers of rums in only three markets in namely Ose-Okwodu, Relief and Bridge Head markets that were purposely sampled for this study.

Questions asked included names background status of respondents and physicochemical characteristics of the products.The administration of questionnaires was supplemented with observation of production techniques for rum spirits among producers of rum in Onitsha metropolis.

Ten rums (distilled alcoholic beverages) four of which were foreign (produced outside Nigeria) and six Nigerian-made for this study were purposively and randomly obtained from three different markets in Onitsha metropolis (Onitsha and Ogbaru Local Government Areas) namely Ose-Okwodu, Relief and Bridge Head markets. These markets were purposively selected as they are generally known to be popular with sales of distilled alcoholic beverages. Details of the six Made-in-Nigeria and four Not-Made-in-Nigeria rum spirits that were selected for this study given in table 1:

One (1no.) rum spirit was self-produced in the laboratory following standard specifications for comparison with market samples. For ethical and legal considerations, the actual names of the sampled rums were not made public (Table 1).

TABLE 1
NAMES AND MARKET SOURCES OF SELECTED RUM SPIRITS FOR STUDY

No	Brand Name of Rum Spirits	Market-Sources	Country of production
1	DCL	Bridge Head market	Nigerian-Made
2	SBR	Ose- Okwodu	Nigerian-Made
3	S5BD	Bridge head market	Nigerian-Made
4	PBC	Ose- Okwodu	Nigerian-Made
5	BWR	Relief market	Nigerian-Made
6	CBD	Relief market	Nigerian-Made
7	KSCR	Bridge Head market	Not-Made-in-Nigeria
8	ELR	Ose-Okwodu	Not-Made-in-Nigeria
9	IVGR	Relief market	Not-Made-in-Nigeria
10	GMCR	Relief market	Not-Made-in-Nigeria
11	SBCR	Self-processed in laboratory	Nigerian-Made

2.3 Production of rum in the laboratory

The self-processed rum in the laboratory was labeled SBCR. The raw materials for the production of SBCR (Laboratory sample); (treated and de- mineralized water, food grade ethanol, sugar syrup, vanilla flavor, caramel, rum flavour and coffee flavour) were measured accurately with the help of weighing balance and calibrated stainless steel vessels. The batch formulation for production of SBCR was strictly maintained. The volume of water and ethanol used for the production of 48% SBCR measured into the mixing vessel. Ten litres of food grade ethanol and 9.7litres of de-mineralized water were measured into a mixing vessel (200L capacity) and the mixture stirred continuously for 3 to 5minutes. Fifty six milliliters of caramel was added, followed by the addition of 4 litres of sugar syrup, 64 ml rum flavour, 4ml vanilla flavor and 192ml coffee flavor making a total volume of 34.016 litres. The mixture was briskly blended for 10 to 20 minutes. The in-process sample was collected for sensorial (aroma, colour, taste and mouth feel and general acceptance) and physico- chemical analysis (p^H, specific gravity, titratable acidity, alcohol content, dissolved solids, suspended solids and total solids). Satisfactory result of the physico-chemical and sensorial analysis on the collected samples gave approval for filtration, aging for 24 hours and bottling (filling and capping) of the product. The products (SBCR) were sighted, labeled with appropriately date marked labels. Quality control checks were carried out on the finished product (SBCR) to ensure that the fill volume, date mark, batch code were properly done and sub-standard labels were not used. The products were also

checked for sensorial and physico-chemical parameters. The satisfactory products were stored on pallets in the laboratory shelf.

2.4 Methods of Laboratory Analyses

Physicochemical and sensory analyses on samples were conducted on market and self-produced samples. Analyses conducted on the samples included pH, titratable acidity, specific gravity and alcoholic content. Others were dissolved solids, suspended solids and total solids.

Standard methods were used in the various laboratory analyses. The P^H of the rums was determined using a P^H meter type pHs-2F, Harris, England (24).

Methods for other determinations included titratable acidity, for coloured spirits (25) and for non coloured spirits (25). Specific gravity (26); alcohol content determination (27); suspended solids (28) dissolved solids (28) and total solids (28). The sensory evaluation of the rum distilled beverage samples was carried out using a 9-point Hedonic scale by ten panelists (29).

2.5 Statistical Analysis

The mean and standard deviation of the data obtained were presented in tables whereas the statistical differences of the obtained data were determined by ANOVA ($P < 0.05$) using SPSS 22 (SPSS Inc., Chicago, and U.S.A). The significant means were compared using Fisher's Least Significant Difference (LSD)

III. RESULTS AND DISCUSSIONS

3.1 Production Techniques of Rum in Onitsha Metropolis

Field studies on the producers of rum spirit beverages in Onitsha metropolis showed that blending technique is used to produce their various rum categories such as blended white rum, blended dark rum, blended light rum/ blended rum and blended café rum. The choice of rum product adopted by each producer is dependent on the specific organoleptic and physico-chemical attributes desired which in turn affected the raw materials (Rum flavor, coffee flavor, sweetener (such as sugar, sugar syrup, sorbitol, fructose and aspartame), vanilla flavor, caramel, ethanol and water) utilized.

Ethanol and de-mineralized water (treated and de-ionized water) are the basic raw materials for any of the categories. It was discovered that two categories of distilled imported ethanol (Food grade sealed drums and refill drums) were available for production of distilled alcoholic beverages. It was also observed that we do not distil alcohol on commercial bases in Nigeria.

The producers of distilled alcoholic beverages all had treated and de-mineralized water sources, stainless steel mixing tank with in- built stirrer for blending the raw materials, ageing tank which gives room for proper reaction of the constituents and/ or continuous filling tank, from where the product flows into the filling machines for filling into desired packaging container. There was also filtration columns with different micron sizes of filters positioned between the mixing tank and prior filling. The producers have laboratory section where the physicochemical and sensorial parameters were determined on the raw materials (Weight, alcohol percentage, colour, taste, aroma, expiration date of the raw materials), in-process (alcohol percentage, colour, taste, aroma, pH, titratable acidity, total solids, dissolved solids, suspended solids, and specific gravity) before commencement of filling, and the finished product (batch coding, date marking, fill volume, colour, taste, aroma, specific gravity, alcohol percentage, pH, titratable acidity, total solids, dissolved solids and suspended solids), once the product was found to be in-line with the specifications for rum in accordance with regulatory standards of NAFDAC and SON.

Table 2 summarized the production techniques of rums produced and sold in Onitsha while table 3 gives a summary of rum spirits produced outside Nigeria and sold within Onitsha metropolis. Among the foreign rums, only ELR is registered with NAFDAC. All the locally produced rums are registered with NAFDAC. Both the foreign and locally produced rums are packaged in bottles. None of the rum spirit brands had nutritional labeling. There was no raw material listing on ELR, IVGR and GMCR. Their alcohol contents as specified on their labels range from 42% to 47%.

TABLE 2

NAMES AND PRODUCT CHARACTERISTICS OF RUMS PRODUCED IN ONITSHA METROPOLIS ANAMBRA STATE, NIGERIA

No	Product characteristics	Types of rum sold in Onitsha					
1	Product Name	DCL	SBD	S5BD	PBC	BWR	CBD
2	Place of manufacture	Ota, Ogun state, Nigeria	Ota, Ogun state, Nigeria	Nkpor, Anambra state Nigeria	Obosi, Anambra state, Nigeria	Ogidi, Anambra state Nigeria	Nkwelle-Ezunaka Nigeria
3	% Alcohol	45	42	45	43	40%	43%
4	Volume	100mls	700mls	120mls	100mls	120mls	120mls
5	Colour	Golden brown	Dark brown	Dark brown	Dark brown	Colourless	Golden brown
6	Nutritional labeling	NIL	NIL	NIL	NIL	NIL	Nil
7	Packaging	Glass bottle	Glass bottle	Pet bottle	Pet bottle	Pet bottle	Pet bottle
8	NAFDAC No.	Yes	Yes	Yes	Yes	Yes	Yes
9	Raw materials used	Ethyl alcohol, de-ionized water, sugar, coffee flavour, cream flavour, whisky flavour,	Demineralized water, ethyl alcohol, rum spirit, sugar, colour(E150(a)	Ethanol, treated water, sugar syrup, caramel, rum flavor	Ethanol, caramel, de-ionized water, rum flavor, coffee flavor, sugar syrup,	Food grade ethanol, treated water, sugar syrup	Ethanol, rum flavor, water, caramel

TABLE 3

NAMES AND PRODUCT CHARACTERISTICS OF FOREIGN-MADE RUMS SOLD IN ONITSHA METROPOLIS ANAMBRA STATE, NIGERIA

No	Product characteristics	Types of rum sold in Onitsha			
1	Product Name	KSCR	ELR	IVGR	GMCR
2	Place of manufacture	India	Spain	India	India
3	% Alcohol	47	47	42.8	42.8
4	Volume	70cl	70cl	750mls	750mls
5	Colour	Amber	Amber	Dark brown	Dark brown
6	Nutritional labeling	NIL	NIL	NIL	NIL
7	Packaging	Glass bottle	Glass bottle	Glass bottle	Glass bottle
8	NAFDAC No.	NIL	Yes	NIL	NIL
9	Raw materials used	Extra neutral alcohol, demineralized water, coffee extract, natural identical flavouring substance, sugar, colour (E150(a)	NIL	NIL	NIL

TABLE 4
PHYSICOCHEMICAL CHARACTERISTICS OF RUM SPIRITS MARKETED IN ONITSHA METROPOLIS

No	Name	% Alcohol	pH	T. - Acidity	Specific gravity	Total solids	Susp-solids	Dissolved solids
1	ELR	50.0±0.20^{d}	3.7±0.17^{ab}	0.16±0.06^{c}	0.92±0.01^{d}	4.40±0.40^{bc}	2.20±0.04^{c}	7.00±0.50^{f}
2	KSCR	48±0.10^{d}	3.7±0.35^{abc}	0.4±0.01^{f}	0.93±0.06^{d}	32.18±0.16^{g}	12.31±0.27^{h}	18.05±0.50^{h}
3	IVGR	47±0.10b^{c}	3.4±0.36^{ab}	0.64±0.00^{i}	0.94±0.00^{e}	10.64±0.99^{f}	5.90±0.05^{e}	6.44±0.41^{ef}
4	PBC-Rum	45±0.10^{b}	4.2±0.36^{cd}	0.47±0.00^{f}	0.99±0.00 ^{h}	7.09±0.30^{a}	5.57±0.21^{d}	1.53±0.21^{a}
5	S5BD-Rum	45±0.17^{b}	4.2±0.26^{cd}	0.56±0.00^{h}	0.97±0.00^{g}	3.03±0.30^{ab}	10.28±0.23^{g}	5.30±0.10^{cd}
6	SBD-Rum	42±0.17^{a}	4.7±0.10^{ef}	0.11±0.00^{a}	0.97±0.00^{g}	4.36±0.31^{bc}	6.43±0.01^{f}	5.59±0.18^{cde}
7	DCL-Rum	42±0. 00^{a}	4.5±0.26^{de}	4.5± 0.26^{de}	0.90±0.60^{c}	5.60±2.50^{de}	5.85±0.04^{e}	3.23±0.22^{b}
8	GMC-Rum	47±0.12^{bc}	3.7±0.38^{abc}	0.45±0.00^{e}	0.88±0.00^{b}	6.83±0.02^{d}	5.71±0.03^{a}	8.95±1.46^{g}
9	CBD-Rum	48±0.10^{cd}	5.1±0.10^{f}	0.31±0.06^{d}	0.99±0.00^{h}	6.35±0.14^{d}	5.90±0.04^{a}	4.57±0.66^{c}
10	CW-Rum	50±0.20^{d}	3.3±0.26^{a}	0.66±0.00^{j}	0.87±0.00a	3.42±0.05^{ab}	1.19±0.02^{b}	5.89±0.03^{de}
11	SBC-Rum	48±0.10^{cd}	3.9±0.10^{bc}	0.54±0.00^{g}	0.95±0.06^{f}	9.04±0.70^{e}	5.59±0.07^{f}	3.44±0.50^{b}

NB:
Values are mean± standard deviation of the three replicates
Values in the same column bearing different superscripts differ significantly ($p \leq 0{,}05$)

3.2 Physicochemical characteristics of rum produced in Onitsha metropolis

Physicochemical characteristics of eleven rum distilled alcoholic beverages (Rum spirits) both Nigerian and foreign makes including the laboratory produced (LBC) were evaluated and results are shown in Table 4.The alcohol content of the eleven rum spirit brands differed among themselves ($P \leq 0.05$). However, ELR and KSCR, CBD, BWL and LBC do not differ significantly, ($P \geq 0.05$) Also, SBD and DCL do not differ significantly ($P \geq 0.05$). PBC and S 5BD do not differ significantly ($P \geq 0.05$).

The alcohol content of the rum spirit brands range from 42% to 50% and average of 46.5%. ELR and BWR have the highest percentage alcohol content of 50% while DCL and SBD have the lowest alcohol percentage of 42%. This work differs with the works of (30) who reported the alcohol contents of native spirituous beverages from Africa including Burukutu (Sorghum beer), Ogogoro (Distilled palm wine) Kunnuzaki, whistle palmwine, Adoyo, Nunu, Fura da nunu, Zobo, and Omi wara to be 37.6%v/v for Ogogoro, 4.6%v/v for Burukutu, 3.1%v/ for Palm wine and others (0.3%).

Similarly, Cachaca, a typical and exclusive cane sugar liqueur of Brazil, is distilled from fermented must of the sugar cane broth with alcohol content from 38 to 48% v/v at 20^{o}c (31). However, this work is in line with the works of IARC *(32*) who reported the alcohol in alcoholic beverages to range from 3.2- 4.0% for beer, 3.2- 7.0% for malt liquor, 7.1-14.0% for table wines, 8.0-14% for sparkling wines, 14- 24% for fortified wines,40- 95% for hard liquor. 4.8% for beer, 10-22% for wines, 40- 50% for brandy and 40 – 55% for whisky and rum. (Shrikant *et al* (33) also revealed that distilled alcoholic beverages contain 40-60% alcohol. This work differs with the works of Yohannes and Siraj (34) who reported traditional spirituous beverages produced in Ethiopia Tella, Tej and Areki to have an average alcohol content of 5.17%.

The pH in conjunction with other storage or aged conditions (such as the oxidation reactions of some of the constituents and reactions with the contact surfaces of the storage container) play a key role in determining the organoleptic properties of spirituous beverages (35 ; 36). The pH of the rum spirit brands differ significantly ($P \leq 0.05$) but ELR, GMCR, BWR and IVGR do not differ significantly ($P \geq 0.05$). The range of the rum spirit varies from pH 3.3 to pH 5.1 with an average of pH 4.03. CBD had the highest pH value of 5.1 while BWR had the lowest pH value of 3.3 respectively IVGR and BWR.

An acidic pH is related to the presence of organic acids in the rum brands (37). Low pH could also be dependent on the oxidation reactions of some constituents in contact with metals (38) Low pH of BWR and IVGR could presumably, be because some of the volatile components that predominate in them are carboxylic acids that evidently contribute to the acidity of the beverage. Other components added along the production process might be responsible for the acidity (39).

The physico-chemical properties of some spirits beverages from Nigeria (Palm wine), Mexico (Tequilas), Ethiopa (Tella), and Cynthiana (Cynthiana) reveals that they have pH values 4.3, 3.5-4.9, 4.0,4.11 respectively (34). Similarly, Muoro, a Greek spirit distilled from fermented fruit of Mulberry tree has a pH equal to4.46 similar to tequila pH (4.7). Similar pH values observed in these spirit beverages may possibly be due to the fact that these spirits share similar raw materials and production procedures (40).

The titratable acidity of the rum spirit brands (Table 4) shows that the rum spirits differ significantly ($P \leq 0.05$). KSCR and PBC do not ($P \geq 0.05$). Similarly, GMCR and DCL do not differ significantly ($P \geq 0.05$).The titratable acidity of the rum spirit brands varies from 0.11 to 0.66 an average value of 0.80. SBD has the lowest titratable acidity value (0.11) while BWR has the highest titratable acidity value of 0.80.

The specific gravity of the rum spirit brands (Table 4), shows that the rum spirit brands differ significantly ($P \leq 0.05$). However, ELR and KSCR do not differ significantly ($P \geq 0.05$). Similarly, S5BD and SBD do not differ significantly ($P \geq 0.05$). PCR and CBD do not differ significantly ($P \geq 0.05$).The specific gravity of the rum spirit brands vary from 0.87- 0.99. PBC and CBD showed the highest specific gravity (0.99.) while BWR has the lowest specific gravity (0.87).

Total solids of the rum spirit brands (Table 4) shows that the brands KSCR differ significantly from the other brands ($P \leq 0.05$). Similarly, IVGR differed significantly ($P \leq 0.05$) from the other brands. The total solids of the rum spirit brands vary from 3.03 to 32.18 and an average of 8.45. S5BD blended dark rum has the lowest total solids (3.03) while KSCR has the highest total solids (32.18). The total solids revealed from this works compares favorably with the works of Oleiveras *et al* (41) on Palm wine (3.4%), Ogogoro (12%), Fura da nono (21.8%), Burukutu (7.9%).

The suspended solids of the rum spirit drinks (Table 4), shows that KSCR differed significantly ($P \leq 0.05$) from the other rum spirit brands. Similarly, IVGR differed significantly ($p \leq 0.05$) from the other brands ($P \leq 0.05$). The suspended solids of the rum spirit brands range from 1.12% and 12.31% with an average of 6.08%. BWR contains the lowest suspended solids

(1.12%) while KSCR has the highest value of suspended solids (12.31%). Adeleke and Abiodun (42) observed total suspended solids in ogogoro (3-10.7%) and Burukutu (0.8%).

The dissolved solids of the rum spirits show that they differ significantly ($P \leq 0.05$). However, ELR and IVGR do not differ significantly ($P \geq 0.05$) but the KSCR differed significantly from the other brands ($P \leq 0.05$). The dissolved solid content of the rum spirit brands vary from 1.53%- 18.05% with an average of 6.36%. PCR showed the lowest dissolved solids content (1.53%) while KSCR contained the highest dissolved solids (18.05%).

3.3 Sensory properties of rum alcoholic spirits

The sensory properties evaluated on the rum spirit brands for consumer acceptance and preference using 10 panelists on a 9-point Hedonic scale on colour, aroma, taste, mouth feel and general acceptance, gave results as presented in Table 5.

TABLE 5
ORGANOLEPTIC ACCEPTABILITY OF RUM SPIRITS MARKETED IN ONITSHA METROPOLIS

No	Name	Aroma	Taste	Colour	Mouth feel	General Acceptance
1	ELR	6.70 ± 1.70^{c}	5.50 ± 2.37^{bc}	6.40 ± 2.41^{b}	6.20 ± 2.25^{bc}	6.60 ± 1.43^{c}
2	KSCR	3.40 ± 2.27^{ab}	2.50 ± 1.96^{a}	4.40 ± 3.24^{ab}	2.60 ± 2.72^{a}	3.30 ± 2.41^{a}
3	IVGR	5.40 ± 1.71^{bc}	4.70 ± 2.67^{abc}	5.60 ± 1.51^{ab}	4.30 ± 2.21^{ab}	5.00 ± 2.54^{abc}
4	PCR	5.70 ± 1.57^{c}	4.50 ± 2.84^{abc}	4.70 ± 1.77^{ab}	4.30 ± 2.41^{ab}	5.90 ± 1.73^{bc}
5	S5BD	3.20 ± 12.82^{a}	3.00 ± 2.36^{a}	4.30 ± 2.95^{a}	3.60 ± 2.17^{a}	3.40 ± 2.01^{a}
6	SBD	4.90 ± 2.38^{abc}	4.50 ± 2.32^{abc}	4.00 ± 2.79^{ab}	3.70 ± 1.95^{a}	4.10 ± 1.80^{ab}
7	DCL	6.70 ± 2.26^{c}	6.80 ± 1.40^{c}	6.40 ± 0.97^{a}	6.70 ± 1.06^{c}	6.30 ± 1.50^{c}
8	GMCR	6.60 ± 2.37^{c}	6.10 ± 2.02^{bc}	5.80 ± 2.15^{ab}	4.60 ± 1.26^{ab}	6.20 ± 1.48^{c}
9	CBD	5.30 ± 1.70^{abc}	4.80 ± 1.55^{abc}	5.10 ± 1.91^{ab}	4.30 ± 1.95^{ab}	4.70 ± 2.06^{abc}
10	BWR	4.80 ± 2.90^{abc}	2.90 ± 2.51^{a}	3.70 ± 3.16^{a}	4.00 ± 2.49^{a}	4.60 ± 2.41^{abc}
11	LBC	5.50 ± 1.72^{bc}	4.10 ± 2.81^{ab}	5.60 ± 2.17^{ab}	3.80 ± 2.53^{a}	5.00 ± 2.10^{abc}

NB:

i. Values are mean± standard deviation of the ten replicates

ii. Values in the same column bearing different superscripts differ significantly ($p \leq 0,05$)

The aroma of the rum spirit brands as presented in Table 5, shows that the aroma of the rum brands do not differ significantly($P \geq 0.05$).S5BDdiffers significantly($P \leq 0.05$) from ELR. However, ELR do not differ significantly ($p \geq 0,05$) from PCR, DCL and GMCR Similarly, S5BD do not differ significantly ($p \geq 0,05$) from KSCR, SBD, CBD and BWR. The panelists' scores of aroma of the rum spirit brands vary from 3.20- 6.70. S5BD has the lowest score (3.20) and was disliked moderately.ELR and DCL on the other hand, scored the highest ((6.70).) and were liked moderately.

The taste scores of the rum spirit brands (Table 5), shows that they differ significantly ($P \leq 0.05$). DCL differed significantly from BWR, S5BD and KSCR. However, ELR, IVGR, SBD, PCR, GMCR, CBD and LBC do not differ significantly ($P \geq 0.05$). Similarly, KSCR did not differ significantly ($P \geq 0.05$) from IVGR, CBD, LBC, and SBD The range of panelist scores for taste of the rum spirit drinks vary from 2.50- 6.80. KSCR has the lowest score (2.50) and was disliked very moderately while DCL has the highest score (6.67).and was liked moderately

The colour of the rum spirit brands (Table 5) shows that the brands differed significantly ($P \geq 0.05$). ELR differed significantly ($P \leq 0.05$) from S5BD, DCL and BWR. However, ELR, did not differ significantly ($P \leq 0.05$) from KSCR, IVGR, PCR, SBD, GMCR, CBD and LBC. Panelists' scores of colour on the rum spirit brands varied from 3.70- 6.40. BWR has the lowest score (3.70) and was disliked slightly. On the other hand, ELR and DCL had the highest scores (6.40) and were liked slightly

The mouth feel of the rum spirit brands (Table 5) shows that the rum spirits differed significantly ($P \leq 0.05$). ELR rum and DCL do not differ significantly ($P \geq 0.05$). However, DCL, S5BD, SBDBWR, LBC, CBD, GMCR, KSCR, IVGR and PCR did not differ significantly ($P \geq 0.05$). Panelists's scores for the mouth feel of the rum spirit brands vary from 2.60 – 6.70. KSCR had the least score (2.60) and was disliked moderately while DCL had the highest score (6.67).and was liked moderately

The sensory scores of the general acceptance of the rum spirit brands (Table5), shows that the brands differed significantly ($p \leq 0,05$). KSCR differed significantly ($P \leq 0.05$) from ELR, IVGR, PCR, SBD, CBD, BWR and LBC, and GMCR. Similarly, SBD differed significantly ($P \leq 0.05$) from IVGR, PCR, CBD and LBC. However, KSCR, S5BD, IVGR, SBD, CBD, BWR, and LBC did not differ significantly ($P \geq 0.05$). Panelists' scores of general acceptance of the rum spirit brands varied from 3.30- 6.60. KSCR scored the lowest (3.30.)and was disliked moderately while ELR scored the highest (6.67) and was liked moderately.

IV. CONCLUSION

This study on the production techniques and quality evaluation of rum distilled alcoholic beverages (Rum spirits): a case study of Onitsha metropolis revealed that there are observable deviations in the physico-chemical and sensorial characteristics of some of the rum spirit brands evaluated, from the standards set by regulatory bodies like NAFDAC and SON. It is therefore evident, that better products can be achieved using optimization of some of the response variables like alcohol content, titratable acidity, total solids, dissolved solids, specific gravity colour and taste, in order to produce rum spirit beverage with better quality attributes.

REFERENCES

[1] Joshi, V.K., and Pandy, A. (1999) Biotechnology: Food Fermentation. In: Biotechnology: Food Fermentation.Joshi V.k. and Pandy, A. (ed.) Vol. 1. Educational Publishers and Distributors, New Delhi, pp. 1-24.fermented foods and beverages: A micro level survey. *Journal of Hill Research, 20 (1) : 1- 37.*

[2] *Sekar*, S., and Mariappan,S. (2007) Usage of Traditional Fermented Products by India in Rural folks and IPR, Indian Journal of Traditional Knowledge,6 () 1: 111-120.

[3] Tamang, J.P, Thapa N, Rai B.,Thapa S, Yonzan H, Dewan S, Tamang B, Sharma R, RaiA, and Chettri R (2007) Food consumption in Sikkim with special reference to traditional.

[4] Joshi, V.K., Thakur,N.S.,Bhatt A andGarg C. (2011) wine and brandy: A perspective in Handbook of Enology. Joshi, V.K (ed.) Vol.1, Asia – Tech Publishers, Inc., New Delhi,pp.3-45.

[5] Kanwar, S.S., Gupta, M.K.,Katoch, C. and Kanwar, P. (2011) Cereal based traditional alcoholic beverages of Lahaul and spiti area of Himachal Pradesh. *India journal of Traditional Knowledge, 10 (2) :251-257*

[6] WHO (2004) Global Status Report on Alcohol (2004) Geneva, World Health Organisation, Department of Mental Health and Substance Abuse.

[7] International Wines and Spirit Records (2019) Worldwide Alcohol Consumption Declines -1.6% Retrieved 2019-06-04.

[8] Grisworld, Max G. et. Al. (2018) Alcohol use and Burden for 195 Countries and Territories 1990 –2016; ASystematic Analysis for the global burden of disease study. The Lancet 392 (10152) :1015-35 doi: 10.1016/S0140- 6736 (18) 31310-2 PMC 6148333 PMID 0146330.

[9] Caton, S. J., Ball, M., Ahern, A. and Hetherington, M.M. (2004). Dose Dependent Effect of Alcohol onAppetite and food Intake, Physiology and Behaviour. 81 (1) : 51- 58.doi:101016/1 Physbeh 2003 12:01PMID 15059684 doi:10.1111/j.1365-2521.2005.00990.

[10] Gama P.M and Shemington K.B (1997).The Science of food. An Introduction to Food Science, Nutrition and Microbiology, UK:.Pergamon Press, Oxford.

[11] Alexis, Lichine. (1987) AlexisLichine's New Encyclopedia of Wines & Spirit (5^{th}edition) NewYork: Alfred A. knopfPp 236,707 - 709.ISBN978-03945626299.

[12] Burroughs D. and Bezzant N. (1990). The new wine Companion, 2^{nd} edition, Wine and Spirit Education Trust, Heinemann Professional Publishing: Oxford.

[13] Britannica.com (2013) Distilled Spirit/Distilled Liqour. Retrieved 2013-02-05.

[14] Encyclopedia Britannica (2019) "Distilled Spirit –alcoholic beverage" Retrieved 5 February 2019

[15] Shrikant,Bashngappa Swami, Thankor N.J, and Divate A.D. (2004). in Fruit Win Production *Journal of Food Research and Technology July- September 2004, Vol.2/Issue 3/ Pages 93-100. JakrayaPublications (P) ltd.*

[16] Nicol, Denis A. (2013) Fermented Beverages Production. Springer.com. Springer, Boston M.A Pp. 263-287.doi10.1007/978-1-4615-0187-9-12 Retrieved 15 January 2018.

[17] Faria, J.B, Loyola, E., Lopez, M.G. and Dufour J.P. (2003) Cachasa, Piso and Tequila. In: Fermented Beverage Production, Second edition, Edited by Lea A.G.H. and Piggot J.R., Kluwer Academic New York Pp. 355- 363

[18] Rosa, C. a., Soares A.M., and Faria J.b. (2009) Cachaca Production In: The Alcoholic Textbook (Ed. Ingledew, W.M, Kelsal, D.R., Aushin, G. D., Kluh Spies, C. 5^{TH} Edition, Nottingham University Press, Lallemand Ethanol Technology, Nolfolk. UK.)

[19] Food Standards Australia New Zealand (FSANZ) (2011) Spirits Standard 2.7.5. Available at: https://www.comlaw.gov..au/Series/F2008B00655. Accessed 8 April 2015

[20] U.S.A Government (2010) 27CFR 5.22.

[21] European Union Regulation (1989) Council Regulation (EEC) No. 1576/89 of 29 May 1989 laying down general rules on the definition, description and presentation of spirit drinks. Available at: wipolex.wipo.int

[22] NAFDAC (2019) National Agency for Food and Drug Administration and Control act 1993 (As amended) Spirit drinks Regulations 2019.

[23] Buglass, Alan J. (2011). Handbook of Alcoholic Beverages: Technical, Analytical and Nutritional Aspects John Willey & SonsLtd. ISBN 978-0-470-51202-9.

[24] AOAC (1990) Official Methods of Analysis, 15TH edition, Association of Official Analytical Chemists Virginia, USA. 692-6937511058 -1061.

[25] AOAC (2000) Official Methods of Analysis of Association of Analytical Chemists, 25th Edition AOAC, Washington D.C

[26] Ayo, J.A. and Agu, H.O. (2012) Simplified Manual of Analysis for Tertiary and Research Institutions Vol. 1. Amana Printing and Advertising Ltd. Kaduna, Nigeria.Pp.77-104

[27] Manual of Methods for Analysis of Alcoholic Beverages (2015) Food Safety and Standards Authority of India. Ministry of Health and Family Welfare, Government of India, New Delhi.

[28] EAS, (2014) Alcoholic Beverages, Method of Sampling and test2nd Edition KSEAS 104:204 ICS 67 160.10.

[29] Iwe, M.O (2003) Handbook of sensory methods and analysis. Rejoint communication, Enugu.

[30] Adeleke R.O and Abiodun O.A. (2010) Physico-chemical properties of commercial local beverages in Osun State, Nigeria." *Pakistan Journal of Nutrition, 9 (9) ;853-855.*

[31] BRAZIL Ministerio da Agricultura (2005) Pecuariae Abastrecimento *Instrucao Normativan°13, 29 June,2005 [cited 2011 Jan. 21] Available from: http://www.receita. Fazenda.gov.br/legislacao/decetos/ant20/01/1992/dec231497.htm>*.

[32] IARC. (1988) Consumption of Alcoholic Beverages; Types and Ethanol Contents of Alcoholic Beverages IARC Monograghs, 96: Pp 41-42.

[33] Shrikant,Bashngappa Swami, Thankor N.J, and Divate A.D. (2004). in Fruit Win Production *Journal of Food Research and Technology July- September 2004. Vol.2/Issue 3/ Pages 93-100. Jakraya Publications (P) ltd.*

[34] Yohannes, T., Melak, K., and Siraj, F., (2013) Preparation and Physico-chemical Analysis of some Ethiopian Traditional Alcoholic Beverages "*African Journal of Food Science.7 (11) 399-403.*

[35] McRae J.M., kassara S., Kennedy J.A, Waters E.J. and Smith P.A. (2013). The Effects of Wine pH and Bottle Closure on Tannins "*Journal of Agriculture and Food Chemistry.61 (4) : 11618-11627.*

[36] Hopfer, H., Buffon, P.A., Ebeler, S.E., and Heymann (2013). "The Combined Effects of Storage Temperature and Packaging on the Sensory, Chemical and Physical Properties of a Cabamet Sauvignon wine *"Journal of Agriculture and Food Chemistry*, 16 (13) : *3320-3334*.

[37] Benn S.M. and Peppard T.L, (1996) Characterization of tequila flavor by instrumental and sensory analysis," *Journal of Agricultural and Food Chemistry, 44 (2) ; 557-566.*

[38] Prado-Ramirez R, Gonzalez-Alvarez V, Pelayo-Ortiz C, Casillas N, Estarron M, and Gomez-Hernandez H.E. (2005) The role of distillation on the quality of tequila, *"Journal of Food Science and Technology, 40 Pp.701-708.*

[39] Ibanez J.G, Carreon-Alvarez A, Barcena-Soto M, and Casillas N. (2008) Metals in alcoholic beverages: a review of sources, effects concentrations, removal, speciation and analysis, "*Journal of Food Composition and Analysis,* vol. 21, no.8, Pp 672-683.

[40] Souflerous, E.H., Mygdalia A, S. and Natskoulis P. (2004) " Characterization and safety evaluation of the Traditional Greek Fruit Distillate 'Muoro' by flavor compounds and mineral analysis" *Food Chemistry, 86 (16) : 4 Pp 625- 636.*

[41] Oleiveras–Lappe P., Moreno- Terrazas, R., Arrizon- Gavino J., Herrera-Suarez T., Garcia Mandoza A. and Gschaedler- Mathis A. (2008) " Yeast associated with the production of Mexican alcoholic non- distilled and distilled Agave beverages. *FEMS Yeast Research 8 (7) :7 Pp 1037- 1052.*

Economic Efficiency and Profitability of Sweet Potato Marketing in Anambra State, Nigeria.

Isibor, C.A.[1]; Nkamigbo, D.C[2*]; Ekeke, N.C.[3]

Department of Agricultural Economics and Extension,
Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Nigeria.
*Corresponding Author

Received:- 10 August 2021/ Revised:- 18 August 2021/ Accepted:- 24 August 2021/ Published: 31-08-2021

Abstract— *The study examined the economic efficiency and profitability of sweet potato marketing in Anambra State, Nigeria. Specifically, it described profitability, economic efficiency and constraints to sweet potato marketing. Multistage sampling procedure which involved purposive and random sampling methods was used to select 240 marketers (120 wholesalers and 120 retailers). Data were collected from primary source using well structured questionnaire and were analyzed by means of descriptive statistics, enterprise budgeting and Sherpherd-Futell techniques. From the result, profitability indicators such as net marketing income, return on investment, net return on investment and coefficient of marketing efficiency of ₦ 8,775,807.4, 1.68, 0.68, 59.6 and ₦7, 892,837.4, 1.89, 0.89, 52.6 for wholesalers and retailers respectively proved the enterprise profitable at both levels. The implication of the net return on investment figures is that the wholesalers and retailers respectively return 0.68 kobo and 0.89 kobo for every 1 Naira invested in the business. Findings also indicated marketing efficiency levels of 59.6% for wholesalers and 52.6% for retailers implying that the retailers are more efficient in the marketing of sweet potato than the wholesalers. Findings on the constraints shows that seasonality of the product, high cost of transportation and rioting militated against sweet potato marketing on the wholesale whereas rioting and inadequate storage facility were perceived at the retail levels. Modern storage facilities and good road transport systems should be made available so that the volume of trade of marketers should increase for optimum profit*.

Keywords— Economic, Efficiency, Profitability, Sweet potato.

I. INTRODUCTION

Agriculture is Nigeria's most assured engine of development, a reliable key to industrialization, food security and a live wire to a well standardized country ensuring sustainability and poverty alleviation Nkamigbo, Ugwumba and Okeke (2019). Central Bank of Nigeria (CBN)(2014) opined that in Nigeria, the agricultural sector contributes about 42% of the gross domestic product (GDP) and provides employment to more than 70% of the people. Okeke and Nkamigbo (2013) and Isibor and Nkamigbo (2019) reported that agricultural sector is an engine room for sustaining growth of Nigeria economy and still remain the mainstay of our economy by providing food for the teeming population, create jobs as well as wealth, raw materials for industrial sector and foreign exchange earnings.

Sweet potato (*Lpomea batatas L*) is an herbaceous, warm-weather creeping plant that belongs to the family of Convolvulaceae and genus Ipomoea. It originated from South America where it was introduced to Europe between 153AD Sanusi and Adesogan (2014) and Udemezue (2019). The family is made up of 45 genera and 1000 species. Sweet potato grows throughout the world from latitude 400N to latitude 350S. It grows best at a temperature of between 24^{o}C with annual rainfall of 1000mm to 7000mm Mbanaso (2010) and Udemezue (2019). Sweet potato is regarded as world most important food crop due to its high yield. It is the fifth most important food crop after rice, wheat, maize and cassava in developing countries like Nigeria and the seventh most important food crop in the world in terms of production Sanusi, Lawal, Sanusi and Adesogan (2016) and Udemezue (2019). Nigeria is one of the largest producers of sweet potato in sub Saharan Africa with annual production estimated at 3.46 million tonnes per year and fourth largest producer in Africa while Egypt is Africa number one producer followed by Malawi. It was introduced into Nigeria in the late 1694-1698 through the early activities of the Portuguese and Spanish explores (Mbanaso (2010). Production of sweet potato was encouraged by the British colonial government during the Second World War as their tubers were needed to feed their armed forces in West Africa. Since then,

the importance of potato has been widely realized such that it is now as an important commodity in both local and international trade Ugonna, Jolaoso and Onwualu (2013).

Sweet potato is a major source of carbohydrate for millions of people especially in developing countries like Nigeria. It a great source of minerals like Manganese, Folate, Copper and Iron. The darker coloured variety is a great source of Carotenes (Precursor of Vitamin A), Vitamin C, B_2, B_6, E and biotin and also a source of dietary fibre, antioxidants which work in the body to prevent inflammatory problems like asthma, arthritis and regulate blood sugar level Sanusi *et al.*, (2016). They also noted that sweet potato can be used to fight against wide spread of vitamin A deficiency that result in blindness and even death of about 25000-500000 African children per year. Vitamin A deficiency is a particular problem with children under five and for pregnant and lactating women. Serious Vitamin A deficiency can weaken the immune system leaving them susceptible to diseases such as measles, malaria, and diarrhea and can also lead to blindness.

Sweet potato plays a great role in developing countries; it provides job opportunity to teeming population by raising their income. The demand for sweet potato is quite higher than the supply Ajakaije and Akande (1999) as cited by Adewumi and Adebayo (2008). This is as a result of its high nutritional value, cheap and inexpensive of the product compared to other root source of carbohydrates and vitamin. The leaf of potato can be use to feed animal either fresh or in the form of silage. The tubers can be consumed by man either boiled, roasted or fried. It can be dehydrated into chips, canned, cooked and frozen, creamed and used as pie fillings. It could also be dried and ground into flour to make biscuits, bread and other pastries. Sweet potato can be pounded together with yam to give a delicious meal Udemezue (2019) .

Baby food has being formulated using sweet potato while some bakeries blend 15-30% of sweet potato flour for making bread and 20-30% for pastries. It is used for brewing of alcoholic drinks and as sweetness in non-alcoholic drinks Agbo and Ene (1992). Sweet potatoes have medicinal value, the leaf decoction is used in folk remedies for asthma, bug bites, burns, catarrh, ciguatera stomach distress, tumor and whit lows. Sweet potato starch can be used in textile, glue, paint and cardboard industries. Industrial potentials of sweet potato have not been exploited due to mainly chronic lack of awareness to the abundance of industrial and commercial benefits.

It was reported by Sanusi *et al*, (2016) that sweet potato production has recorded good profit margin and is suitable for income generation. It has the potential for food security as well as serving as a cash crop. Sweet potato is becoming a thriving business in the State due to its economic, nutritious and commercial value and it is readily available in every market called sweet potato market. They further reported that sweet potato marketing has a large potential to enhance agribusiness development, generate income and employment opportunities that will lead to significant impact in the rural sector and non-producing areas. Sweet potato from the farm reaches the consumers through the marketing system. Nkamigbo and Isibor (2019) reported that marketing involves all processes in the movement of products that consumers need from the point of production to the point of purchase. Marketing is concerned with all stages of operation which facilitate the movement of commodities from the farm to the consumers. Marketing has economic value because it gives form, time and place utility (Asogwa and Okwoche (2012) Osondu, Nwadike, Ijeoma, *et al.*(2014) further explained that efficient marketing plays a crucial role in an economy. This role becomes more evident in areas where there is high rate of urbanization. The marketing system enables producers as well as middlemen to earn income with which they purchase other useful goods and services Ebe (2007) as cited by Nkamigbo, Ugwumba and Okeke (2019) .

Ejechi, Anyagbunam, Okoye and Eleodinmuo (2014) noted that there has been growing activities in the marketing of sweet potato due probably to increasing consumer/marketer's awareness of its economic, nutritional and medicinal values. Sweet potato is either sold as a whole, roasted or fried. The State has several daily markets both in the urban and rural areas where agricultural produce are sold especially sweet potato known as sweet potato market.

II. MATERIALS AND METHODS

The study was carried out in Anambra State, Nigeria. Several raw industrial materials and agro products are produced in various parts of the State. Some of the crops grown in the State include oil palm, maize, rice, yam, groundnut, cassava, sweet-potato, cucumber, watermelon, melon, green-beans (akidi), pigeon pea, soybean, livestock such as goats, sheep, poultry and cattle are also raised. It is an agrarian State and majority of the people are subsistence farmers. It is situated on a generally low elevation on the Eastern side of the River Niger sharing boundaries with Delta State to the west, Imo, Abia and Rivers State to the South, Enugu State to the East and Kogi State to the North. The State occupies an area of about 4844 Km^2. Geographically, the State lies within longitude 5^o55^1 and 6^o42^1N. The population of the State is 4,182,232 with 863 Sqkm density (NPC, 2016).

The State has several daily markets both in the rural urban areas where agricultural goods are sold especially sweet-potato known as sweet potato markets. Sweet potato is a thriving business in the State due to its nutritional, medicinal, industrial value, population and economic returns. It is either sold as a whole, fried or roasted.

The State consists of twenty one (21) Local Government Areas (LGAs) and four agricultural zones. The state is drained by five major River, Ezu River, Idemili River and Ulasi River. In addition to these, there are smaller perennial streams like the Oji, Nkisi and Obizi. In-land valley ponds and lake occur with the Agulu Lake draining a collection of towns in the State Nwalieji (2016). The state has two distinctly marked seasons: rainy and dry seasons. The rainy seasons occurs from the month of March through October. The dry season occupies the months of November to February. The annual rainfall ranges from 1400 mm in the North to 2500 mm in the South with temperature of 25^0C-35^0C.

2.1 Population and Sampling Procedure

The study population was made up of all sweet potato marketers in Anambra State, Nigeria. Multistage, purposive and random sampling methods were used to select 12 Local Government Areas, 12 daily sweet potato markets and 240 intermediaries (120 wholesalers and 120 retailers) for the study. The respondents were selected based on size of the markets. Details of the selection process are given as:

Stage 1: Three agricultural zones were randomly selected from the four agricultural zones of the State. These are Onitsha, Anambra and Aguata zones.

Stage 2: Four Local government areas were randomly selected from each of the three selected agricultural zones, totaling 12 LGAs. The LGAs selected were Ogbaru, Onitsha North, Onitsha South, Ihiala, Anambra North, Anambra West, Oyi, Ayamelum, Aguata, Nnewi North, Orumba North and Orumba South.

Stage 3: This involved purposive selection of one daily market with large number of intermediaries and consumers from each selected LGAs. The selection was based on opened dairy nature, large number of intermediaries and volume of produce handled per month as revealed by pre-test survey. A total of 12 markets were selected, the market were Afor Atani, Ose-Okwaodu, Ochanja, Nkwo-Okija, Eke-Otuocha, Nkwo-Otupu, Eke-igwe Nteje, Nkwo-Omor,, Nkwo-Igboukwu, Nkwo-edo Nnewi,Afor-Ufuma and Nkwo-Umunze.

Stage 4: Ten sweet potato markets, consisting of five wholesalers and five retailers were randomly selected from each of the selected twelve markets in stage iii, thus making a total of 240 respondents for the study as shown in Table 1.

TABLE 1
SAMPLING OF MARKETS AND RESPONDENTS

Agricultural zone	LGAs selected	Markets selected	Intermediaries selected
Onitsha	Onitsha North	Ose-Okwaodu	5 Wholesalers 5 Retailers
	Onitsha South	Ochanja-Market	5 Wholesalers 5 Retailers
	Ihiala	Nkwo-Okija	5 Wholesalers 5 Retailers
	Ogbaru	Afor Atani	5 Wholesalers 5 Retailers
Anambra	Anambra East	Eke-Otuocha	5 Wholesalers 5 Retailers
	Anambra West	Nkwo-Otupu	5 Wholesalers 5 Retailers
	Oyi	Eke-Igwe Nteje	5 Wholesalers 5 Retailers
	Ayamelum	Nkwo-Omor	5 Wholesalers 5 Retailers
Aguata	Aguata	Nkwo Igboukwu	5 Wholesalers 5 Retailers
	Nnewi North	Nkwo-edo Nnewi	5 Wholesalers 5 Retailers
	Orumba North	Nkwo-Umunze	5 Wholesalers 5 Retailers
	Nnewi South	Afor-Ukpor	5 Wholesalers 5 Retailers
Total	12 LGA	12 Markets	240 Respondents

Source : Field survey, 2021.

III. METHOD OF DATA ANALYSIS

The objectives of the study were realized using budgetary technique, Sherpherd-Futrel technique and relative index ranking. The budgeting technique was used to determine the profitability of sweet potato marketing. The budgeting technique is expressed as:

$$\text{NER/Profit} = P_{yi}Y_{yi}\sum(P_{XIJ}X_{ij} + F_{ij})$$

Where

$\sum$ = sum

$P_{yi}Y_i$= unit price of i^{th} respondents sales + Total revenue (TR) for i^{th} respondent.

$P_{xi}Y_i$ = Prices X qualities of i^{th} respondents variable inputs= total variable cost (TVC) for j^{th} respondent. The marketing efficiency of farmers' using social network to advance agribusiness was determined using Sherpherd-Futrell technique.

$$ME = \frac{TC}{TR} \times 100$$

Where:

ME= Coefficient of marketing efficiency,

TC= Total marketing cost incurred

TR= Total value of product sold

IV. RESULT AND DISCUSSION

4.1 Profitability of sweet potato marketing by the intermediaries

The enterprise budgeting analysis was used to estimate the monthly profitability of sweet potato marketing by the intermediaries as shown in Table 2. Result of the analysis, indicating total cost (TC), total revenue (TR), total variable cost (TVC), total fixed cost (TFC), gross margin (GM), net marketing income (NMI), mean net marketing income (MNMI) and net return on investment (NROI), is presented in Table 2. It could be seen from the Table that out of the total cost of ₦12, 974,192.6 spent by the wholesalers, purchases constituted 87.3% while the least expense was miscellaneous cost 1.03%. Similarly, the retailers spent 91, 06% of their total cost on purchase and 0.58% on miscellaneous cost as the least expense. By this, cost of purchasing marketing stock is the most important cost of business while the cost on miscellaneous is the least. This result is in tandem with Nkamigbo and Isibor (2019) who reported that the cost of stock/purchase constituted 94.2% and 89.76% of the total cost of marketing dry maize and watermelon respectively to become the most important cost to consider in starting the marketing enterprise.

On enterprise profitability, the wholesalers realized ₦21,750,000 after spending a total of variable cost of ₦12,795,168.4 and total cost ₦12,974,192.6. This transaction generated a gross margin of ₦8,954,831.6, net marketing income of ₦8,775,807.4 and net return on investment of 0.68. The retailers on the other hand realized ₦16,669,906.5 after spending a total variable cost of ₦8,675,540 and total cost of ₦8,777,069.1, with gross margin of ₦7,994,366.5, net marketing income of ₦7,892,837.4 and net return on investment 0.89. The implication of the net return on investment figures is that the wholesalers and retailers respectively return 0.68 kobo and 0.89 kobo for every 1 Naira invested in the business. Overall, the profitability indicators (gross margin, net marketing income and net return on investment values) showed that sweet potato marketing was a profitable at both wholesale and retail levels. Adewumi and Adebayo (2008) and Udemezue (2019) attested to the profitability of sweet potato marketing in Nigeria.

TABLE 2

ESTIMATED MONTHLY PROFITABILITY OF SWEET POTATO MARKETING BY THE INTERMEDIARIES

Variables	Wholesalers	% TC	Retailers	%
TOTAL REVENUE	21,750,000		16,669,906.5	
VARIABLE COST (VC)				
Purchases	11,174,655	87.3	7,900,356.5	91.06
Transportation	965.000	7.54	422000	4.86
Loading	317,753.4	2.4	177,000	2.04
Off-loading	205,200	1.60	95,153.5	1.09
Miscellaneous cost (Recharge card, Cement bag, Nylon bag)	132,560	1.03	51,030	0.58
TOTAL VARIABLE COST (TVC)	12,795,168.4		8,675,540	
FIXED COST (FC)				
Monthly shop rent	97,800	54.62	39000	38.41
Ground levy	31,000	17.31	41000	40.38
Depreciation on equipment (wheelbarrow, tarpaulin, knife, Table, Chair)	28,931.6	16.16	11835	11.65
LGA charges	11,292.6	6.30	5000	38.41
Interest on Loan	10,024.2	5.59	4694.1	4.62
TOTAL FIXED COST (TFC	179,024.2		101,529.1	
TOTAL COST TC= TVC+TFC	12,974,192.6		8,777,069.1	
Gross Margin	8954831.6		7,994,366.5	
Net Marketing Income NMI=GM-TFC	8775807.4		7,892,837.4	
Return on Investment TR/TC	1.68		1.89	
Net return on Investment NMI/TC	0.68		0.89	
Gross Ratio TC/TR	0.596		0.526	
Marketing Efficiency TC/TR x 100/1	59.6		52.6	

Source: Field survey, 2021

4.2 Marketing Efficiency of sweet potato.

The Shepherd-Futrel method was used to determine the co-efficient of marketing efficiency. The method expresses marketing efficiency as the ratio of total cost to total revenue expressed as percentage. The lower percentage, the better the marketing efficiency, since less proportion of the revenue will be expanded on total cost of marketing.

The model is slated as

$$ME = \frac{TC}{TR} \times \frac{100}{1}$$

Wholesalers $ME = \frac{TC}{TR} \times \frac{100}{1} = \frac{12974192.6}{21,750,000} \times \frac{100}{1} = 59.6\%$

Retailers $ME = \frac{TC}{TR} \times \frac{100}{1} = \frac{8777069.1}{16,669,906.5} \times \frac{100}{1} = 52.6\%$

Where:

ME = Marketing efficiency

TC = Total cost

TR = Total revenue

The result of the analyses revealed that none of the intermediaries attained efficiency of 100% in the marketing of sweet potato implying the existence of good level of inefficiencies among the intermediaries (wholesalers and retailers). The level of inefficiency was higher 59.6% among the wholesalers than the retailers implying that the retailers were more efficient in the marketing of sweet potato than the wholesalers. The retailers do not spend much on transportation and miscellaneous because they source their product from nearby market which resulted to reduced cost. Nkamigbo and Isibor (2019) confirmed that none of the intermediaries attained optimal efficiency of 100%.

TABLE 3
CONSTRAINTS TO SWEET POTATO MARKETING

Constraints	Wholesalers mean score	Rank	Retailers mean score	Rank
Rioting	3.10	3rd	3.11	1st
Breakage on Transit	2.80	5th	2.58	5th
Low market price	2.50	6th	2.74	4th
Seasonality of the product	3.36	1st	3.01	3rd
Inadequate storage facility	2.90	4th	3.05	2nd
High transport cost	3.20	2nd	2.50	6th

Source: field survey, 2021.

V. CONCLUSION AND RECOMMENDATION

The result established by profitability indicators (gross margin, net marketing income and net return on investment values) that sweet potato marketing was a profitable venture both at wholesale and retail levels. Also, the retailers are more efficient in the marketing of sweet potato than the wholesalers although inefficiencies existed among their activities due to marketing constraints. The level of profitability would improve if adequate measures are taken by government and marketers to address marketing constraints. It was recommended that modern storage facilities and good road transport systems should be made available so that the volume of trade of marketers should increase for optimum profit.

REFERENCES

[1] Nkamigbo, D.C, Ugwumba, C.O.A., Okeke, Uche. (2019). Market structure, conduct and volume of trade among watermelon marketers in Anambra State, Nigeria. *International Journal of agriculture and Biosciences*, 8(2), 112-116.

[2] Central Bank of Nigeria, (CBN, 2014). Statistical Bulletin: Financial Statistics, Second Quarter, Abuja, Nigeria.Retrived from http:www.cenbank,org/documents/stabulletin.asp.

[3] Okeke, C.O., Nkamigbo, D.C. (2013). Costs and revenue of different legume based intercrop combination in Awka North LGA, Anambra State, Nigeria. *Journal of vocational and Technical Education*, 8(2), 164-174.

[4] Isibor, A.C,Nkamigbo, D.C.(2019). Economic Assessment of the Total Amounts of Repayments and Default by Large and Small Scale farmer-Beneficiaries of Bank of Agriculture Loans from 2010-2016 in Anambra State, Nigeria.. *Journal of Natural Science Research*, 9 (14), 9-13.

[5] Sanusi, M.M., Adesogun, A.O.(2014). Resource use efficiency in sweet potato production in Odeda LGA, Ogunu State, Nigeria. *Journal of Basic and Applied Science*, 22(3&4),111-117.

[6] Udemezue, J.C. (2019). Profitability and constraints to sweet potato production in Nigeria. *Current Trends Biomedial Eng & Biosciences*,19(2),1-5.

[7] Mbanaso, E.O. (2010). Adoption and dis-adoption of sweet potato production and processing technologies by farmers in South-Eastern Nigeria. Unpublished Ph D thesis, Department of Agricultural Extension, University of Nigeria, Nssuka.

[8] Sanusi, M.M., Lawal, O.I., Sanusi, R.A., Adesogan, A.O.(2016). Profitability of sweet potato production in derived Savannah zone of Ogun State, Nigeria. *Journal of Agriculture and Social Research*,16(1), 16-27.

[9] Ugonna, C.U., Jolaso, M.O., Onwualu, A.P. (2013). A Technological Appraisal of Potato Value Chain in Nigeria. International Research Journal of Agriculture, Science and Soil Science, 3(8), 767-769.

[10] Adewumi, M.A., Adebayo, F.A.(2008). Profitability and technical efficiency of sweet potato production in Nigeria. *Journal of Rural Development*,31 (5), 105-120.

[11] Agbo, I., Ene, C.S.O.(1992). Status of sweet potato production and research in Nigeria. Sweet potato situation and Priority in Research inWest Africa. Proceedings of a workshop held in Dovia, Cameroon from July 27th to 29th. International Sweet potato Research Center, Lima, Peru.

[12] Nkamigbo, D.C., Isibor, A.C.(2019). Inter-Market Price Spread and Determinants of Net Farm Income in Watermelon marketing in Anambra State, Nigeria. . *International Journal of Agricultural Policy and Research*,7(4), 100-107.

[13] Asogwa, B.C., Okwoche, V.O. (2016). Marketing of Agricultural produce among rural farm household in Nigeria. The case of Sorghum marketing in Benue State. *International Journal of Business and Social Science*,3(13), 2609-2770.

[14] Osundu, C.K., Nwadike, F.C, Ijeoma, J.C., Udah, S.C, Ugboaja, C.I.(2014). Marketing performance of salad vegetables. The case of cabbage marketing in Abia State, Nigeria. *International Journal of agricultural science Research and Technology in Extension and Educational Systems*, 4(3),151-162.

[15] Ejechi, M.E, Anyaegbunam, H.N, Okoye, B.C, Eleodinmuo, P.O. (2016). Determinants of marketing efficiency of sweet potato market structure in Nasarawa State and FCT, Nigeria. *Agricultural Journal*, (2), 23-28.

[16] National Population Commission.(2006). Population Census Report national Population Commission of Nigeria, Abuja.

[17] Nwalieji, H.U.(2015). Comparative analysis of rice production enterprises among farmers in Anambra and Eboyi States, Nigeria. *Asian Journal of agricultural extension, economics and sociology*, 3(2), 23-27.

Mesquite (*Prosopis Juliflora*) Pod Meal to Goats Feed: Ruminal Parameters and Molecular Diversity of Ruminal Bacteria and Methanogenic Archaea

Lizziane Argôlo-Batista[1*], Mara Lúcia Albuquerque Pereira[2], João Carlos Teixeira Dias[3], Herymá Giovane de Oliveira Silva[4]

[1]Instituto Federal de Educação, Ciência e Tecnologia Baiano, Itaberaba *Campus*, Zipcode 46880-000, Itaberaba, Bahia, Brazil.
[2,4]State University of Southwest Bahia, Itapetinga *Campus*, BR 415, Km 03, Zipcode 45700-000, Itapetinga, Bahia, Brazil
[3]State University of Santa Cruz, Soane Nazaré de Andrade *Campus*, Rod. Jorge Amado, Km 16 - Salobrinho, Zipcode 45662-900, Ilhéus, Bahia, Brazil
*Corresponding Author

Received:- 09 August 2021/ Revised:- 18 August 2021/ Accepted:- 24 August 2021/ Published: 31-08-2021

Abstract**—** *This study aimed to evaluate the effects of mesquite (Prosopis juliflora) pod meal (MPM) replacing corn in concentrate feeds on ruminal parameters and microbial diversity. MPM was used in 0.0, 33.3, 66.7 and 100% levels in isonitrogenous diets, and elephant grass (Pennisetum purpureum) silage as forage. For the experiment we divided the animals into 4x4 Latin square. The intake of dry matter, crude protein, neutral detergent fiber and acid detergent fiber were not affected by the MPM levels. The pH varied linearly, increasing according to the levels of MPM and remained at adequate range between 6.32 and 6.85 for 8 hours after feeding. The ammonia concentration showed a peak of 14.01 mmol L^{-1} 2 hours after the morning feeding and the acetate, propionate and butyrate concentrations did not show any effect. The genetic diversity of bacteria and archaea was determined by PCR-DGGE. The analyses showed variations in banding pattern, indicating changes in the populations studied as a result of the treatments and a reduction in methanogenic after the addition of up to 66.7% of MPM. MPM can be used at levels of 33.3% and 66.7% of corn replacing without reducing the nutrients intake. The reduction of archaea has a possible contribution in reducing methanogenesis, since it also reduces the acetate:propionate ratio. Mesquite is a source of food for goats in small holdings, with potential reduction in methanogenesis.*

Keywords**— ecology, microbial, multivariate analysis, PCR-DGGE, ruminal fermentation.***

I. INTRODUCTION

Frequently there is needed to search for alternative feeds as substitutes for those commonly used in the ruminants' diets. According to [1], the mesquite tree (*Prosopis juliflora*), introduced in Northeast Brazil in the 1940's to serve as animals feed during dry season, is highly palatable and productive, and its chemical composition reveals 25-28% glucose, 11-17% starch, 7-11% protein and 14-20% organic acids and pectins. These qualities render the mesquite an important species with high potential as animal feed in the semi-arid regions.

The use of mesquite in concentrate feeds for ruminants is limited due to the presence of toxins and anti-nutritional factors, such as polyphenols, nitrogenated compounds and lectin [2]. Mesquite pods have low tannins levels that are toxic to animals [3]. However, alkaloids have been isolated, such as julifloricine, which has significant antimicrobial activity, especially on Gram-positive bacteria. This effect was compared to the action of benzyl penicillin, gentamicin and trimethoprim [4, 5].

In studies by [6], some ruminal fermentation parameters were analyzed non-fistulated lactating Saanen goats fed with different levels of mesquite pod meal (MPM). Their results evidenced that the intestinal flow of microbial nitrogenous

compounds decreased linearly with the increase of MPM levels in the diets with no change to the intake of dry matter and milk production by the animals, although the assessed feed efficiency had a linear reduction.

The study of ruminal microbial diversity tries to clarify the transformations that occur in the rumen, to explain the nature of fermentation and how it affects the ruminant nutrition, as well as the fluctuations in the degree of feed supply, for example the changes in the levels of a concentrate diet. The quality and quantity of fermentation products depends on the type and activity of microorganisms that are part of the population and these, in turn, depends on the diet [7].

Therefore, our objective for this research was to assess the effect on the intake of nutrients after substituting corn for MPM in the concentrate feeds, as well as on ruminal parameters and microbial diversity of bacteria and archaea.

II. MATERIAL AND METHODS

Four crossbreds Anglo-Nubian adult goats were used for this study. They were fistulated, non-lactating and average live weight of 31 kg, confined at individual raised sheds on slatted floors measuring 1.5 x 1.0 m. The animals were distributed into a 4 x 4 Latin square for assessment of the effects of different levels of mesquite pod meal (MPM) in isonitrogenous diets, based on elephant grass silage forage at 43% of the dry matter (DM). The substitution of corn for MPM in the concentrate was the independent variable used to characterize the treatments, consisting of four levels of substitution (0, 33.3, 66.7 and 100% of natural matter) (Table 1). Sources of protein were soy meal and cotton meal.

TABLE 1
PROPORTION OF NUTRITIONAL INGREDIENTS OF CONCENTRATE FEED (g kg^{-1}).

Ingredients	Replacement level of milled corn by mesquite pod meal (%)			
	0	**33.3**	**66.7**	**100**
Mesquite pod meal	0.0	254.2	512.8	776.0
Milled corn	781.0	525.1	264.8	0.0
Soybean meal	133.0	132.4	131.9	131.3
Cottonseed meal	49.2	49.6	50.0	50.5
Mineral mixture	36.8*	38.5†	40.3‡	42.0§
Total	1000.0	1000.0	1000.0	1000.0

*Dicalcium phosphate 399.0 g kg^{-1}, Common salt 201.0 g kg^{-1}, Commercial mineral salt 400.0 g kg^{-1}. †Dicalcium phosphate 423.0 g kg^{-1}, Common salt 192.0 g kg^{-1}, Commercial mineral salt 385.0 g kg^{-1}. ‡Dicalcium phosphate 444.0 g kg^{-1}, Common salt 186.0 g kg^{-1}, Commercial mineral salt 370.0 g kg^{-1}. §Dicalcium phosphate 464.0 g kg^{-1}, Common salt 179.0 g kg^{-1}, Commercial mineral salt 357.0 g kg^{-1}.

The experiment was performed in four experimental periods, each one lasting 14 days: nine days for adaptation and the last five days to collect data. The goats had free access to food and water. They were fed at 8 am and 4 pm. The diet supplied and the leftovers of all animals were daily weighed in order to intake estimate. The leftovers were kept at around 10% of the total feed offered.

During the data collection period, from the 10th to the 14th day of each experimental period, samples of forage, concentrate feed and the leftovers of each animal were collected daily, packaged into plastic bags and stored at -20°C for further analysis and determination of the nutrients intake.

The chemical composition of the food offered (Table 2) and leftovers was determined according to methods described by [8]. For the determination of neutral detergent fibers (NDF) level we used thermostable alpha-amylase prior to extraction with neutral detergent. The total carbohydrates (TCH) levels in the feed offered and leftovers were calculated according to [9]: TCH = 100 - (Crude Protein (%) + ethereal extract (%) + ash (%)) and the levels of non-fibrous carbohydrates (NFC) were estimated by subtracting the concentrations of NDF from TCH, according to [10]: NFC = TCH – NDF (Table 2).

TABLE 2
CHEMICAL COMPOSITION OF ELEPHANT GRASS SILAGE, MESQUITE POD MEAL, CONCENTRATES AND DIETS (g kg^{-1} of DM).

	EGS*	MPM†	Concentrate			
			Replacement level of milled corn by MPM (%)			
			0	33.3	66.7	100
Dry Matter	281.0	927.5	727.0	849.0	843.0	830.0
Organic Matter	906.0	958.3	950.0	935.0	935.0	925.0
Crude Protein	40.9	78.2	135.4	134.7	134.5	133.0
Ethereal Extract	38.7	16.4	40.0	35.0	28.0	23.0
Neutral Detergent Fiber	789.0	296.5	340.0	314.0	332.0	346.0
Non-Fibrous Carbohydrates	106.4	56.72	464.6	496.3	480.5	458.0
Total Carbohydrates	895.4	86.37	804.6	810.3	812.5	804.0
Acid Detergent Fiber	488.5	24.15	133.0	141.0	148.0	191.0
Mineral Matter	25.0	4.17	20.0	20.0	25.0	40.0
			Diet			
Dry Matter			535.2	604.8	601.3	593.9
Organic Matter			931.1	922.5	922.5	916.8
Crude Protein			94.8	94.4	94.3	93.4
Ethereal Extract			39.4	36.6	32.6	29.8
Neutral detergent fiber			533.1	518.3	528.5	536.5
Non-Fibrous Carbohydrates			310.6	328.6	319.6	306.8
Total Carbohydrates			843.6	846.9	848.1	843.3
Acid Detergent Fiber			285.9	290.4	294.4	318.9
Mineral Matter			22.2	22.2	25.0	33.6

*EGS - Elephant grass silage. †MPM - Mesquite pod meal.

At the 14th day of each experimental period the ruminal liquid was collected via fistula, in periods of 0, 2, 4, 6 and 8 hours after the morning feed. Time 0 (zero) refers to the sampling that preceded the feed supply. The ruminal fluid collected from its ventral sac was filtered in four layers of gauze. The pH was measured immediately after collect. To determine the concentration of ammonia (N-NH3) and volatile fatty acids (VFA) in the rumen, the samples were fractioned and immediately acidified with sulfuric acid 20% and phosphoric acid 25% (1 mL acid: 10 mL ruminal fluid), respectively. A portion of the samples collected four hours after the morning feed was immediately frozen at -20ºC for further microbial diversity analyses. A portion of the samples were defrosted and centrifuged at 2,000 x g during 10 minutes and 2 mL of the supernatant were collected to determine the N-NH3 concentration after distillation with 5 mL of KOH 2N. The other samples were centrifuged at 800 x g for 15 minutes for determination and quantification of VFA. This experiment was carried out by gas chromatography according to the method by [11]. A gas chromatograph model GC-2010 (Shimadzu Corporation) equipped with an Rtx-Wax 30 m x 0.25 mm x 0.25 mm column was used. The temperatures used for operation of the injector, separation column and flame ionization detector were 210, 90 to 170 and 230ºC, respectively. Solutions with 20 mM of acetic, propionic and butyric acids were prepared as standard VFA solution. For each determination 1.0 µL of sample was injected and the result was obtained through an integrator that used the standard solution as the base to calculate the VFA concentrations in the sample.

Total DNA was extracted and isolated using the methodology described by [12, 13] of the rumen content collected 4 hours after morning feeding, on the 14th day of each experimental period. Briefly, the samples were submitted to a rinsing process with 1x PBS (Buffered Saline Solution). 600 µL of TESC (10 M Tris base, 1 M EDTA, 0.1 M NaCl, pH 8.3) and 30 µL of Tween 80 (Merck®) were added. The sample was submitted to a Magiclean 1600 ultrasonic bath (Unique®) for 2 minutes to promote the release of bacterial cells, due to the possibility of being adhered to organic matter. Then, the samples were again centrifuged for 3 minutes at 50 x g and the supernatant was collected and later centrifuged at 7,000 x g for 5 minutes. The sample was resuspended in 700 µL of Cell Lysis Buffer (50 mM Tris-HCl (pH 8.5) containing 500 mM NaCl) with 12 µL of proteinase K (20 mg mL^{-1}) and 12 µL of 10% SDS (Sodium Dodecyl Sulfate). The samples were incubated at 65ºC for 30 minutes.

Physical lysis was promoted by thermal shock in three cycles of 10 minutes at -80ºC and 5 minutes in a water bath at 80ºC. An equivalent volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added and the mixture was gently homogenized. The mixture was centrifuged for 10 minutes at 1800 x g and the aqueous phase recovered. DNA was precipitated by the addition of 0.7 volume of cold isopropanol and 0.1 volume of 3M sodium acetate. The solution was gently mixed and kept at -20°C. The pellet was resuspended in 50-100 µl of TE buffer (10 M Tris-HCl, 0.1 M EDTA, pH 8.0). The extracted DNA was purified on Sephadex G-200 mini columns according to [14].

For analysis of microbial diversity, the polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) were performed. Approximately 200 bp of the bacterial 16S rDNA was amplified using primers 357F (5'-CCCGGGGTACGGGAGGCAGCAG -3') and 518R (5'- ATTGCTGCTACCGCGGG-3') with a GC clamp on the 5' end of the forward primer [15]. The primers 1100F (5' - AACCGTCGACAGTCAGGYAA CGAGCGAG - 3') with the GC clamp and 1400R (5' - CGGCGAATTCGTCGTA GGAGCAGG GAC -3') described by [16] were used for identification of members of domain Archaea.

The PCR mixture for domain Bacteria consisted of 17.65 µL of sterile Milli-Q water, 1 x of Taq polymerase buffer, 2.5 mM of MgCl2, 10 pmol of each primer, 200 µM of each dNTP, 0.5 U of Platinum Taq DNA Polymerase (Invitrogen) and 2 µL of DNA (c. 20 ng) in a final volume of 25 µL. The cycling conditions were 5 min at 95°C, plus 35 cycles of 60 s at 95°C, 60 s at 55°C, and 60 s at 72°C, ending with an extension step at 72°C for 3 min. The reagents concentrations for domain Archaea in a final 25 µL reaction were 16.85 µL sterile Milli-Q water, 1 x Taq polymerase buffer, 2.1 mM of MgCl2, 280 µM of each primer (1100F with GC-clamp and 1400R), 280 µM of each dNTP and 2.5 U of Platinum Taq DNA Polymerase (Invitrogen), and 2 µL of DNA (c. 20ng). The cycling conditions were 2 min at 94ºC, plus 35 cycles of 30 s at 94ºC, 30 s at 55ºC, 90 s at 72ºC, and a final extension at 72ºC for 3 minutes. The reactions were analyzed in agarose gel at 2% (w/v), stained with ethidium bromide, and photographed on UV transillumination.

The DGGE was performed using the Mutations Analysis System CDC 20 x 20 cm (Bio-Rad). 20 µL of the PCR products were placed in 8% (w/v) polyacrylamide gel in TAE buffer (2 M Tris-base, 1 M Acetic acid, 0.5 M EDTA, pH 8.0) 0.5 mmol L^{-1} with denaturing gradient ranging between 30 and 55% for domain Bacteria and between 35 and 65% for domain Archaea. 100% denaturant is defined as 7 M urea and 40% (v/v) formamide [15]. Gels were electrophoresed at 85 V for 20 minutes and then at 200 V for 5 h at 60ºC. The gels were visualized by silver staining.

The data analysis was performed using MIXED procedure by SAS [17]. The treatment effects and the experimental times were decomposed in linear polynomial contrasts at 4th degree. Furthermore, we examined the interaction of the treatments along time using the MIXED slice option and contrasts. The lowest configuration of the Akaike information criterion (AIC) was obtained by using the variance component (VC):

$$Y_{ijk} = \mu + Tr_i + T_j + Tr_iT_j + \varepsilon_{ijk} \; ; \; NID\,(0; \sigma^2) \quad (1)$$

Where: Tr = levels of substitution (0; 33.3; 66.7 and 100%) and T = time (0; 2; 4; 6 and 8 h).

In order to choose the best equation, factors Tr and T as well as non-significant interactions in $p > 0.05$ were removed from the model. The interaction contrasts were used to compare the effect of levels of extracts and interactions along time in pH, N-NH3, VFA.

Multivariate statistical techniques were performed to evaluate the multivariate structure contained in the original data. From the DGGE profiles, binary matrixes of presence and absence of bands were performed [18]. From these matrixes we performed a cluster analysis in dendrograms for which we used the calculation of similarity among individuals and the clustering method UPGMA (average distances method) with the Raup-Crick similarity index [19] using statistical program PAST.

The Biplot analysis applied to the Principal Components (PC) was made to verify correlations between the assessed variables and to identify which had more influence from the diets. The PAST program was used for this analysis. The Microsoft Excel 2010 was used to prepare the species richness graph and the Venn diagrams, using the banding profile generated in the DGGE and considering only the levels of substitution of corn by mesquite pod meal.

III. RESULTS AND DISCUSSION

No effect was observed ($p > 0.05$) for replacement levels of corn by MPM in the intake of dry matter (DM), organic matter (OM), mineral matter (MM), Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) or Total

Carbohydrates (TCH) according the linear (L), quadratic (Q) and cubic (C) contrasts analysis (Table 3). However, the non-fiber carbohydrates intake (NFCI) showed a quadratic effect ($p < 0.05$) regarding levels of MPM. The maximum estimated relative NFCI was 0.382 kg day^{-1} at the level of 35.4% of corn replacement by MPM. The ether extract (EE) intake showed a linear decrease. Replacing corn by MPM also obtained a significant effect ($p < 0.0001$, with quadratic fit) on NDF intake. The data was described as a percentage of dry matter intakes (NDFI-DMI). The estimation of a minimum relative intake was 47.7% at 26.8% of corn replacement level (Table 3).

TABLE 3
AVERAGE INTAKE RESULTING FROM THE REPLACEMENT LEVELS OF CORN BY MESQUITE POD MEAL IN CONCENTRATED FEED FOR GOATS

	Levels of mesquite mod meal (%)				SEM	Effect*		
	0	33.3	66.7	100		L	Q	C
	Intake (kg day^{-1})							
Dry Matter	0.713	0.728	0.742	0.753	0.060	0.77	0.98	0.99
Organic Matter	0.667	0.673	0.685	0.690	0.056	0.84	0.99	0.97
Crude Protein	0.072	0.074	0.077	0.077	0.005	0.62	0.87	0.95
Ethereal Extract	0.028	0.028	0.027	0.026	0.002	0.73†	0.90	0.95
Mineral Matter	0.028	0.026	0.023	0.021	0.002	0.23	0.97	0.85
NDF	0.356	0.349	0.355	0.395	0.031	0.61	0.66	0.93
ADF	0.201	0.194	0.196	0.230	0.017	0.48	0.46	0.84
NFC	0.365	0.315	0.326	0.350	0.012	0.57	0.03‡	0.43
NDFI-DMI (%)	49.82	47.80	46.72	52.37	0.830	0.30	0.02§	0.36

SEM - Standard Error of Mean; NDF - Neutral Detergent Fiber; ADF - Acid Detergent Fiber; NFC - Non-Fibrous Carbohydrates; NDFI-DMI - Percentage of NDF intake in relation to DM intake.
*L - linear, Q - quadratic and C - cubic effects.
† $\hat{Y} = (0.032 \pm 0.0028)^{+} - (0.00013 \pm 0.000045)Tr^{+++}$
‡ $\hat{Y} = (0.3761 \pm 0.0072)^{+} - (2.81^{-6} \pm 1.31^{-6})Tr^{2+++}$
§ $\hat{Y} = (48.38 \pm 0.6)^{++} - (0.054 \pm 0.029)Tr^{ns} + (0.0010 \pm 0.00028)Tr^{2+++}$
$^{+}(p < 0.0001)$; $^{++}(p < 0.001)$; $^{+++}(p < 0.05)$.

The DM intake was not influenced by the diets (Table 3). This result was expected because the similarity between nutrient levels (Table 2). In another study, with unpublished data, we also observed this behavior when assessing the same levels of MPM in the concentrate supplied to lactating Saanen goats. The explanation for the reported results is that the use of MPM did not increase NDF to levels that restrict the food intake. Furthermore, isonitrogenous diets also does not affect DM intake by animals [20].

The CP intake was not affected ($p > 0.05$) when corn replaced by MPM for all replacement levels used due isonitrogenous diets used. Just as there was no significant variation in the DM intake (Table 3).

The non-fiber carbohydrates intake (NFCI) showed a quadratic effect ($p < 0.05$) regarding levels of MPM. The maximum estimated relative NFCI was 0.382 kg day^{-1} at the level of 35.4% of corn replacement by MPM. Sugars and organic acids belong to fraction 'A' of rapid ruminal degradation, according to the Cornell Net Carbohydrate and Protein System [21]. The starch represents 91.45% of non-structural carbohydrates in corn and belongs to fraction 'B1' of intermediate degradation, likewise pectin. However, the main components of MPM are mono-and oligosaccharides (28%) followed by organic acids and pectin (20%). Starch with only 11-17% is not the main energy component. [22] reported that the value of fraction A + B1 of MPM (59.92%) was lower than corn (72.20%).

The ether extract (EE) intake showed a linear decrease. This behavior is possibly due to the lower concentration of this nutrient in MPM. Other authors also observed the same behavior [6, 23].

Replacing corn by MPM obtained significant effect ($p < 0.0001$, with quadratic fit) on NDF intake. The data was described as a percentage of dry matter intake (NDFI-DMI). The estimation of a minimum relative intake was 47.7% at 26.8% of corn substitution level. This increase in ingestion is possibly due to the higher concentration of NDF in MPM when compared to corn. The maximum level of NDF in diets that does not have inhibitory effect on intake is not well defined. But, in this work, at the level of fiber ingestion, it seems that the intake control mechanism was not caused by the ruminal fill.

The principal components analysis (PCA) allowed us to resize the space of the original information into a new space formed by two latent variables named principal components 1 and 2 (PC1 and PC2). It was created by linear combinations of the original variables in the region that had the higher concentration of the original variance. These new variables arranged orthogonally generated a bidimensional distribution of variables as can be observed in Fig. 1. The biplot analysis applied to the principal components explained 97.29% (PC1 = 72.61% and PC2 = 24.68%) of the total variability observed in intake of nutrients data (Fig. 1). Fig. 1 presents the correlations between each variable and their respective principal components, indicating those variables that discriminated the most in each axis. PC1 (horizontal axis or X axis) explained 72.61% of the total variation, showing a clear discrimination of levels 66.7 and 100% of substitution of corn by MPM in relation to the others 0.0 and 33.3%.

The variables with positive correlations were responsible for the discrimination of treatments in right PC1 and those with negative correlations were responsible for the discrimination of treatments in left PC1 (Fig. 1). Therefore, variables DMI, OMI, CPI, NDFI, ADFI, TCI and NDFI-DMI, in this order, were responsible for the discrimination of levels 66.7 and 100% of corn substitution by MPM, whereas variable EEI was responsible for the discrimination of levels 0.0 and 33.3% of substitution. The variables with positive correlations were responsible for the discrimination of treatments above the zero and those with negative correlations were responsible for the discrimination of treatments below the zero in PC2 (vertical axis or Y axis). Therefore, variable CPI was responsible for the discrimination of level of substitution 66.7%, whereas variables NFCI and NDFI-DMI discriminated level 100% of substitution of corn by MPM, in this order (Fig. 1).

For lactating Saanen goats fed a similar diet, [6] did not observe a significant effect of collection time and levels of MPM substitution on rumen pH, having found a mean of 6.95. [24] Working with non-pregnant and non-lactating Alpine goats fed with diets consisting of different ratios of forage and concentrate, observed pH values of 6.5 to 6.9 in the animals that received 40 and 20% of concentrate, respectively. The authors observed that there was a quadratic decrease ($p > 0.05$) of the concentrate level in relation to pH and that it achieved its minimum point between 2 and 4 hours following each feed.

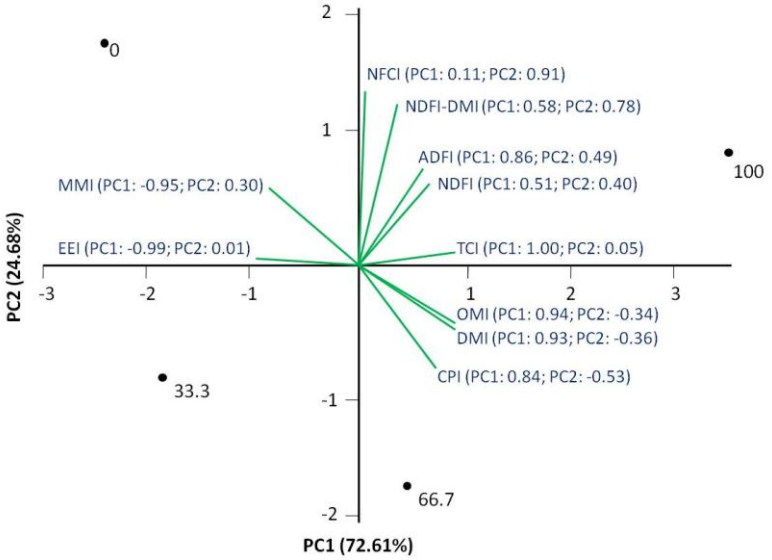


FIGURE 1: Biplot graph resulting from the Principal Components (PC) Analysis showing the distribution of treatments and nutrients intake and its correlations. DMI – dry matter intake; OMI – organic matter intake; CPI – crude protein intake; EEI –ethereal extract intake; MMI – mineral matter intake; NDFI – neutral detergent fiber intake; ADFI – acid detergent fiber intake; TCI – total carbohydrates intake; NFCI – non-fibrous carbohydrates intake; NDFI-DMI -percentage of NDF intake in relation to DM intake.

According to [25], this is due to the higher rate of volatile fatty acid production from the fermentation of the fibrous fraction of the feed. However, in the present study the rumen pH showed minimum estimated values for treatments 0; 33.3; 66.7; and 100% of MPM of 6.33, 6.34, 6.46 and 6.52, respectively, at 2.1 h and maximum values of 6.68, 6.74, 6.80 and 6.86 between 6.5 at 7 h after feeding (Fig. 2). The high pH value observed for treatment 100%, almost 7 h after ingestion, owes to the rumination time, which increased linearly between diets. The rumination increases both the superficial area and the rate of fermentation of the feed. It also increases the flow of saliva, which keeps the pH favorable for microorganisms and animals [7].

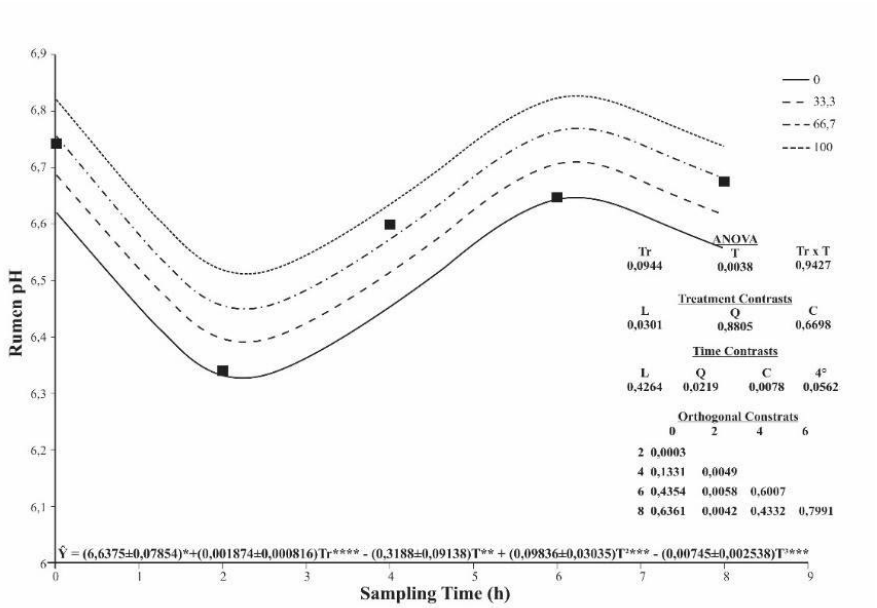


FIGURE 2: Ruminal pH estimative in non-lactating fistulated goats, fed a concentrate containing mesquite pod meal as substitute for corn in different levels. Lines correspond to replacement levels (%) of mesquite pod meal. *(p < 0.0001); **(p < 0.001); *(p < 0.01); ****(p < 0.05).**

The concentration of N-NH3 showed a quartic behavior ($p < 0.05$) as result of sampling times. There was no influence of the diets on the concentration of N-NH3, with means of 7.95, 7.68, 8.88 and 8.36 mmol L^{-1} for treatments 0, 33.3, 66.7 and 100% of MPM as substitute for corn. A peak of N-NH3 production was observed 2 h after feeding (Fig. 3). The values of N-NH3, for all levels of MPM, were higher than 5 mmol L^{-1} of rumen liquid, which is the minimum level necessary to keep the ruminal normal function [26]. However, the level of ammonia should be higher than 10 mmol L^{-1} to increasing ruminal dry matter digestion, and higher than 20 mmol L^{-1} to increasing dry matter intake [27]. The concentration of ruminal ammonia usually varies according to the time elapsed since feeding, the sampling spot in the rumen, the balance between protein and energy in the diet, the solubility, and the level of protein in the feed [28].

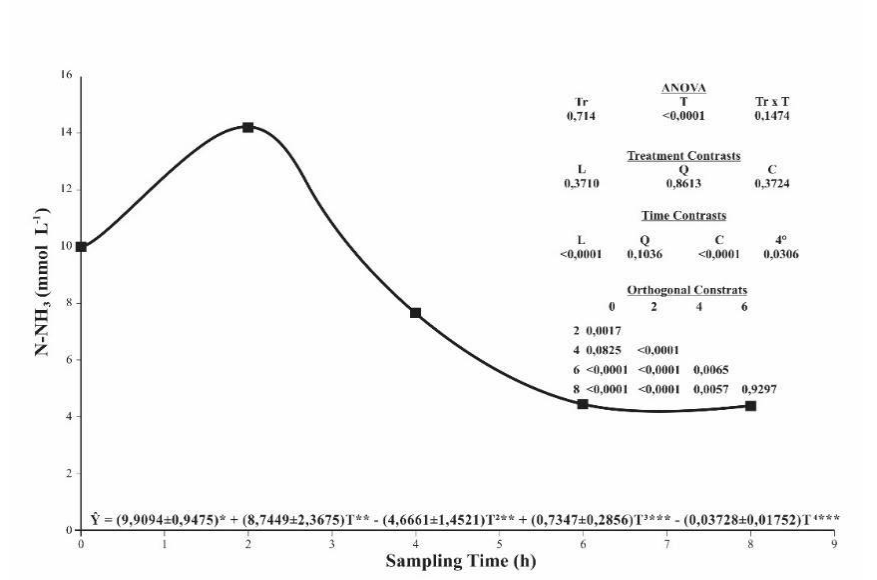


FIGURE 3: Concentration of N-NH_3 in fistulated, non-lactating goats, fed concentrate containing mesquite pod meal as substitute for corn. *(p < 0.0001); **(p < 0.001); *(p < 0.05).**

The relative concentrations of acetate, propionate and butyrate (Table 4) indicate that diets "with added" MPM resulted in higher values of acetate in relation to propionate. The concentrations of total or individual volatile fatty acids (VFA) in the rumen are highly variable and depend on feed frequency, time after feeding and diet composition. In the present work, we obtained a relative proportion of VFA produced of 69:20:11 (mols of acetate:propionate:butyrate). This proportion is near to

65:15:10 for diets with high proportions of forage [29]. There was significant cubic effect of replacement levels by MPM on acetate:propionate ratio, being the lowest ratio found for the replacement level of 33.3%. This report corroborates the hypothesis of that mesquite use reduces the acetate:propionate ratio in ruminal liquid.

TABLE 4
LEAST SQUARE MEANS, MEAN STANDARD ERROR AND SIGNIFICANCE INDICATORS FOR THE EFFECTS OF CONTRASTS OF THE CONCENTRATIONS OF ACETATE, PROPIONATE AND BUTYRATE IN GOATS FED CONCENTRATE CONTAINING MESQUITE POD MEAL AS SUBSTITUTE FOR CORN.

Item	Levels of Mesquite pod meal (%NM)				SEM	Effect♦		
	0	33.3	66.7	100		L	Q	C
Acetate (mM)	17.96	13.43	17.54	14.84	1.45	0.70	0.77	0.29
Propionate (mM)	4.52	4.66	5.27	4.35	0.40	0.98	0.55	0.61
Butyrate (mM)	2.40	2.69	2.60	2.55	0.24	0.85	0.76	0.89
Acetate:Propionate (mM)	4.04	2.85	3.28	3.47	0.18	0.52	0.06	0.05[1]

♦L, Q and C – linear, quadratic and cubic effect.
[1]$\hat{Y} = 4.0418^{*} - 0.07894Tr^{***} + 0.0016Tr^{2***} - 8.43^{-6}Tr^{3***}$
*significant ($p < 0.0001$); **significant ($p < 0.001$); ***significant ($p < 0.05$).

The sum of the two initial components shows the 86.57% of data variation for fermentation parameters, showing that the horizontal axis (PC1) is the most important for the results interpretation, since it cluster the most data variation as show in Fig. 4. There was a shift of treatment 0% to the negative part of the graph in PC1, indicating that this treatment shows differences in relation to the others. PC1 shows a separation of treatments with MPM (33.3, 66.7 and 100%) and without MPM (0%) as replacement of corn. The variables with positive correlations that most influenced the discrimination of treatments to the right of the graph in PC1 were, in this order Butyrate, pH, N-NH_3 and Propionate. Those with negative correlations and responsible for the discrimination of treatment 0% to the left of PC1 were acetate:propionate and acetate, in this order (Fig. 4). The treatment with 66.7% of corn replacing by MPM appears distant from the other treatments, shifting positively along the Y axis, with the other levels of substitution (0, 33.3 and 100%) shifting in the opposite direction. PC2 was responsible for 33.2% of data variation, especially for the shift in treatment 66.7% of MPM, influenced by variables with positive correlation, Propionate, Acetate and N-NH_3, in this order.

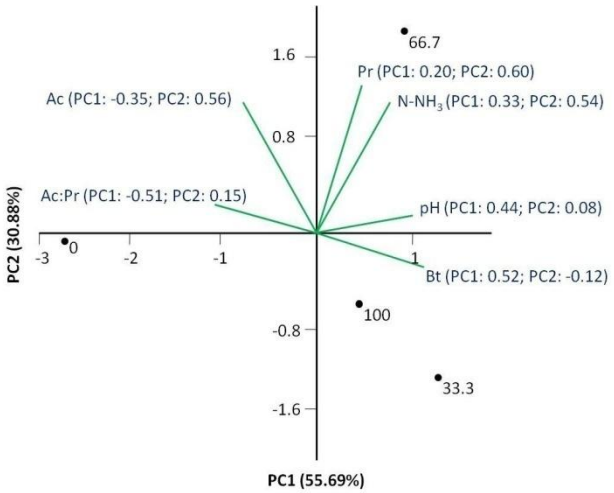


FIGURE 4: Biplot graph resulting from the Principal Components (PC) Analysis showing the distribution of treatments and nutrients intake and its correlations. N-NH3 – ammoniacal nitrogen; Ac – acetate; Pr – propionate; Bt – butyrate; A:P – acetate: propionate ratio.

DGGE is normally used to determine the number or differences between genus and species of microorganisms present in the sample. In the present work, it was possible to observe differences between the patterns of PCR products in DGGE banding, both for domain Bacteria and Archaea demonstrated by the different number of bands, which possibly indicate different genus or species. The DGGE banding patterns obtained were used to analysis of microorganism community structure. The microbial diversity was estimated by cluster analysis based on a data matrix of bands presence and absence verified in each sample (Fig. 5 and 6).

In the Raup-Crick dendrogram (Fig. 5A) generated from DGGE considering the replacement levels of corn by mesquite pod meal (0, 33.3, 66.7 and 100%) shows that the point of union of all groups has similarity of approximately 50%. The high similarity (88%) was observed between treatments 0 and 33.3% of MPM which can be explained due the similarity between both ruminal environments. The other treatments showed a lower similarity rate. The Venn diagram clearly demonstrates the clustering of bands (supposed species) that are shared or that are specific to each "sampled group" (Fig. 5B). It is observed that treatments 0, 33.3%, 66.7 and 100% share the same number of bands (3 bands), demonstrating possible similarity in terms of species distribution.

Diet components and changes to ingestive behavior can cause changes to gastro-intestinal microbial ecology, which plays a fundamental role on the animal's health and productivity. With the inclusion of MPM in the diets of crossbred Anglo-Nubian goats, an increase in NDF was observed in the diets because of the increase in substitution levels (51.83; 52.85 and 53.65%). Although there was no evidence of this increase in NDF intake, it is suggested that increments to the level of NDF in the diet increased time spent chewing (TSC), leading to high rumen pH because of the higher flow of saliva to the rumen, and high flow of buffering substances.

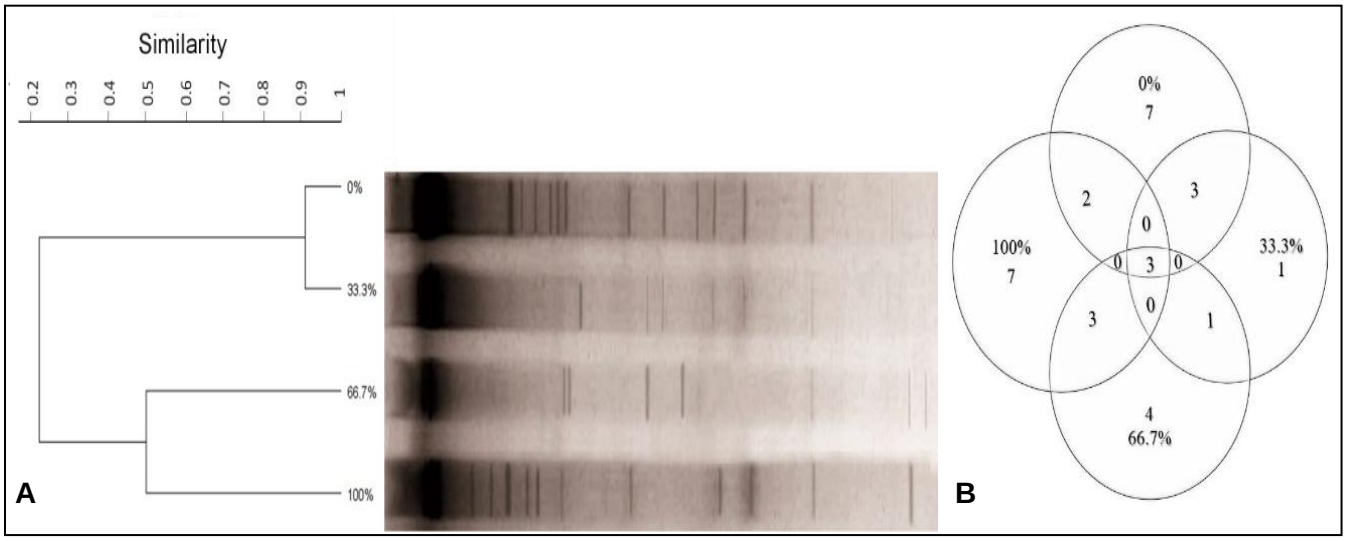


FIGURE 5: Assessment of Bacteria ruminal communities in four levels of corn replacement by mesquite pod meal (0, 33.3, 66.7 and 100%) determined by PCR-DGGE. (A) Raup-Crick dendrogram generated from PCR-DGGE analyses of fragments amplified with specific Bacteria primers (V3 region from 16S rRNA). (B) Venn diagram generated from the DGGE banding profile indicating numbers of shared bands across different treatments.

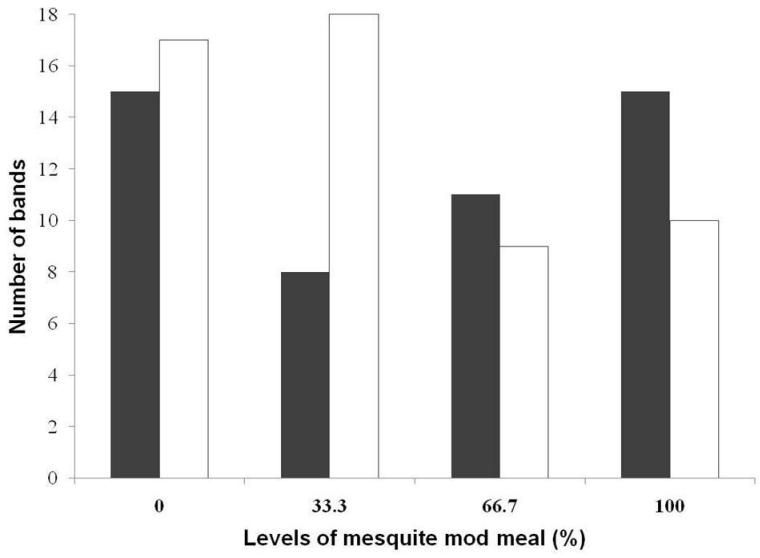


FIGURE 6: Richness of bands for Bacteria (■) and Archaea (□) considering the levels of corn substitution by mesquite pod meal.

Ruminal pH is entirely related with the final products of fermentation, as well as with the growth rate of rumen microorganisms [30]. Thus, pH is the main environmental factor in the distribution of microorganisms, playing a regulating role in the definition of their environment and diversity. It is positively correlated with ammoniacal nitrogen and acetate:propionate ratio. This reflected clearly on the richness of bands for bacteria, where it is possible observe that treatment with 33.3% of MPM, which has the lowest pH value 4 h after feeding, is also the one that shows the lowest diversity of bacteria (Fig. 7) and the lowest number of rare bands (Fig. 5).

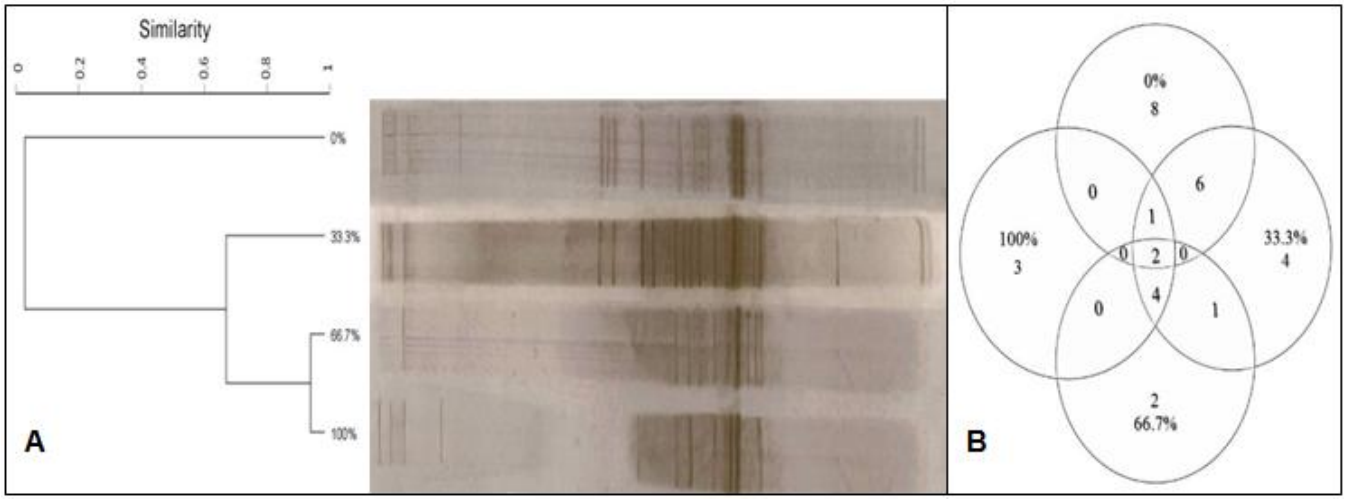


FIGURE 7: Assessment of Archaea ruminal communities in four levels of corn replacement by mesquite pod meal (0, 33.3, 66.7 and 100%) determined by PCR-DGGE. (A) Raup-Crick dendrogram generated from PCR-DGGE analyses of fragments amplified with specific Archaea primers (1100 and 1400 from 16S rRNA). (B) Venn diagram generated from the DGGE banding profile indicating numbers of shared bands across different treatments.

As done to domain Bacteria, a Raup-Crick dendrogram and Venn diagram were generated for domain Archaea from DGGE. The point of union of all groups showed low similarity around 0.08% (Fig. 6). We can see that there was more similarity between levels 66.7 and 100% of MPM (96.2%) than other treatments. This observation is clearly confirmed by Biplot analyses applied to the Principal Components, discussed earlier, that evidenced the same variables exerting influence on the ruminal environment. This influence can have reflected on the richness of archaea species viewed on DGGE banding profile, generating a lower diversity of species for these two substitution levels (Fig. 6 and 7).

The diet supplied with 66.7 and 100% of corn replacement by MPM were the treatments that showed the least number of methanogenic Archaea. This can have occurred due to a reduction of protozoa which has led to a reduction in methanogens associated with these microorganisms, either by adherence or endosymbiosis [31, 32]. These also were the treatments for which we observed the highest levels of $N\text{-}NH_3$ (10.24 and 8.32 mmol L^{-1}, respectively) 4 h after feeding, still exist synergy between levels of acidity (pH). Through them it is possible to increase microbial efficiency, biomass production and degradation of fiber in the feed, thus obtaining increased of feed intake. Such changes to the ruminal environment were sufficient to reduce the population of methanogenic Archaea, also suggesting a hydrogen deviation in the medium towards the production of propionate, as visualized in the treatment that received 66.7% of MPM (Table 4).

IV. CONCLUSION

The use of mesquite pod meal at levels of 33.3% and 66.7% as a replacement for corn does not reduce nutrient intake but alters the bacterial population in the rumen of crossbred goats and decreases the population of archaea present in the fluid. This replacement reduces methanogenesis, contributing to production efficiency and reducing methane emissions into the environment.

ACKNOWLEDGEMENTS

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel, CAPES, for the financing of the doctoral scholarship, the Bahia State Research Support Foundation (FAPESB) for financial assistance.

REFERENCES

[1] Lima, P. Leucena. In: Kill, L.H.P., and Meneses, E.A. Espécies vegetais exóticas com potencialidades para o semiárido Brasileiro. Brasília, DF: Embrapa Semiárido; Embrapa informação tecnológica, 2005. pp.158-205.

[2] Andrade-Montemayor, H.M., Cordova-Torres, A.V., García-Gasca, T., and Kawas, J.R. Alternative foods for small ruminants in semiarid zones, the case of mesquite (*Prosopis laevigata* spp.) and Nopal (*Opuntia* spp.). Small Ruminant Research, vol. 98, 2011, pp. 83–92.

[3] Sawal, R.K., Ratan, R., and Yadav, S.B.S. Mesquite (*Prosopis juliflora*) pods as a feed resource livestock – A Review. Asian-Australasian Journal of Animal Sciences, vol. 17, n. 5, 2004, pp. 719-725.

[4] Aqeel, A., Khursheed, A.K., Viqaruddin, A., and Sabiha, Q. Antimicrobial activity of julifloricine isolated from *Prosopis juliflora*. Arzneimittelforschung, vol. 39, n. 6, 1989, pp. 652-655.

[5] Nakano, H., Nakajima, E., Fujii, Y., Shigemori, H., and Hasegawa, K. Structure-activity relationships of alkaloids from mesquite (*Prosopis juliflora* (Sw) D.C.). Plant Growth Regulation, vol. 44, 2004, pp. 207-210.

[6] Argôlo, L.S., Pereira, M.L.A., Dias, J.C.T., Cruz, J.F., Del Rei, A.J., and Oliveira, C.A.S. Farelo da vagem de algaroba em dietas para cabras lactantes: parâmetros ruminais e síntese de proteína microbiana. Revista Brasileira de Zootecnia, vol. 39, n. 3, 2010, pp. 541-548.

[7] Russell, J.B., and Rychlik, J.L. Factors that alter rumen microbial ecology. Science, vol. 292, 2001, pp. 1119-1122.

[8] Detmann, E., Souza, M.A., Valadares Filho, S.C., Queiroz, A.C., Berchielli, T.T., Saliba, E.O.S., Cabral, L.S., Pina, D.S., and Ladeira, J.A.G. Métodos para análise de alimentos. Visconde do Rio Branco, MG: Suprema, 2012. 214p.

[9] Sniffen, C.J., O`Connor, J.D., Van Soest, P.J., Fox, D.G., and Russell, J.B. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science, vol. 70, n. 11, 1992, pp. 3562-3577.

[10] Hall, M.B., Hoover, W.H., Jennings, J., and Webster, T.K.M. A method for partitioning neutral detergent soluble carbohydrates. Journal of the Science of Food and Agriculture, vol. 79, n. 15, 1999, pp. 2079-2086.

[11] Erwin, E.S., Marco, G.J., and Emery, E.M. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. Journal of Dairy Science, vol. 44, n. 9, 1961, pp. 1768-1771.

[12] McSweeney, C.S., Denman, S.E., Wright, A.-D.G., and Yu, Z. Application of recent DNA/RNA-based techniques in rumen ecology. Asian-Australasian Journal of Animal Science, vol. 20, n. 2, 2007, pp. 283-294. http://dx.doi.org/10.5713/ajas.2007.283

[13] Green, G.L., Brostoff, J., Hudspith, B., Michael, M., Mylonaki, M., Rayment, N., Staines, N., Sanderson, J., Rampton, D.S., and Bruce, K.D. Molecular characterization of the bacteria adherent to human colorectal mucosa. Journal of Applied Microbiology, vol. 100, n. 3, 2006, pp. 460-469. https://doi.org/10.1111/j.1365-2672.2005.02783.x

[14] Tsai, Y., and Olson, B.H. Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. Applied and Environmental Microbiology, vol. 58, 1992. pp. 2292-2295.

[15] Muyzer, G., Waal, E.C., and Uitterlinden, A.G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology, vol. 59, n. 3, 1993, pp. 695-700.

[16] Kudo, Y., Nakajima, T., Miyaki, T., and Oyaizu, H. Methanogen flora of paddy soils in Japan. FEMS Microbiology Ecology, vol. 22, 1997, pp. 39-48.

[17] Littell, R.C., Milliken, G.A., and Stroup, W.W. SAS® system for mixed models. Cary, 1996. 633p.

[18] Kozdrój, J., and Elsas, J.D. Van. Structural diversity of microbial communities in arable soils of a heavily industrialised area determined by PCR-DGGE fingerprint and FAME profiling. Applied Soil Ecology, vol. 17, 2001, pp. 31-42.

[19] Raup, D., and Crick, R.E. Measurement of faunal similarity in paleontology. Journal of Paleontology, vol. 53, 1979, pp. 1213-1227.

[20] Argôlo, L.S., Albuquerque Pereira, M.L.P., Silva, H.G.O., and Oliveira, L.N. Comportamento ingestivo de cabras anglo nubianas alimentadas com dietas contendo farelo de vagem de *Prosopis juliflora* (algaroba). Acta Veterinaria Brasilica, vol. 7, n. 3, 2013, pp. 205-211.

[21] CNCPS (Cornell Net Carbohydrate and Protein System). version 5.0.18. (software). Ithaca: Cornell University, 2002.

[22] Figueiredo, M.P., Cruz P.G., Costa, S.S, Rodrigues, C.S., Pereira, L.G.R., Ferreira J.Q., Sousa F.G., and Irmão, J.N. Fracionamento dos carboidratos e componentes nitrogenados do farelo e diferentes partes integrantes da vagem de algaroba (*Prosopis juliflora* (Swartz D.C.). Revista Brasileira de Saúde e Produção Animal, vol. 8, n. 1, 2007, pp. 24-31.

[23] Pereira, T.C.J., Pereira, M.L.A., Oliveira, C.A.S, Argôlo., L.S., Silva, H.G.O., Pedreira, M.S., Almeida, P.J.P., and Santos, A.B. Mesquite pod meal in diets for lactating goats. Revista Brasileira de Zootecnia, vol. 42, n. 2, 2013, pp. 102-108.

[24] Gonçalves, A.L., Lana, R.P., Rodrigues, M.T., Vieira, R.A.M., Queiroz, A.C., and Henrique, D.S. Padrão nictemeral do pH ruminal e comportamento alimentar de cabras leiteiras alimentadas com dietas contendo diferentes relações volumoso:concentrado. Revista Brasileira de Zootecnia, vol. 30, n. 6, 2001, pp. 1886-1892.

[25] Van Soest, P.J. Nutritional ecology of the ruminant. 2 ed. Ithaca: Cornel University Press, 1994. 476p.

[26] Satter, L.D., and Slyter, L.L. Effect of ammonia concentration on rumen microbial protein production in vitro. British Journal of Nutrition, vol. 32, n. 2, 1974, pp. 199-208.

[27] Leng, R.A. Factors affecting the utilization of "poor-quality" forages by ruminants particularly under tropical conditions. Nutrition Research Reviews, vol. 3, 1990, pp. 277-303.

[28] Eardman, R.A., Proctor, G.H., and Vandersall, J.H. Effect of rumen ammonia concentration on 'in situ' rate an extent of digestion of foodstuffs. Journal of Dairy Science, vol. 29, 9, 1986, pp. 2312-2320.

[29] Owens, F.N., and Goetsch, A.L. Ruminal fermentation. In: Church, D.C. (ed.). The ruminant animal digestive physiology and nutrition. 1993, pp.145-171.

[30] Yokoyama, M.T.; Johnson, K.A. Microbiología del rumen e intestino. In: Church, D.C. (ed.). El Ruminant fisiologia disgestiva e nutrición. Zaragoza: Editorial Acribia, S.A., 1988. pp. 137-157.

[31] Moss, R.A. Methane: global warming and production by animals. Chalcombe Publications, Canterbury, 1993. 105p.

[32] Tokura, M., Changan, I., Ushida, K., and Kojima, Y. Phylogenetic study of methanogens associated with rumen ciliates. Current Microbiology, vol. 39, 1999, pp. 123-128.

Quantitative Growth Analysis of Tomato (*Lycopersicon esculentum Mill.)*

Mohd Ashraf Lone[1], Sabzar Ahmad Kuchay[2*]

[1]Department of Botany, Bhagwant University Rajasthan, 305004
[2]Department of Education (J&K)
*Corresponding Author

Received:- 14 August 2021/ Revised:- 20 August 2021/ Accepted:- 25 August 2021/ Published: 31-08-2021

Abstract— *The plant growth analysis parameters like Fresh Mass, Dry Mass, Resource Allocation, Leaf Area, Leaf Area Ratio (LAR), Net Assimilation Rate (NAR), Relative Growth Rate (RGR), Leaf Weight Ratio (LWR) and Root Shoot Ratio and relation between these parameters was studied in Tomato (Lycopersicon esculentum Mill.) during entire life span i.e. from sowing till senescence in the field conditions. The values of growth analysis parameters like RGR and NAR were highest for the period of vegetative growth showing gradual decline towards the senescence. Leaf Weight Ratio (LWR) in general followed a declining trend but the decline was sharp during the transition from vegetative phase to reproductive phase. More resources were allocated towards leaves during vegetative phase to increase the photosynthetic efficiency whereas there was a shift towards reproductive parts during reproductive phase for fruiting. Leaf area followed an increasing trend with time reaching at its peak just before senescence and thereafter leaf area declined with the progress of senescence.*

Keywords— Growth Analysis Parameters, Root-Shoot Ratio and Resource Allocation.

I. INTRODUCTION

The tomato is the edible, often red, fruit/berry of the plant *Lycopersicon esculentum*, commonly known as a tomato plant. The plant belongs to Angiosperms of nightshade family, Solanaceae. The species originated in western South America. Its use as a cultivated food may have originated with the indigenous peoples of Mexico. The tomato (*Lycopersicon esculentum* Mill.) is commercially important throughout the world both for the fresh fruit market and the processed food industries. Tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. While tomatoes are botanically berry-type fruits, they are considered culinary vegetables as an ingredient or side dish for savory meals. Numerous varieties of tomato are widely grown in temperate climates across the world, with greenhouses allowing its production throughout the year. In the recent decades, the consumption of tomatoes has been associated with prevention of several diseases (Willcox et al. 2003 and Sharoni et al. 2006) mainly due to the content of antioxidants including carotenes, (Lycopene as well as β-carotene), ascorbic acid, and phenolic compounds (Jesus Periago et al. 2009). The plants typically grow to 1–2.8 meters (100– 280 cm) in height and have a weak stem that sprawls. It is a perennial in its native habitat, and cultivated as an annual. Fruit size varies according to cultivar, with a width range of 0.5–4 inches (1.3–10.2 cm).

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important and has the highest acreage of any vegetable crop in the world (Jensen et al., 2010). In 2010, its global production was approximately 145.6 million tons of fresh fruit and Brazil ranks ninth, with 2.7% of the world production (Matos et al. 2012). Tomato growing is considered a high-risk activity due to the great variety of environments and systems in which it is grown, high susceptibility to pests and diseases, and high demand for inputs and services, which lead to high financial investment per unit area. Furthermore, Monte et al. (2013) remark that good productivity requires availability of water throughout the cycle, as the tomato plant is very sensitive to water stress. The commercial value of the table tomato is defined by the characteristics and quality of the fruit (Ferreira et al., 2004).

All the living organisms are, at various stages in their life history, capable of growth. Given suitable conditions, this means change in size, change in form and/or change in number. These three processes together form an important part of the phenomenon of life. Among natural systems they help to distinguish the living from the non-living though, in a sense, many non-living systems also grow. Understanding the principals involved in plant growth requires a systematic approach using the tools of mathematics, physics, and other sciences along with common sense knowledge of biological variability. This teaching resource illustrates the method of interpreting plant development referred to as growth analysis (Kuchay and Zargar, 2016). The exercise can be used jointly with a series of related problems in a crop science course. Plant growth analysis refers to a useful set of quantitative methods that describe and interpret the performance of whole plant systems grown under natural, semi-natural, or controlled conditions (Kuchay and Zargar, 2016). Plant growth analysis provides an explanatory, holistic and integrative approach to interpret plant form and function. A technique of investigating growth and yield by use of growth functions was developed by British plant physiologists and has been commonly termed growth analysis (Watson, 1952 and Williams, 1946). Plant growth analysis is considered to be a standard approach to study the plant growth and productivity (Wilson, 1981). Plant growth analysis uses simple primary data like weight, area, volumes and contents of plants or plant parts to investigate processes within and involving the whole plant or crops (Hunt, 2003). Growth analysis has proved to be highly effective in studying the reaction of particular plant species to different environmental conditions and cultivation/management practices. The growth analysis studies not only help us in understanding how plant accumulates dry matter, but also discloses the underlying principles and events which can make a plant more or less productive (Ahad, 1986). The procedure for analyzing growth in terms of dry weight changes was first made by Blackman 1919, when he pointed out that growth could be regarded as a process of continuous compound interest. Any increment produced in any interval would add to the "capital" for growth in subsequent periods. The classical approach is one of the oldest methods in plant growth analysis studies introduced in the beginning of this century (Blackman 1919, West et al. 1920, Briggs et al. 1920) where the relative growth rate (RGR) is calculated by dividing the difference in loge transformed plant weight at two harvests by the time difference between those harvests. Growth analysis parameters help in studying differences in the performance of different varieties or cultivars of crops under similar or varied conditions. The growth analysis parameters like RGR and NAR directly influence the economic yield of crops (Srivastava and Singh, 1990). Similarly, dry matter production; LAR, NAR and RGR are ultimately reflected in higher yield of crops (Thakur and Patel, 1996). Karim and Fattah (2007) reported that NAR gets increased during fruiting stage. The shading of plant populations to varied degrees can be done in order to determine the effect of light intensity and the reaction of individuals can be monitored through growth analysis, like the shading of leaves can reduce NAR (Net Assimilation Rate) but the plants becomes more leafy. The reductions of light intensity can double the leaf area ratio (LAR) and similarly, more leaf surfaces can compensate the reduction in NAR so that relative growth rate (RGR) remains more or less constant.

II. MATERIALS AND METHODS

The study was carried out to have a detailed analysis of growth parameters and to determine their underlying relationships. To meet the aims and for having better understanding of plant growth analysis, we have to analyze the plant right from germination up to the senescent stage and finally the death.

2.1 Sowing and Transplantation

In order to carry out the growth analysis of tomato, the plant is to be cultivated under the field conditions. The seeds were sown in field beds of 1.5m x 1.5m dimensions to raise the nursery plantlets. When nursery plantlets attained a sufficient size after 15-20 days time interval and were easy to handle, they were transferred to main beds. The plantlets were transplanted into five beds with equal and enough spacing in between the plantlets so that the desired sized plants can be raised for better analysis.

2.2 Sampling

Sampling was done with equal time interval gaps of 15 days and the last sampling was done at the time of senescence. First samples were collected after 15 days of transplantation. Twenty plants were harvested randomly from five beds and were carried directly in polythene bags to laboratory to avoid any water loss.

2.3 Growth indices

Various growth analysis parameters (growth indices) were studied at each sampling which is enlisted below:

2.3.1 Plant height

Due to the photosynthesizing capability of plants there is an increase in the biomass of the plants which is revealed by increase in plant size, thickness etc. The height of our study plant was measured by using thread and ruler. The length of individual parts of plant like stem, roots etc. were also measured by the same technique.

2.3.2 Fresh mass

In order to measure fresh mass, it is important that the standard moisture level is maintained before and during the measurement. The fresh mass for all the plant parts such as roots, stem, leaves, fruits etc. was individually weighed by digital balance.

2.3.3 Dry mass

It is the simplest index of plant growth analysis. Dry mass is a rate of change in size, an increment in dry weight per unit time. For determining dry weight, under and over-drying must be shunned. Dry mass is the mass of the plant or plant parts after removal of moisture. For dry weight estimation the individual parts were placed in paper bags and stored in oven at 25±5°C for a week. After drying the dried individual parts where weighed separately and the dry weights were recorded. Finally, the mean weight of each part was calculated.

2.3.4 Root-Shoot Ratio

An index of the balance of growth between root and shoot components of the plant integrated over a period of time. It was determined by using the following formula:

$$K = \frac{DW_R}{DW_S} \quad (1)$$

Where "K" is Root-Shoot Ratio, "DW_R" is Dry weight of Root and "DW_S" is Dry weight of Shoot (aerial parts).

2.3.5 Moisture content

It is the measure of water present in the plant tissue. For determining the moisture content, the fresh mass of stem, leaves and fruits was subtracted by their respective dry masses. The results were expressed as percentage by dividing the resultant moisture content by fresh mass (Kuchay and Zargar, 2016):

$$Percent\ Moisture\ Content\ (PMC) = \frac{Fresh\ Mass - Dry\ Mass}{Fresh\ Mass} * 100 \quad (2)$$

2.3.6 Resource allocation

The food synthesized during the photosynthetic activity of the plant is allocated to different parts of plant on the basis of requirement. The percentage of resources allocated to different parts of plant was determined by the following formula (Kuchay and Zargar, 2016):

$$Resource\ Allocation = \frac{Resources\ \ or\ Dry\ Mass\ allocated\ \ towards\ \ particular\ \ plant\ \ part}{Total\ \ plant\ \ biomass\ \ or\ Dry\ Mass} * 100 \quad (3)$$

2.3.7 Leaf area

In order to quantify the leaf area all the leaves from each and every sampled plant were drawn on uniformly thick paper to serve as replicas of leaves and were weighed individually for each plant. In addition to it 30 paper chits (10*10 cm^2) of same paper were also weighed in order to determine the leaf area as follows (Kuchay and Zargar, 2016):

Let weight of 100$cm^2$of paper = X g

1g of paper = 100/X cm^2 of area

Let weight of leaf replicas on paper = Y g

Therefore

$$Leaf\ Area = \frac{Y * 100\,cm^2}{X} \quad (4)$$

2.3.8 Fruit volume

The volume is determined either by calculation, if the geometry of the fruit is simple, or, if not, by the displacement of water (employing Archimedes principle). In our study the volume of fruits was calculated by the mathematical formula:

$$Volume\ of\ Fruit = \frac{4}{3}\boldsymbol{\pi r^3} \quad (5)$$

2.3.9 Relative growth rate (RGR)

RGR is the increase in plant dry matter per unit of plant dry matter per unit time. It was calculated by using the formula given by Fisher (1921):

$$RGR = \frac{(loge\ W_2 - loge\ W_1)}{t_2 - t_1} \quad (6)$$

W_1 and W_2 represent dry weights of plant at time intervals t_1 and t_2 respectively.

2.3.10 Net assimilation rate (NAR)

It is the index used to calculate the productive efficiency of plants in relation to total leaf area. NAR was calculated according to formula given by Williams (1946):

$$NAR = \frac{(W_2 - W_1)}{t_2 - t_1} * \frac{(loge\ LA_2 - loge\ LA_1)}{LA_2 - LA_1} \quad (7)$$

LA_1 and LA_2 are leaf areas &W_1 and W_2 represent dry weights of plant at time intervals t_1 and t_2 respectively.

2.3.11 Leaf area ratio (LAR)

This morphological index was devised by G. E. Briggs and co-workers for describing the leafiness of the plant. It takes into consideration the photosynthesizing and respiring components of the plant. Mean leaf area ratio was calculated according to the formula given below:

$$LAR = \frac{(\frac{LA_1}{W_1} + \frac{LA_2}{W_2})}{2} \quad (8)$$

LA_1 and LA_2 are leaf areas of plant & W_1 and W_2 are dry weights of plant at time intervals t_1 and t_2 respectively.

2.3.12 Specific leaf area (SLA)

This index highlights the concept of "leafiness of the leaf". It is a measure of density which involves an assessment of leaf area in relation to its dry weight. Mean SLA was calculated according to the formula:

$$SLA = \frac{(\frac{LA_1}{LW_1} + \frac{LA_2}{LW_2})}{2} \quad (9)$$

LW_1 and LW_2 are leaf dry weights of plant at time intervals t_1 and t_2 respectively.

2.3.13 Leaf Weight ratio (LWR)

It is an index of leafiness of the plant on the dry weight basis; a measure of the "productive investment of the plant, dealing with the relative expenditure on potentially photosynthesizing organs. Mean LWR was calculated by the formula:

$$LWR = \frac{(\frac{LW_1}{W_1} + \frac{LW_2}{W_2})}{2} \quad (10)$$

LW_1 and LW_2 are leaf dry weights & W_1 and W_2 are dry weights of plant at time intervals t_1 and t_2 respectively.

III. RESULTS AND DISCUSSION

3.1 Plant Height

In general, the plant height and the length of stem and roots progressively increased throughout the study. However, the plant height and stem length showed exponential increase during the reproductive phase and diminishing towards senescence. The root length increased fairly constantly but shows sprout towards senescence due to shift in resource allocation pattern (Table 1 & Figure 1).

TABLE 1
PLANT HEIGHT (cm) AND DIMENSIONS OF VARIOUS PLANT PARTS OF *Lycopersicon esculentum* AT DIFFERENT TIME INTERVALS (DAYS).

Parameters	Time Interval (Days)									
	0	15	30	45	60	75	90	105	120	135
Stem length	-	3.6	4.7	10.5	17.6	29.3	38.5	52.3	75.2	81.2
Root length	-	3.5	4.8	7.9	10.9	11.5	13.3	16.4	18.3	25.2
Plant height	-	7.1	9.5	18.4	28.5	40.8	51.7	68.6	93.5	106.4

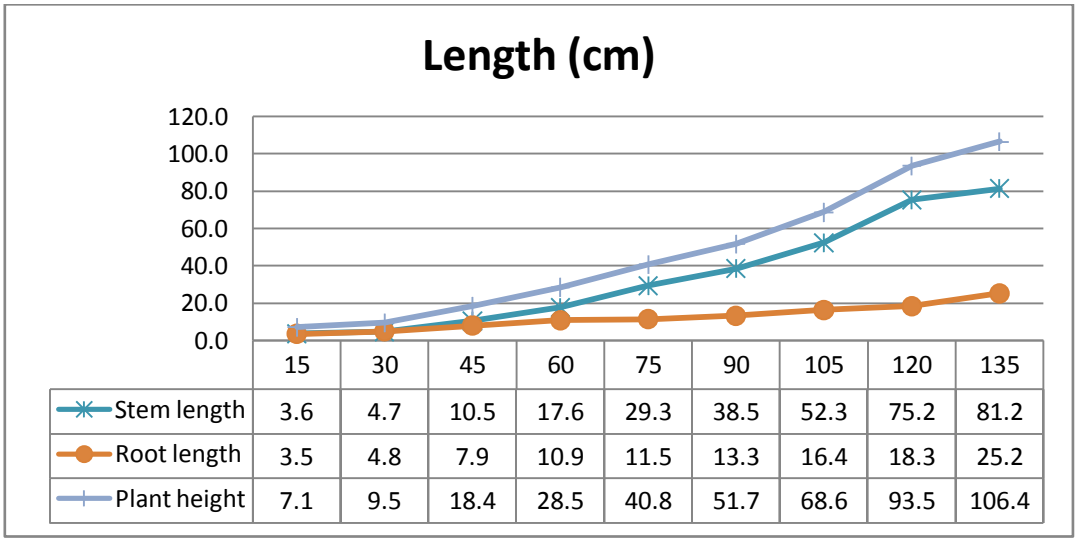


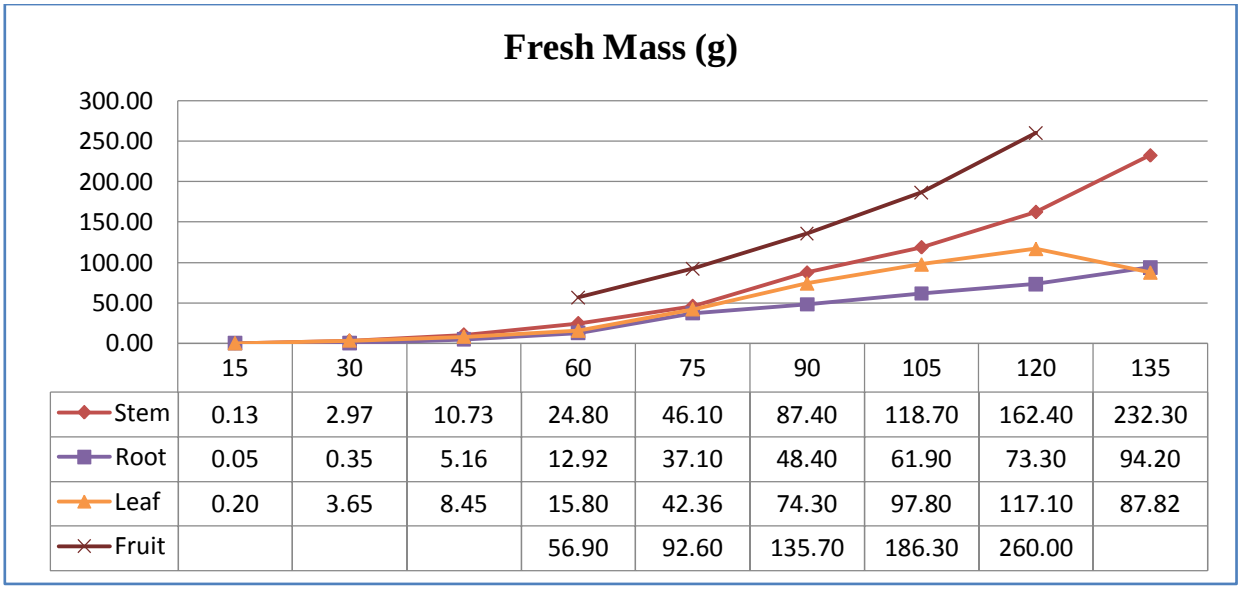
FIGURE 1: Plant height (cm) and dimensions of various plant parts *Lycopersicon esculentum* at different time intervals (days)

3.2 Fresh Mass

Fresh mass of vegetative parts showed an increasing trend from germination to senescence stage. Though the leaf fresh mass also followed the same trend but it showed a decline after the onset of senescence (Table 2 and Figure 2a & 2b). The fresh mass of reproductive parts increased during reproductive stage until senescence of the plant.

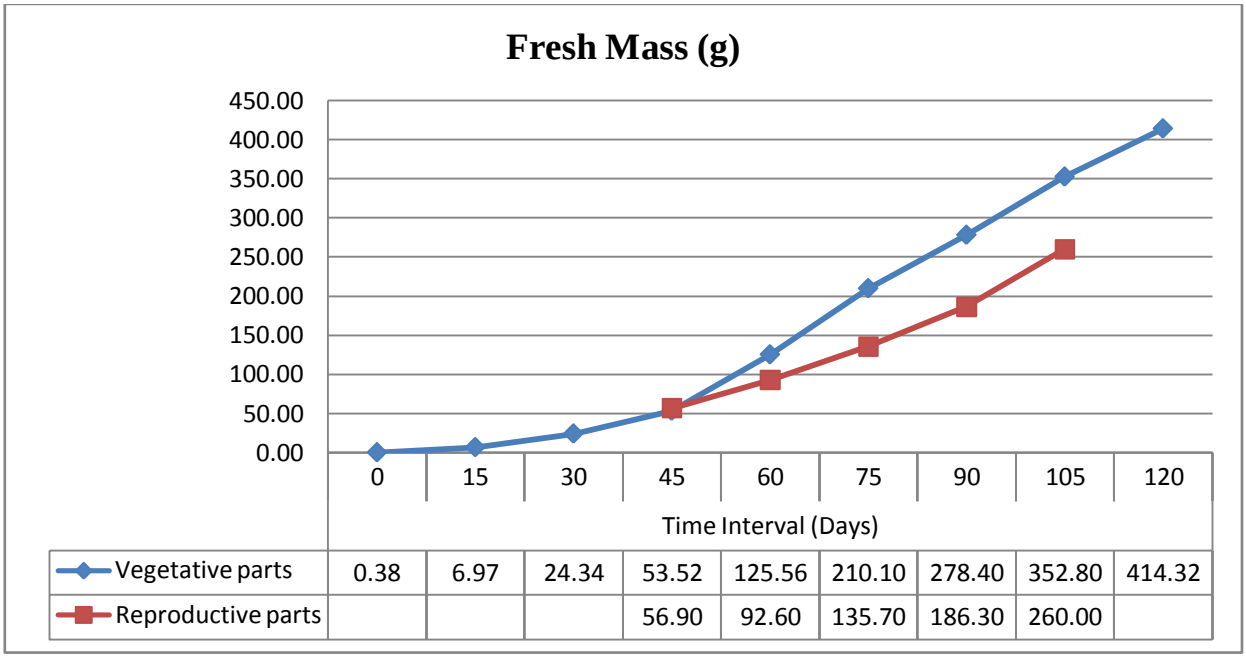
TABLE 2
FRESH MASS (g) OF VARIOUS PLANT PARTS OF *Lycopersicon esculentum* AT DIFFERENT TIME INTERVALS (DAYS)

Parameters	Time Interval (Days)									
	0	15	30	45	60	75	90	105	120	135
Stem	-	0.13	2.97	10.73	24.80	46.10	87.40	118.70	162.40	232.30
Root	-	0.05	0.35	5.16	12.92	37.10	48.40	61.90	73.30	94.20
Leaf	-	0.20	3.65	8.45	15.80	42.36	74.30	97.80	117.10	87.82
Fruit	-	-	-	-	56.90	92.60	135.70	186.30	260.00	-
Vegetative parts	-	0.38	6.97	24.34	53.52	125.56	210.10	278.40	352.80	414.32
Reproductive parts	-	-	-	-	56.90	92.60	135.70	186.30	260.00	-



	15	30	45	60	75	90	105	120	135
Stem	0.13	2.97	10.73	24.80	46.10	87.40	118.70	162.40	232.30
Root	0.05	0.35	5.16	12.92	37.10	48.40	61.90	73.30	94.20
Leaf	0.20	3.65	8.45	15.80	42.36	74.30	97.80	117.10	87.82
Fruit				56.90	92.60	135.70	186.30	260.00	

FIGURE 2a: Fresh mass (g) of various plant parts of *Lycopersicon esculentum* at different time intervals (days).



	0	15	30	45	60	75	90	105	120
Vegetative parts	0.38	6.97	24.34	53.52	125.56	210.10	278.40	352.80	414.32
Reproductive parts				56.90	92.60	135.70	186.30	260.00	

FIGURE 2b: Fresh mass (g) of various plant parts of *Lycopersicon esculentum* at different time intervals (days).

3.3 Dry Mass

The dry mass of stem and root progressively increased throughout the study time. Though the dry mass of leaves also followed the same trend but it showed a decline after the onset of senescence (Table 3 & Figure 3a). The total dry mass of whole plant increases gradually during vegetative phase and sharply during reproductive phase finally declining rapidly during senescent phase (Table 3 & Figure 3a and 3b). As compared to aerial parts the dry mass of underground parts shows an increasing trend throughout (Table 3 & Figure 3b). The total dry mass of vegetative as well as reproductive parts (fruits) increases (Table 3 & Figure 3b).

3.4 Root-Shoot Ratio

Root-Shoot ratio shows an unpredictable trend. However, the ratio decreases sharply as the plant undergoes transition from vegetative phase (from 0 to 45 days) to reproductive phase (from 60 to 120 days) as more resource are allocated towards fruits. After the onset of senescence, it again shows an increase (Table 3 & Figure 3c).

TABLE 3

DRY MASS (g) OF VARIOUS PLANT PARTS OF *Lycopersicon esculentum* AT DIFFERENT TIME INTERVALS (DAYS)

Parameters	Time Interval(Days)									
	0	15	30	45	60	75	90	105	120	135
Stem	-	0.003	0.032	1.382	9.850	28.210	47.600	83.480	96.870	161.260
Root	-	0.001	0.012	0.860	2.980	16.390	25.890	37.300	49.500	62.300
Leaf	-	0.007	0.380	2.720	7.780	24.800	46.960	64.800	79.400	60.830
Fruit	-	-	-	-	16.300	34.030	73.200	99.300	154.400	-
Vegetative parts	-	0.011	0.424	4.962	20.610	69.400	120.450	185.580	225.770	275.390
Reproductive parts	-	-	-	-	16.300	34.030	73.200	99.300	154.400	-
Aerial parts	-	0.010	0.412	4.102	33.930	87.040	167.760	247.580	330.670	213.090
Underground parts	-	0.001	0.012	0.860	2.980	16.390	25.890	37.300	49.500	62.300
Total plant	-	0.011	0.424	4.962	36.910	103.430	193.650	284.880	380.170	275.390
Root/Shoot Ratio	-	0.100	0.029	0.210	0.088	0.188	0.154	0.151	0.150	0.292

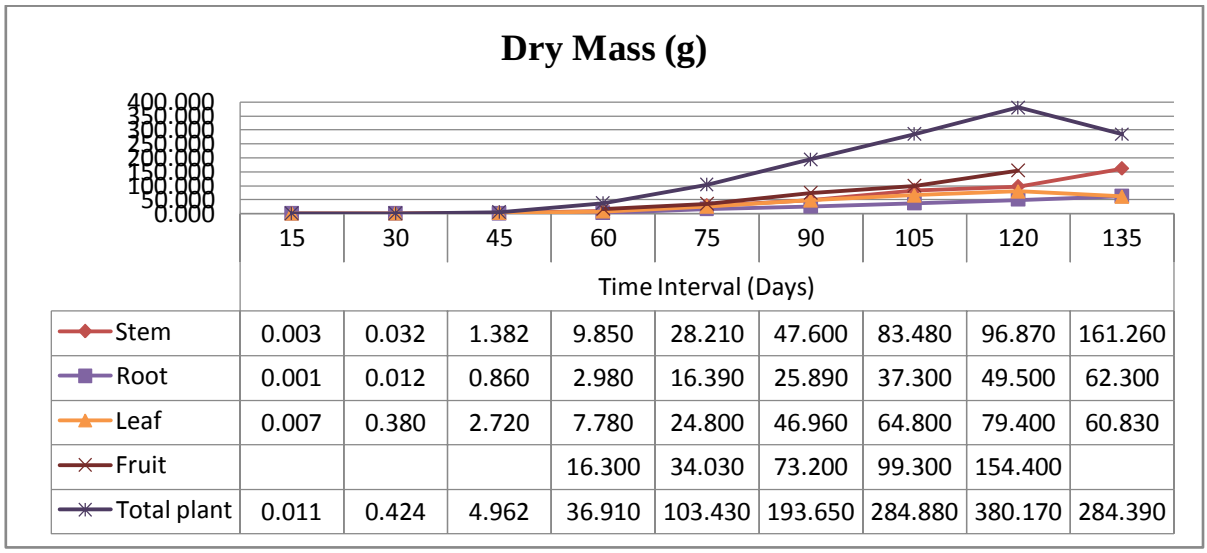


FIGURE 3a: Dry mass (g) of various plant parts of *Lycopersicon esculentum* at different time intervals (days).

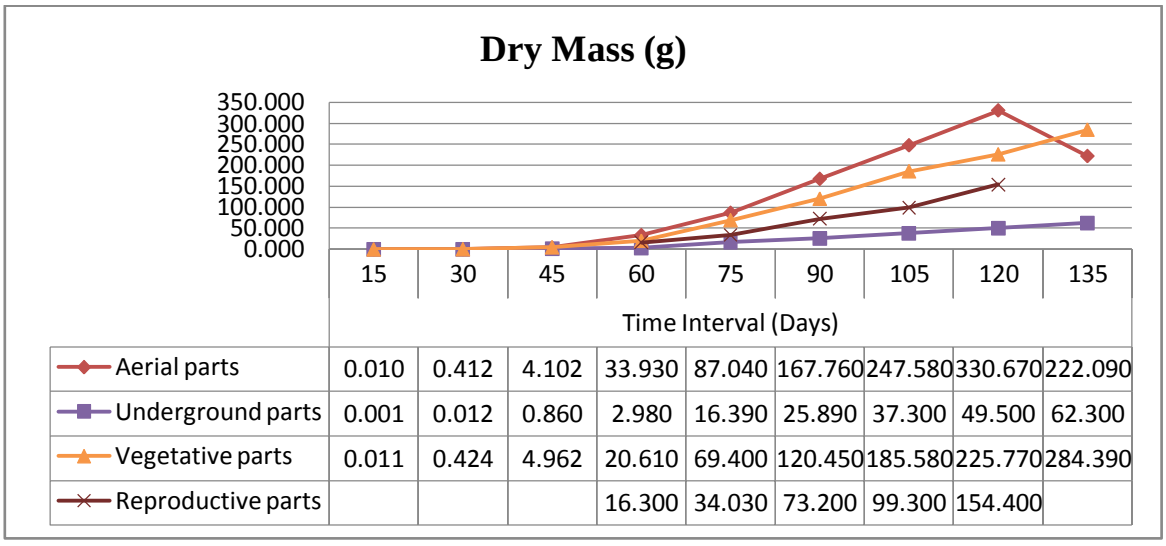


FIGURE 3b: Dry mass (g) of various plant parts of *Lycopersicon esculentum* at different time intervals (days).

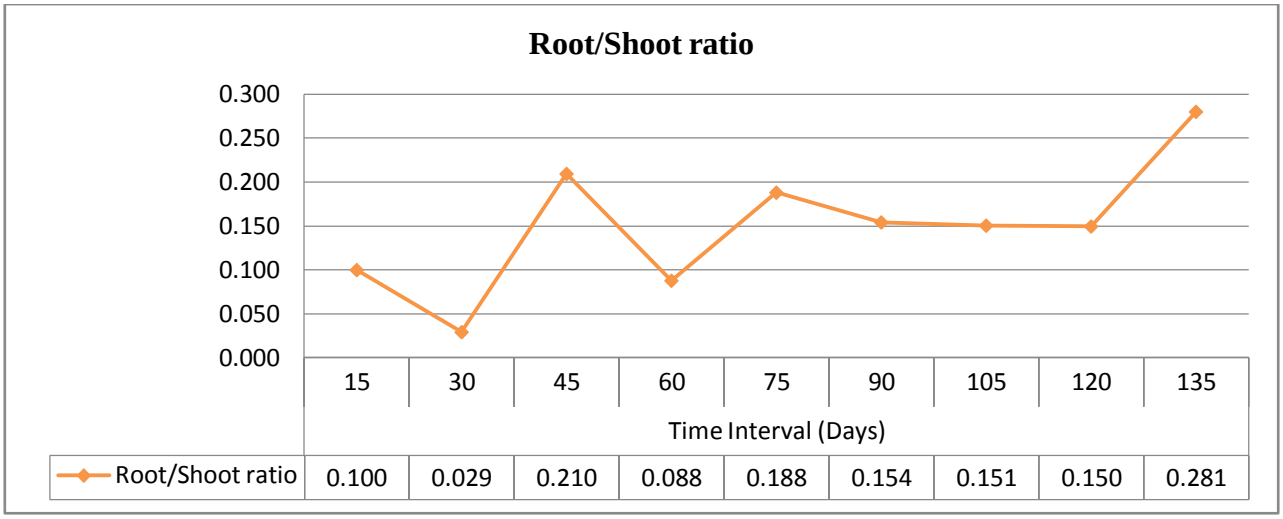


FIGURE 3c: Root/Shoot ratio (on dry mass basis) of *Lycopersicon esculentum* at different time intervals (days).

3.5 Percentage Moisture Content

The moisture content of stem, roots and leaves shows a decreasing trend along the course of growth due to buildup of more matter and maturation of plant. In case of reproductive structures (fruits) the moisture content also shows decreasing trend as they proceed towards maturity (Table 4 & Figure 4).

TABLE 4
PERCENTAGE MOISTURE CONTENT OF VARIOUS PLANT PARTS OF *Lycopersicon esculentum* AT DIFFERENT TIME INTERVALS (DAYS).

Parameters	Time Interval (Days)									
	0	15	30	45	60	75	90	105	120	135
Stem	-	97.692	98.923	87.120	60.282	38.807	45.538	29.671	40.351	30.581
Root	-	98.000	96.571	83.333	76.935	55.822	46.508	39.742	32.469	33.864
Leaf	-	96.500	89.589	67.811	50.759	41.454	36.797	33.742	32.195	30.733
Fruit	-	-	-	-	71.353	63.251	46.057	46.699	40.615	-

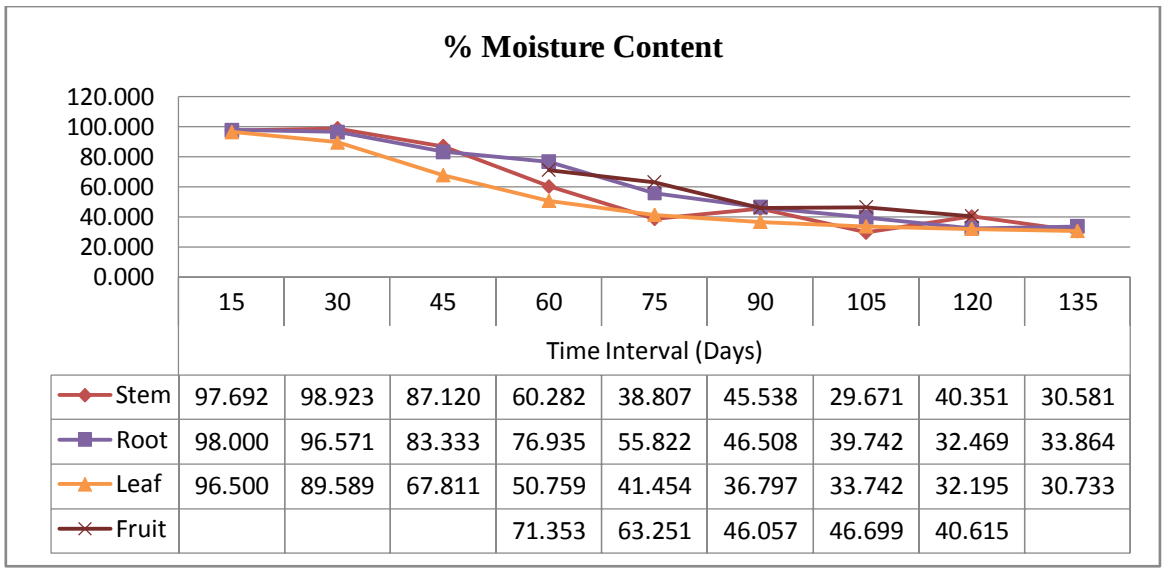


FIGURE 4: Percentage Moisture Content of various plant parts of *Lycopersicon esculentum* at different time intervals (days).

3.6 Resource Allocation

During vegetative growth more resources are allocated towards leaves followed by stem and then roots but during reproductive phase more resources are allocated towards fruits followed by leaves, stem and then roots (Table 5 & Figure 5).

TABLE 5
RESOURCE ALLOCATION (PERCENT) OF *Lycopersicon esculentum* AT DIFFERENT TIME INTERVALS (DAYS)

Parameters	Time Interval (Days)									
	0	15	30	45	60	75	90	105	120	135
Stem	-	27.27	7.55	27.85	26.69	27.27	24.58	29.30	25.48	56.70
Root	-	9.09	2.83	17.33	8.07	15.85	13.37	13.09	13.02	21.91
Leaf	-	63.64	89.62	54.82	21.08	23.98	24.25	22.75	20.89	21.39
Fruit	-	-	-	-	44.16	32.90	37.80	34.86	40.61	-

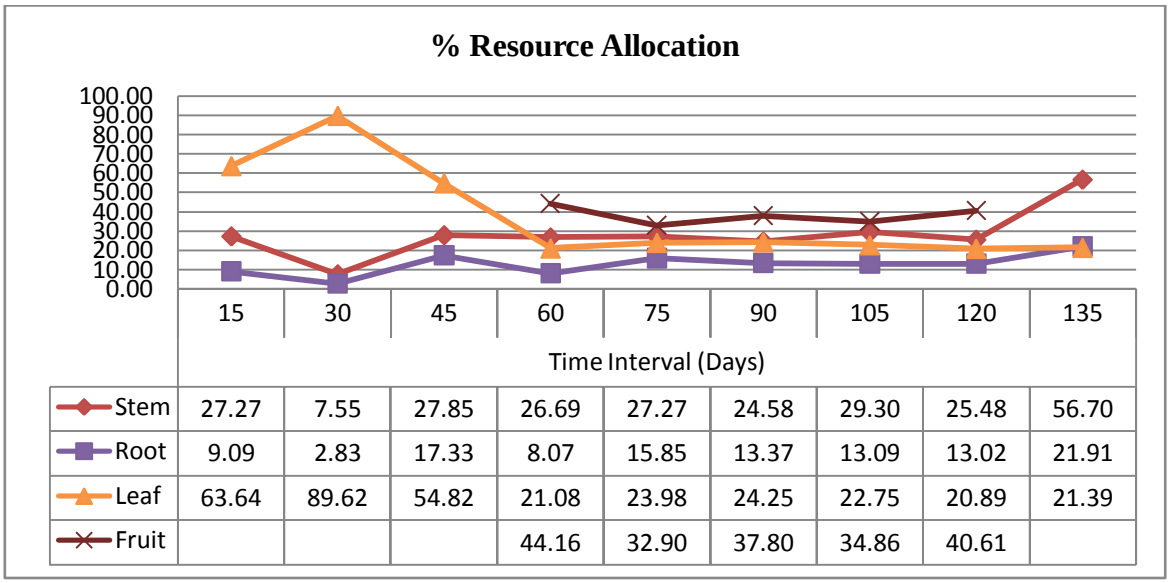


FIGURE 5: Resource Allocation (percent) of *Lycopersicon esculentum* at different time intervals (days).

3.7 Leaf Area

The leaf area increases with time reaching its peak at 120th day of sowing and after that due to onset of senescence it starts decreasing with time (Table 6 & Figure 6).

3.8 Fruit Volume

Fruit volume gradually increases over the entire growing season after fruiting as more resources are allocated towards fruits and also due to decrease in the moisture content as fruits proceed towards maturity (Table 6 & Figure 6).

TABLE 6
LEAF AREA (cm2) AND FRUIT VOLUME (cm3) OF *Lycopersicon esculentum* AT DIFFERENT TIME INTERVALS (DAYS)

Parameters	Time Interval (Days)									
	0	15	30	45	60	75	90	105	120	135
Leaf Area (cm2)	-	35.2	58.1	120.2	205.45	397.7	506.9	728.3	910.4	802.76
Fruit Volume (cm3)	-	-	-	-	11.02	25.3	34.12	49.15	60.4	-

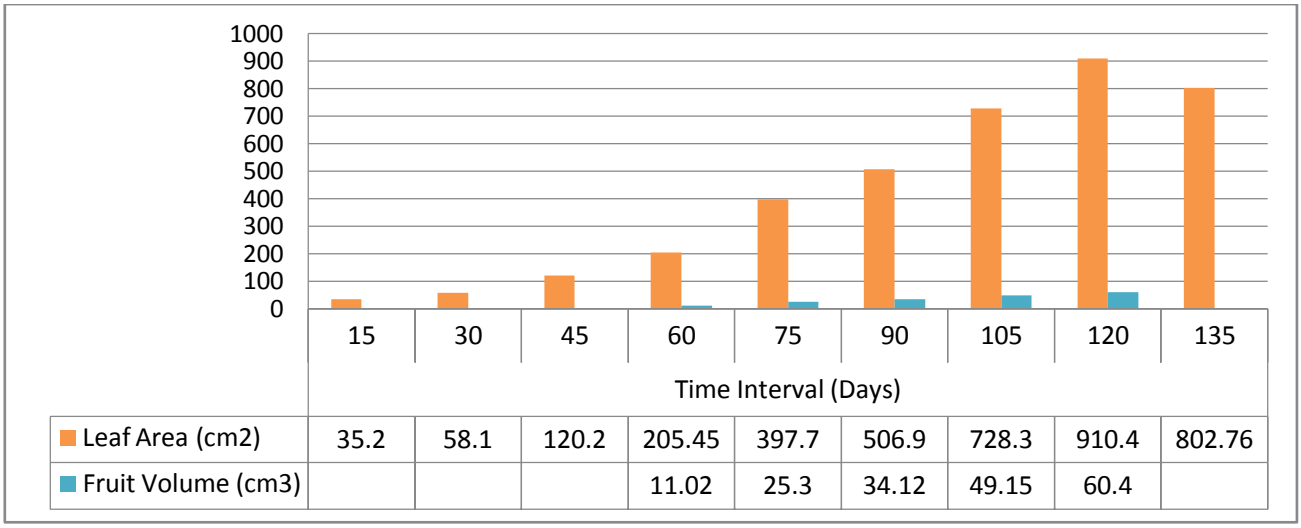


FIGURE 6: Leaf area (cm2) and Fruit Volume (cm3) of *Lycopersicon esculentum* at different time intervals (days).

3.9 Relative Growth Rate (RGR)

Mean RGR is highest during the first intervals thereafter decreasing and becoming negative (relative decay rate) at senescent stage. RGR is at its peak at the start of vegetative phase (Table 7 & Figure 7).

3.10 Net Assimilation Rate (NAR)

Mean NAR remains relatively constant. It follows more or less the same path as that of RGR becoming negative at the senescent stage (Table 7 & Figure 7).

3.11 Leaf Area Ratio (LAR)

Mean LAR was maximum at first time interval after that it sharply decreases during vegetative phase. It remains more or less constant during the reproductive phase as during this phase more resources are allocated to the developing fruits than to any other plant part (Table 7 & Figure 7).

3.12 Specific Leaf Area (SLA)

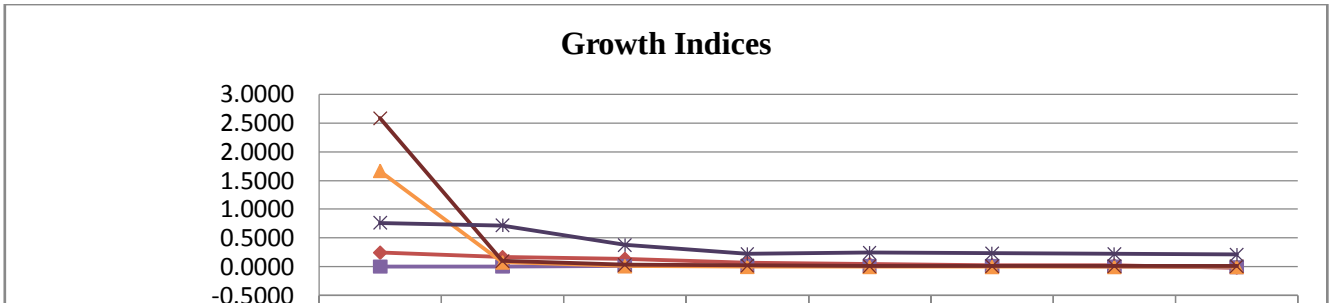
It shows a declining trend throughout the study period but the decrease is quite rapid during vegetative phase (Table 7 & Figure 7).

3.13 Leaf Weight Ratio (LWR)

It is highest at the initial stages showing a sharp decline during transition from vegetative to reproductive phase and then declining constantly (Table 7 & Figure 7).

TABLE 7
GROWTH INDICES OF *Lycopersicon esculentum* AT DIFFERENT TIME INTERVALS (DAYS).

Growth Indices	Time Interval (Days)							
	15-30	30-45	45-60	60-75	75-90	90-105	105-120	120-135
RGR (Day-1)	0.2435	0.1640	0.1338	0.0687	0.0418	0.0257	0.0192	-0.0194
NAR (g/cm2/day)	0.0006	0.0035	0.0134	0.0152	0.0134	0.0100	0.0078	-0.0075
LAR (1000cm2/g)	1.6685	0.0806	0.0149	0.0047	0.0032	0.0026	0.0025	0.0026
SLA (1000cm2/g)	2.5907	0.0985	0.0353	0.0212	0.0134	0.0110	0.0114	0.0123
LWR	0.7663	0.7222	0.3795	0.2253	0.2411	0.2350	0.2182	0.2114



	15-30	30-45	45-60	60-75	75-90	90-105	105-120	120-135
RGR (Day-1)	0.2435	0.1640	0.1338	0.0687	0.0418	0.0257	0.0192	-0.0194
NAR (g/cm2/day)	0.0006	0.0035	0.0134	0.0152	0.0134	0.0100	0.0078	-0.0075
LAR(1000cm2/g)	1.6685	0.0806	0.0149	0.0047	0.0032	0.0026	0.0025	0.0026
SLA (1000cm2/g)	2.5907	0.0985	0.0353	0.0212	0.0134	0.0110	0.0114	0.0123
LWR	0.7663	0.7222	0.3795	0.2253	0.2411	0.2350	0.2182	0.2114

FIGURE 7: Growth Indices of *Lycopersicon esculentum* at different time intervals (days).

IV. CONCLUSION

The study made it evident that growth occurred during all the growing stages but crop yield is extremely related to reproductive stage. The values of growth analysis parameters like Relative Growth Rate and Net Assimilation Rate were highest for the period of vegetative growth showing gradual decline towards the senescence and becoming negative at senescent stage. More Resources (Photosynthates) were allocated towards leaves during vegetative phase to increase the photosynthetic efficiency whereas there was a shift towards reproductive parts during reproductive phase for fruiting. The productivity of crop and the dry mass of fruits are dependent on the Leaf area of plants.

REFERENCES

[1] Ahad, M. A. (1986). Growth analysis of Rice bean (Vignaumbellata Thunb.) under different management practices and their agronomic appraisal. *PhD Diss in Agro*.

[2] Blackman, V. H. (1919). The compound interest law and plant growth. *Annals of Botany*, *33*(131), 353-360.

[3] Ferreira, S. M. R., Freitas, R. J. S. D., &Lazzari, E. N. (2004). Identity and quality standards of tomatoes (Lycopersicon esculentum Mill.) for fresh consumption. *Ciência Rural*, *34*(1), 329-335.

[4] Fisher, R. A. (1921). Some remarks on the methods formulated in a recent article on "The quantitative analysis of plant growth.". *Annals of Applied Biology*, *7*(4), 367-372.

[5] Hunt, R. (2003). Growth and development/growth analysis, individual plants. In 'Encyclopaedia of applied sciences. (Eds B Thomas, DJ Murphy, D Murray.) pp. 579–588.

[6] Jensen, C. R., Battilani, A., Plauborg, F., Psarras, G., Chartzoulakis, K., Janowiak, F., ... & Liu, F. (2010). Deficit irrigation based on drought tolerance and root signalling in potatoes and tomatoes. *Agricultural Water Management*, *98*(3), 403-413.

[7] Jesus Periago, M., García-Alonso, J., Jacob, K., Belén Olivares, A., José Bernal, M., Dolores Iniesta, M., ... &Ros, G. (2009). Bioactive compounds, folates and antioxidant properties of tomatoes (Lycopersicum esculentum) during vine ripening. *International Journal of Food Sciences and Nutrition*, *60*(8), 694-708.

[8] Karim, M. F., & Fattah, Q. A. (2007). Growth analysis of chickpea cv. Bari chhola-6 as affected by foliar spray of growth regulators. *Bangladesh Journal of Botany*, *36*(2), 105-110.

[9] Kuchay, S. A., & Zargar, M. A. (2016). Analysis of growth in some cultivars of *Solanum melongena* grown in Kashmir. *Imperial Journal of Interdisciplinary Research (IJIR)*, *2*(5), 397-405.

[10] Matos, E. S., Shirahige, F. H., & de Melo, P. C. T. (2012). Performance of tomato hybrids of indeterminate growth depending on training systems. *Horticultura Brasileira*, *30*(2), 240-245.

[11] Monte, J. A., Carvalho, D. F. D., Medici, L. O., da Silva, L. D., & Pimentel, C. (2013). Growth analysis and yield of tomato crop under different irrigation depths. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *17*(9), 926-931.

[12] Sharoni, Y., & Levi, Y. (2006). Cancer prevention by dietary tomato lycopene and its molecular mechanisms. *Tomatoes, lycopene & human health*, 111-125.

[13] Srivastava, B. K., & Singh, R. P. (1990). Morpho-physiological response of garden pea (*Pisumsativum L.*) cultivars to sowing dates II. Growth and development pattern. *Vegetable Science*, *17*(2), 140-148.

[14] Thakur, D. S., & Patel, S. R. (1996). Growth and Sink Potential of Rice as Influenced by the Split Application of Potassium. *Journal of Maharashtra Agricultural Universities*, *21*(3), 460-461.

[15] Watson D. J. "The Physiological Basis of Variation in Yield," Advances in Agronomy, Vol. 4, 1952, pp. 101-145.

[16] West, C.,Briggs, G. E. and Kidd, F. 1920. Aquantitative analysis of plant growth-II. *Annals of Applied Biology* 7: 202-223.

[17] West, C., Briggs, G. E., & Kidd, F. (1920). Methods and significant relations in the quantitative analysis of plant growth. *New Phytologist*, *19*(7), 200-207.

[18] Willcox, J. K., Catignani, G. L., & Lazarus, S. (2003). Tomatoes and cardiovascular health.

[19] Williams, R. F. (1946). The physiology of plant growth with special reference to the concept of net assimilation rate. *Annals of Botany*, *10*(37), 41-72.

[20] Wilson, J. W. (1981). Analysis of growth, photosynthesis and light interception for single plants and stands. *Annals of Botany*, *48*(4), 507-512.

Review on Barley Scald Disease Management

Worku Abebe

Ethiopian Institute of Agricultural Research, Holleta Agricultural Research Center (HARC), P.O. Box 2003, Holeta, Ethiopia

Received:- 06 August 2021/ Revised:- 17 August 2021/ Accepted:- 24 August 2021/ Published: 31-08-2021

Abstract— *Barley (Hordeum vulgare L.) is one of the ancient grain crops cultivated and used worldwide. In Ethiopia, barley is among important staple crops next to tef, maize, wheat and sorghum mainly grown on about 1 million ha of land with average yield of 2.1t ha. Leaf scald is one of the most important diseases of barley in the worldwide where the crop is grown and it causes significant reduction in yield and quality. In Ethiopia, barley is the predominant cereal in the high altitudes and it accounts nearly 25% of the total production in Africa. In addition, Ethiopia is the second largest barley producer in Africa.*

Leaf scald is one of the most important diseases of barley in the worldwide wherever the crop is grown and it causes significant reduction in yield and quality. Yield loss due to scald disease reaches up to 100% in susceptible cultivars under severe epidemics. In Ethiopia, scald is among widely distributed and destructive diseases in cool highland areas and yield losses reaching about 67% have been recorded. This review discusses recent information on economic importance, epidemiology, life cycle, geographical distribution and disease management of barley leaf scald disease. It also presents the barley leaf scald disease management methods such as cultural, chemical, use of host resistance methods as well as integrated barley leaf scald disease management. Under host resistance method, information on types of resistance, sources of resistance have been presented.

Keywords— Barley, Scald disease, Management, Methods, Cultural, Chemical, Host resistance.

I. INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the ancient grain crops cultivated and used worldwide (; Baik and Ullrich, 2008). It has been grown in the Middle East about 10,000 years ago (Zohary and Hopf, 2000) and is mainly produced for feeding and malting worldwide. Moreover, barley occupies 57 million hectares of the world's agricultural land area, and is a staple food for many people globally, in addition to its uses in malting and as an animal feed (Newton *et al.,* 2011).

In Ethiopia, barley is among important staple crops next to tef, maize, wheat and sorghum mainly grown on about 1 million ha of land with average yield of 2.1t ha (CSA, 2017). Ethiopia is the second largest barley producer in Africa (FAO, 2014). Ethiopia accounts nearly 25% of the total production in Africa (FAO, 2014). Scald is a serious foliar disease in barley (*Hordeum vulgare* L.) and occurs worldwide wherever barley is grown (Shipton, 1974). It can cause up to 40% yield loss in susceptible cultivars and also has a detrimental impact on grain quality. Thus, scald is considered as one of the most economically important barley disease worldwide predominantly in the cool and semi-humid barley growing areas (Zhan *et al.,* 2008).

II. ECONOMIC IMPORTANCE OF SCALD DISEASE OF BARLEY

Scald, caused by the fungus *Rhynchosporium commune*, occurs wherever barley is grown from northern and central Europe to the Middle East, Central Asia, North and South Africa, the Americas, Australia and New Zealand (Shipton *et al.,* 1974; Beigi *et al.,* 2013) and is one of the most economically important diseases of barley worldwide (Beer, 1991), causing yield and grain quality reduction (Zhan *et al.,* 2008). The disease is particularly significant in cool, semi-humid areas, where crop canopies are aggregated and leaves are exposed to prolonged wet conditions (Shipton *et al.,* 1974). This pathogen can cause dramatic yield reductions, up to 40%, along with reductions in grain quality (Jenkins & Jemmett, 1967) and losses of nearly 100% can occur on susceptible barley cultivars (Yahyaoui, 2004). Yield losses occur mainly through reduced 1000 grain weights, although other above ground parts may be reduced as well (James *et al.,* 1968). There is also a report that indicates

the national barley yield loss of UK in 2005, due to scald disease was estimated£ 10.8million per annum (at a price of £225/tonne) despite fungicide treatment (HGCA, 2011). It affects barley on several million hectares in North Africa, West Asia, East Africa (Eritrea and Ethiopia), Yemen, Central Asia, the Andean countries (Peru, Colombia, Bolivia, and Ecuador), and the Far East (Yahyaoui *et al.,* 1999). Scald is particularly damaging in areas where barley is cultivated continuously such as in West Asia, in central and southern parts of North Africa, the highland regions of Peru and Nepal, and large areas in Eritrea and Ethiopia. In Ethiopia, it can reduce yields by up to 67% (Chilot *et al*., 1998).

III. EPIDEMIOLOGY OF BARLEY SCALD DISEASE

Rhynchosporium commune is a polycyclic barley disease causing pathogen that involves several pathogen generations during a growing season (Fitt *et al.,* 1989). It is splash dispersed and mainly overwinters on crop debris, seed and infected soil surfaces. Thus, Sources of primary inoculum for infection of barley plants include, crop debris (Fitt *et al.,* 2012), infected soils (Zhan *et al*., 2008), infected seeds (Topp *et al.,* 2019), barley volunteers infected from the debris of previous crops(Jenkins and Jemmet, 1967) as well as wind-borne ascospores (Fountaine *et al.,* 2010). The inoculum from these sources infects barley plants during the next growing season (Stefansson *et al*. 2013). Nevertheless crop debris is considered the most important source of primary inoculum (Nielsen & Jensen, 2001) and sporulation potential of *Rhynchosporium commune* on crop debris in the field could survive for up to 12 months (Murray *et al*., 1999). Unlike to crop debris, soil surface is not a major source of primary inoculum because the fungus has limited survival ability in soil due to its weak competitive saprophytic ability (Shipton *et al,* 1974). On the other hand, Fountaine *et al.* (2010) indicated that infected seeds might play an important role in the introduction of scald disease to new geographical areas (Fountaine *et al.* 2010). Interestingly it has been recently reported that the pathogen can infect seeds without the appearance of visual symptoms (Fountaine *et al.,* 2010).

Scald on barley (*Hordeum vulgare* L.) is a hemibiotrophic fungal disease caused by *Rhynchosporium commune*, which was recently verified to be a distinct species from the rye and triticale pathogen *R. secalis* through phylogenetic analysis (Linde *et al.,* 2003; Zaffarano *et al.,* 2011). In the previous years, there was uncertainty regarding the host specificity of what was known as *R. secalis*, although it had been noted in the early 20th century that isolates taken from one species tended only to cause disease on the same species (Caldwell 1937). Now it is understood that *R. commune* belongs to a group of closely-related cereal pathogens, each with a relatively narrow host range (Zaffarano *et al.,* 2011). *R. commune* has also been observed to cause disease in various other wild barley (*Hordeum*), brome (*Bromus*), and ryegrass (*Lolium*) species (Zaffarano 2011).

In addition, *Rhynchosporium commune*has also long been considered to be exclusively asexual and is known only from its conidial morph and host specialization nature. In recent findings, scholars reported that *Rhynchosporium commune* is heterothallic (Linde *et al.*, 2003), which imply that sexual reproduction does take place in this species but no sexual fruiting bodies have been described. Based on phylogenetic analyses of the ITS-rDNA region, it was predicted that, if a sexual morph of *R. commune* exists, it would be a species of *Oculimacula* (Goodwin 2002). Analyzing distribution and frequencies of mating type alleles within populations are good indications of the occurrence of a sexual cycle in fungal species. In populations with approximately equal frequencies of mating alleles, it is possible that sexual recombination does take place (Linde *et al*. 2003). Mating type idiomorphs have been cloned and characterized in *R. commune* and a multiplex polymerase chain reaction (PCR) method has been developed for one-step determination of its mating type identity in *R. commune* (Linde *et al*. 2003). Analyses of distribution and frequencies of mating type alleles among *R. commune* populations have revealed the occurrence of *MAT1-1* and *MAT1-2* types at broadly similar ratios in most barley growing regions (Linde *et al*. 2003).

Rhynchosporium commune fungal growth in barley occurs in four phases: germination (occurring approximately 12 hrs. after inoculation), then penetration (from approximately 24 hrs. after inoculation), leaf colonization with a slow increase in fungal biomass, followed by exponential growth with a massive gain of biomass (at around 10 days after inoculation), and a late stationary phase during which a dense stroma forms producing sporulation (Ayesu-Offei and Clare, 1970; Zhan *et al*., 2008).

Conidia are two-celled and characteristically beak-shaped and germination optimum is reported between 15-21°C and at least 95 % air humidity (Beer, 1991). The conidia can germinate with several germ tubes and appressoria develop at the tips of the germ tubes (Shipton, *et al*., 1974; Ayesu-Offei, 1970).Germination of up to 80 % of spores occurs within 24 hours (Ryan & Clare, 1975) when conditions are dark and moist (Jackson, 1997). After a conidium germinates on a leaf surface, *R. commune* penetrates the epidermis of the host with an appressorium and penetration peg directly by penetrating the cuticle (Ayesu-Offei, 1970) and initially grows subcutaneously and intracellularly (Caldwell1937; Zhan 2008. The developed

hyphae penetrate the epidermal cell layer, particularly at the junction of guard and epidermal cells and this action causes stomata to open more to light, due to an alteration of the turgor relations between guard cells and the surrounding epidermal cells. Conidia on the stroma results in the separation and eventual cracking of the cuticle, thus superficially exposing the stroma. Approximately nine days after infection starts, mesophyll cells in contact with the mycelium collapse and the fungus begins to grow inter-cellularly. The timing of the mesophyll collapse corresponds with the appearance of water-soaked grayish lesions (Caldwell 1937). Four days later, the lesions dry out, and become first chlorotic and then necrotic (Jackson 1997). Furthermore, the scald like lesions of the disease is visible on the leaf blades and sheaths. Thereafter, the water-soaked appearances of the lesions soon fade to a bleached, scalded appearance and are surrounded by a brown-pigmented ring (Bockelman, *et al*., 1981). New *R. commune* conidia are produced on conidiophores, which erupt through the leaf cuticle in apparently healthy leaf regions Davis *et al*., 1994).

The latent period has been reported between 8 and 14 days at 20°C and about twice as long at 5°C (Beer, 1991; Jackson, 1997). Lesion growth rate has been observed at 2 mm day-1 (Xue & Hall, 1991). Conidia production is reported to be poor beyond 5 and 30°C and retarded between 27 and 37°C with optimum between 15 and 20°C (Jackson, 1997). Ayesu-Offei (1971) counted conidia production, and recorded 0.5-1.3 × 106 conidia produced in 48 hours, from groups of 2-3 lesions collected in the field and allowed to sporulate in the laboratory under optimal conditions. Spore release is favoured by rainfall and wind following rainfall, confirming that the conidia are splash dispersed and with rain may be picked up by the wind (Ayesu-Offei & Carter, 1971).

Sources of secondary inoculum (infection) are splash-dispersed conidia from infected leaves (Fitt et al., 1989). The disease is spread from leaf to leaf by rain splash (Skoropod, 1960). During each generation these conidia germinate and infect new host tissues. Spread of the disease is associated with rainfall rather than wind as the conidia are embedded in mucilage (Skoropad, 1959). Long-distance transmission can occur by sowing infected seed or movement of stubble as hay, spreading R. commune to new geographical locations. As yet, the sexual form of R. commune is unknown, thus the possibility of long-distance dispersal of ascospores remains as another pathway for the spread of disease.

Although splash dispersal of *R. commune* conidia contributes to the short-distance spread in the field (McDonald *et al*., 1999; Shipton *et al*., 1974), transport of infected seeds may be responsible for the long-distance dispersal of inoculum in general, as well as the spread of new physiological races.

Initial symptom of scald disease of barley is oval, water-soaked, grayish-green spots, 1.0-1.5 cm long. As the disease develops the centers of the lesions dry and bleach, becoming light gray, tan, or white with a dark brown margin (Avrova and Knogge, 2012). The lesions are not delimited by the leaf veins and often coalesce, allowing them to appear as large blotches anywhere on the leaf (Jackson1997. In extreme cases, the disease can completely kill the leaf tissue of the plant (Jackson1997). The typical disease symptoms, necrotic lesions, occur after a latent period lasting from a few days up to several weeks, when mesophyll cells in leaf regions that are heavily colonized by the fungus collapse (Kirsten *et al*., 2012).

IV. GEOGRAPHIC DISTRIBUTION OF BARLEY SCALD DISEASE.

Scald is a globally-distributed disease. The fungus causing the disease on rye was originally described in 1897 in Holland and, as early as the 1920s, was noted to have a range including North America, Europe and Australia (Brooks 1928). A decade later, Africa and South America had been added to its recorded range (Caldwell 1937). It is hypothesized that *Rhynchosporium* species became pathogens of barley and rye about 2,500 years ago in Scandinavia, the center of *Rhynchosporium* diversity, and then traveled southwards to the Fertile Crescent and Africa. Limited genetic diversity in *Rhynchosporium* populations in North America, Australia, and New Zealand indicate that infected seed probably traveled with European colonists to these locations within the past 500 years (Linde *et al.*, 2009).

V. LIFE CYCLE OF BARLEY SCALD PATHOGEN (RHYNCHOSPORIUM COMMUNE)

Rhynchosporium commune, the causal agent of barley scald disease has been thought that it has no sexual stage of reproduction and in the absence of a sexual life stage the fungal life cycle is comprised by conidia production, host infection and hyphal growth. Skoropad & Grinchenko (1957) observed micronidia produced in flask-shaped branches of older parts of the mycelium but attempts to germinate these microconidia failed (Skoropad & Grinchenko, 1957) and no function has been reported. However, recent findings, which indicate that the fungus is heterothallic (Linde *et al*., 2003), imply that sexual reproduction does take place in this species but no sexual fruiting bodies have been described. In the absence of any known sexual structures, the fungi probably survives between cropping seasons as mycelia in infected host residues, but may also by transmitted via seeds (Jackson, 1997). However, left over residues from previous year's crops are considered the most

important source of primary inoculum (Nielsen & Jensen, 2001). Sporulating potential of fungal material on crop residues left in the field could survive for up to 12 months (Murray *et al.,* 1999). Overwintering mycelia will produce spores when environmental conditions are favourable, serving as primary inoculum to initiate an epidemic.

Conidia are two-celled and characteristically beak-shaped and germination optimum is reported between 15-21°C and at least 95 % air humidity (Beer, 1991).Germination of up to 80 % of spores occurs within 24 hours (Ryan & Clare, 1975) when conditions are dark and moist (Jackson, 1997). The conidia can germinate with several germ tubes from one or both cells and appresoria develop at the tips of the germ tubes (Ayesu-Offei, 1970; Shipton *et al.,* 1974). The optimum temperature for germ tube growth is 15-21^{0}c (Caldwell, 1937; Fowler and Owen, 1971) within the range of 2^{0}c to 31^{0}c (Reed, 1957). Penetration takes place by penetrating the cuticle with the help of penetration peg on the appresoria (Ayesu-Offei, 1970). Infection is followed by formation of a subcuticular mycelium, which develops into a stroma, one to several cells in thickness (Ayesu-Offei and Clare, 1970; Caldwell, 1937). Later, hyphae penetrate the epidermal cell layer, particularly at the junction of guard and epidermal cells (Ayesu-Offei and Clare, 1969). Infection causes stomata to open more to light, due to an alteration of the turgor relations between guard cells and the surrounding epidermal cells (Ayres, 1972). Infection causes mesophyll cells to collapse, which is evident on the leaf surface as water soaking and scalding of the tissues (Caldwell, 1937; Ayesu-Offeiand Clare, 1970). The optimum temperature for the hyphal growth of *Rhynchosporium commune* in barley leaves is 16-18^{0}C, with a maximum at 25-30^{0}C and a minimum of 0^{0}C (Fowler and Owen, 1971). The latent period has been reported between 8 and 14 days at 20°C and about twice as long at 5°C (Beer, 1991; Jackson, 1997). Similarly, optimum temperature for spore production (sporulation) is 15-20^{0}C.

VI. BARLEY SCALD DISEASE MANAGEMENT

Scald is a stubble and seed-borne disease which is favoured by high rainfall environments. This disease is most damaging in the high rainfall. However, severe epidemics have been observed in medium rainfall areas under favourable conditions. Disease management is best achieved by knowledge of the pathogens involved and manipulation of the interacting factors. Scald of barley is more likely to be a problem when infected trash remains from a previous barley crop, or when infected barley grass is present.

Based on the complexity of the pathogen, control of the disease requires an integrated and multifaceted approach, including application of fungicides, manipulation of sowing date, cultural disease management, and the use of resistant cultivars (McLean and Hollaway, 2018); though using resistant varieties provide the easiest and most effective option to manage the disease. Thus, for effective management of the disease, it is important to use the integrated disease management practices that focus on the factors affecting the disease. Furthermore, the high genetic variability of *Rhynchosporium commune* can result in rapid adaptation of pathogen populations to render some of these control strategies ineffective when they are used alone (Shipton *et al.,* 1974)). Therefore, sustainable control of *Rhynchosporium* needs to integrate major-gene-mediated resistance, partial resistance and other strategies such as customized fungicide programmes, species or cultivar rotation, resistance gene deployment, clean seed and cultivar mixtures.

6.1 Cultural Practice

Different cultural practices such as crop rotation, cultivation, use of cultivar mixtures, manipulation of sowing date or sowing rate or agronomic treatments and planting clean seeds can be used to manage scald disease in barley. Moreover, the disease can be managed using an integrated approach that includes growing resistant varieties, avoiding early sowing, using seed dressings and not sowing into infected crop residues. Furthermore, the amount of primary inoculum available for initiating epidemics may be decreased by rotation, so that there is a break of several seasons with non-susceptible crops between successive barley crops, or by ploughing after harvest to bury infected debris and diseased volunteer barley (Shipton et al., 1974). By contrast, short rotations and minimum or reduced tillage practices which leave infected debris on the soil surface may result in severe *Rhynchosporium* epidemics in crops exposed to more primary inoculum.

Crop rotation with a non-host crop will minimise initial inoculum levels for next season's crop. Continuous cropping with the same susceptible host plant will result in the inoculum build-up of the pathogen population. Crop rotation avoids this and is often associated with a reduction in crop diseases. Similarly, crop rotation is useful in reducing inoculum of *R. commune* which can be spread from crop debris (Oxley and Burnett 2009). Rotations involving consecutive barley crops should be avoided. A minimum of 2 years is required between crops for residue to break down sufficiently. Rotating any crop other than barley between barley crops in a field will significantly reduce the potential for barley scald disease. Continuous barley cultivation leads to the accumulation of crop debris in the field and, with it, to a build-up of inoculum (Elen, 2002). Over 40

years ago, Hansen and Magnus reported an increase in scald that might have been caused by the shift from crop rotation to continuous barley cultivation (Hansen and Magnus, 1969). Reports showed that crop rotation, or even a 1-year interruption with oats, is effective in controlling the occurrence of the disease on barley (Elen, 2002).

Cultural practices such as incorporating the residue into the soil or removing it completely by burning will reduce the abundance of the pathogen and the disease pressure. Stubble may be reduced by baling and grazing; however, these methods only result in a small reduction in the disease pressure. Stubble reduction must be balanced against the increased risk of soil erosion by wind or water. Tillage has indirect effects on pathogen spread and can also be used to reduce pathogen inoculum in the soil. Deep tillage can bury pathogens deeper in the soil where they are less likely to become a problem. Reduced tillage or no-tillage is often associated with higher microbial biomass and activity in upper soil layers compared to regular tillage (ploughing) (van Diepeningen *et al.,* 2005). This concentration of crop debris in the top layers of the soil can promote the over-wintering and survival of numerous pathogens, prompting concern that reduced tillage practices might lead to increased disease and reduced yields. Indeed, this has proved to be true under certain circumstances, although there have been reports of reduced incidence of soil-borne pathogens following reduced tillage (Sturz *et al.,* 1997). Moreover, reduced tillage leads to the accumulation of crop debris in the field and, with it, to a build-up of inoculum. More recently, reduced tillage and continuous spring barley cultivation have led to an increase in the occurrence of *Rhynchosporium* in the Nordic countries (Arvidsson, 1998).

In barley scald disease, infected straw provides a reservoir of inoculum for splash dispersal when weather conditions favour the development of *R. commune* infection (Fitt *et al*., 1987). *Rhynchosporium commune* can survive on straw for about 1 year, depending on the ambient conditions, but cannot over summer in straw left in the open field or buried in soil (Skoropad, 1959). Thus, Reducing infected stubble and barley grass by grazing, burning or cultivation decreases the carry-over of the fungus between crops (Mayfield and Clare, 1984). Furthermore, stubble management can also have an impact on reducing inoculum of all stubble-borne diseases of cereals and burning or cultivating stubble can significantly reduce the level of inoculum prior to sowing and will reduce or delay disease development in the subsequent crop. More recently, reduced tillage and continuous spring barley cultivation have led to an increase in the occurrence of *Rhynchosporium* in the Nordic countries (Arvidsson, 1998).

One of the important cultural practices in management of scald disease of barley is green bridge management. A green bridge of self-sown barley leading into the cropping season provides host material for the pathogen increases the risk of its early onset. Removing this green bridge as early as practicable before seeding will greatly reduce the risk of early crop infection. Managing 'Green Bridge' volunteers reduces inoculum sources. Volunteers should be controlled before sowing to ensure that spores produced on these plants are not viable by the time of crop emergence.

Another important cultural practice used to manage scald disease of barley is use of mixtures of resistant and susceptible isogenic lines, cultivars or even species. This method of managing scald disease of barley may decrease severity of epidemics through decreasing the rate of secondary disease spread by spores dispersed from affected susceptible plants. Classically, such mixtures have been used to control diseases such as mildews and rusts caused by biotrophic pathogens with gene-for-gene interactions with their hosts, and wind-dispersed spores. Simulation modelling suggests that use of mixtures will be most effective against such wind-dispersed pathogens with shallow spore dispersal gradients. However, it has also been shown that mixtures can provide effective control of diseases with secondary spread by splash-dispersed spores with steep spore dispersal gradients and less clear gene-for-gene interactions, such as barley *Rhynchosporium* (McDonald *et al*., 1988), although this was not always the case (Abbott *et al*., 2000). Use of highly heterogeneous cultivar or cereal species mixtures can decrease severity of *Rhynchosporium* epidemics by half, and correspondingly increase yield by up to 15% (Newton *et al.,* 1997).

Likewise, altering the time of sowing to avoid high levels of pathogen inocula or conditions conducive for development of a particular disease can lead to reduced severity of several diseases. For example, it has been reported that in the UK, late sowing may be recommended for autumn-sown barley crops, in order to decrease exposure of newly emerging seedlings to inoculum of *Rhynchosporium commune* produced on previous barley crops in the area . Moreover, early sown crops develop higher levels of scald. Early sown crops may be exposed to the heaviest release of spores from infected residues. The disease can develop in the upper leaves of the plant when conditions favour spread of disease. Therefore, it is important to avoid early plantings because scald is worse in early-sown crops and when conditions favour disease development late plantings are less damaged. So, avoiding early sowing of susceptible varieties, especially in high-rainfall areas, will reduce the loss caused by scald.

Correspondingly, scholars reported that planting healthy seeds is equally important to other cultural practices used to manage scald disease of barley. Scald of barley disease can be seed-borne. Sowing infected seed can introduce disease into a new crop. Therefore clean seed should be used wherever possible. Fungicide seed dressings can reduce the risk associated with sowing infected seed. Seed treatments that suppress early scald infection are an essential part of effective scald management. A seed dressing or fungicide applied in-furrow with fertiliser should be used in medium to high rainfall areas or if the seed is from an infected crop. It is important to use good quality seed with high germination and vigour and if the seed is from an infected crop it should be treated with recommended treatment at proper rates. Moreover, seed treatments with fungicides based on maneb, prochloraz, and thiabendazole is important in decreasing the impact of early season *Rhynchosporium* epidemics (Lee *et al*., 2002). Likewise, physical seed treatment (hot water at 51°C for 12 min) is also found effective in removing *R. commune* infection (Habgood, 1971). The location of the fungal pathogen on the seed can have important consequences for the ease of control. Leaf scald symptoms have been observed on the outer structures of the seed and lesions have been used to re-isolate the pathogen (Kay and Owen 1973).

6.2 Chemical Control

The aim of foliar fungicide application in the crop is to delay disease development and to maintain green leaf area which reduces disease impact on yield and grain quality. In barley, the most important contributors to yield are leaf 2 (flag-1), leaf 3 (flag-2), the ear and upper stem. Protecting leaf 2 and leaf 3 is the highest priority in effective disease control. Fungicides can provide very high levels of disease control and are widely used to protect crops. Nevertheless, indiscriminate fungicide use, together with pathogen adaptability can reduce fungicide efficacy considerably. Foliar fungicides are used on most barley crops in Europe. However, the long-term effectiveness of fungicides depends on the ability of pathogens to evolve fungicide resistance.

The cost effectiveness of foliar fungicide applications depends on disease severity, susceptibility of the variety, yield potential of the crop, grain quality outlook and the environment where the crop is growing. When susceptible varieties are grown in conditions favorable for disease development, such as disease prone areas or high rainfall seasons, fungicide can be cost effective in reducing the disease impact where yield potential is over 2.0 t/ha. Reliance on fungicide is much greater in medium to high rainfall areas than in low rainfall regions due to higher disease pressure and longer growing seasons during which the disease epidemic may increase. For instance, in the medium rainfall region a single application of fungicide may be required at late stem elongation to flag leaf emergence stage. In a long season, high rainfall area two fungicide sprays are often required: one at early stem elongation and a follow-up spray at or just prior to flag leaf emergence. Moreover, in Europe, in winter barley crops, fungicides applied in the early spring at GS25-30 can greatly decrease disease development and therefore increase yield, but the best fungicide timing is generally at GS31-32 (Young *et al*., 2006). In spring barley crops, fungicide treatments are generally recommended where the disease is found on the upper three leaves of a susceptible cultivar. Under high disease pressure, using higher fungicide rates will give longer residual protection. In addition, it is important to apply fungicide before head emergence if hot spots within the crop are frequently observed during stem elongation or active infections are present on middle canopy leaves. Fungicides should be applied in mixtures or using alternation in modes of action to limit the rapid development of fungicide resistance**.** In order to minimise the risk of fungicide resistance, it is recommended that two-or three-way fungicide mixtures are used for controlling *R. commune* (Home-Grown Cereals Authority HGCA 2011). For example, at GS 31, a mixture of an effective triazole, a strobilurin, and a succinate dehydrogenase inhibitor (SDHI), will provide a good foundation for disease control (Home-Grown Cereals Authority HGCA 2011).

Use of fungicide mixtures has helped to impede selection for resistance to triazole fungicides in *Rhynchosporium commune* populations (Cooke *et al*., 2004), and fungicides such as epoxiconazole continue to be useful for control of *Rhynchosporium*, when used in mixtures. Fungicides with different modes of action from the strobilurin (Quinone outside Inhibitor, QoI) and anilinopyrimidine group are the most effective mixture for controlling the disease and maintaining yield. During the 1970s and 1980s, *R. commune* was effectively controlled by the application of the methyl benzimidazole carbamates (MBCs) and demethylation inhibitors (DMIs; 'triazoles'), alone or in mixtures. Since the first detection of resistance to MBC fungicides in the early 1990s, the frequency of resistant isolates has increased rapidly (Kendall *et al*., 1994; Taggart*et al*., 1998, 1999). Resistance to MBCs is now widespread in *R. commune* populations in the UK (Locke and Phillips, 1995; Taggart *et al*., 1999). Exposure to flusilazole, tebuconazole and epoxiconazole can result in a 10-fold decrease in the sensitivity of the *R. commune* population to these fungicides (Cooke *et al*., 2004), indicating erosion in their effectiveness. Although there is cross-resistance between the different triazoles, no cross-resistance between the imidazole and triazole (DMI) has been found (Kendall *et al*., 1993). Despite the partial loss of DMI efficacy in some parts of the UK and Europe, DMIs remain one of the

most important fungicide groups for the control of barley diseases (Walters *et al*., 2012). However, it is recommended that they be used mixed with other fungicides with a different mode of action.

In Ethiopia, of several fungicides evaluated, Tilt 250EC and Bayleton 25WP were registered for official use in cereals including barley (Abdurahman, 1997; Abdurahman and Berhanu, 1999). Similarly, in Kenya, scald is controlled by use of fungicides such as Bayleton (Triadimefon), Cercobin, Propiconazole (Tilt), Carbendazim (Bravocarb), Triadimenal (Bayfidan), Frutriafol (Impact) and Prochloraz (Sportak) (Kenya Breweries Ltd., 1990). In Britain, fungicides Captafol, Chlorothanil, Prochloraz, Propico-nazole Tridemefon and Benzimidazoles (alone or in combination with other materials) has been found effective in controling of this disease (Atwood, 1985

6.3 Host Plant Resistance

Resistant varieties are the simplest, most effective, economical and eco-friendly means of managing crop diseases including barley scald disease (Wenzel, *et al*., 1996). *R. commune* is highly variable pathogenically and this enables it for rapid selection to overcome newly released resistance genes (Genger *et al.* 2003). Thus, the most sustainable strategies for *R. commune* management are to develop and deploy disease-resistant barley cultivars through the introgression and pyramiding of different resistance genes (major or minor).

6.3.1 Types of Host Plant Resistance

Barley leaf blotch or scald is one of the most destructive diseases of barley crops. The disease occurs in all of major barley growing regions in the world and can cause significant reductions in barley yield and malting quality (Shipton et al., 1974). *R. commune* is a highly variable pathogen (Goodwin, *et al.,* 1993; Salamati *et al.*, 2000), possibly attributing to its large effective population size (McDermott *et al.,* 1989) high gene flow (Goodwin, *et al.,* 1993; McDonald *et al*., 1999), high mutation rate (Goodwin et al., 1994;), frequently sexual reproduction (Salamati *et al*., 2000) and somatic recombination (Forgan *et al.,* 2007). Recently, the pathogen was re-named as *R. commune* (Zaffarano *et al.,* 2011). Barley resistance to *R. commune* can be race-specific or race-nonspecific. Both types of resistance affect pre- and post- penetration stages of pathogen development but in different ways.

6.3.1.1 Race-specific resistance

Active non-host resistance (NHR) of plants to potential pathogens is based on the recognition of race-nonspecific, microbe associated molecular patterns (MAMPs) by pattern recognition receptors (PRRs) present in the plant cell membrane. Race-specific resistance arises after successful suppression of NHR by a pathogen. It involves major plant resistance (*R*) genes, which directly or indirectly recognize the products of certain pathogen effector genes, termed avirulence (*Avr*) genes. This triggers a qualitative resistance response called effector-triggered immunity (ETI) (Jones and Dangl, 2006).

In barley, several major *R* genes against *R. commune* have been described (Shipton *et al*., 1974). Of these, seven different *R* genes [*Rrs1* (11 alleles), *Rrs2* (two alleles), *Rrs3*, *Rrs4* (two alleles), *Rrs12*, *Rrs13* and *Rrs14*], as well as four unconfirmed *R* genes (*Rh5, rh8*, *Rh10* and *rh11*) have been reported. The major scald resistance genes discovered so far have been mainly identified through experiments with seedlings via inoculation with specific isolates and the problem with using major scald resistance genes in breeding programs is a lack of durability. Overall, at least 17 major resistance gene loci have been described (Wagner *et al.,* 2008).

6.3.1.2 Race non-specific resistance

Race non-specific resistance which is also termed as minor gene, quantitative, horizontal, partial or adult plant resistance is based on multiple genes with partial effects, which may control different mechanisms (Poland *et al*., 2009). Quantitative resistance may affect different stages in the life cycle of *R. commune*. It can influence the development of scald epidemics in barley crops by decreasing the leaf area affected by lesions (Williams and Owen, 1975) or by affecting sporulation (Kari and Griffiths, 1993). Although less obviously differentiated by specific gene-for-gene interactions, partial resistance (Kari and Griffiths 1993) also involves genetic interactions between the host and pathogen, but displays a more continuous distribution. The partial scald resistance found in barley landraces and many improved cultivars is thought to be reasonably durable because there is less selection on the pathogen population (Walters *et al*., 2012) and it has been suggested that pyramiding these genes could reduce the ability of *R. communes* to rapidly acquire new virulence combinations. However, it is possible that strains with higher aggressiveness will increase in frequency due to directional selection on partially resistant cultivars that are grown over a large area for many years, eroding the effectiveness of the resistance (McDonald and Linde,

2002). Several quantitative trait loci (QTLs) have been mapped, occurring on all of barley's chromosomes except for 5H (Wagner *et al.* 2008).

VII. CONCLUSION

Barley is one of the world's most important crops providing food and related products for millions of people. Diseases continue to pose a serious threat to barley production and one of the most economically important diseases of barley is leaf scald which is caused by a fungus known as *Rhynchosporium commune*. *Rhynchosporium* is one of the most destructive diseases of barley worldwide, especially in areas with cool temperate climates. It can cause yield losses up to 100% and decrease grain quality, thus discounting prices for quality uses such as malting. Therefore, there should be sustainable management strategies to tackle the impact of barley leaf scald on barley production. Sustainable management strategies of barley leaf scald needs to integrate major-gene-mediated resistance, partial resistance and other strategies such as customized fungicide programmes, species or cultivar rotation, resistance gene deployment, clean seed and cultivar mixtures. In general, barley leaf scald disease is best managed by integrated and multifaceted approach, including application of fungicides, manipulation of sowing date, cultural disease management, and the use of resistant cultivars.

REFERENCES.

[1] Abbott, D.C., Brown, A.H.D. and Burdon, J.J. (1992) Genes for scald-resistance from wild barley (Hordeum vulgare ssp. spontaneum) and their linkage to isozyme markers. Euphytica, 225–231.

[2] Abbott DC, Lagudah ES, Brown AHD (1995) Identification of RFLPs flanking a scald resistance gene on barley chromosome 6. J Hered 86:152-154

[3] Abbott DC, Burdon JJ, Brown AHD, Read BJ, Bittisnich D, 2000. The incidence of barley scald in cultivar mixtures. *Australian Journal of Agricultural Research* 51, 355–60.

[4] Arvidsson, J.(1998) Effects of cultivation depth in reduced tillage on soil physical properties, crop yield and plant pathogens. *Eur. J. Agron.* 9, 79–85

[5] Atwood, P., 1985. Crop protection handbook of Cereals.- Lavenhan press LTD. Lavenhan, Suffolk UK. 229pg. 390.

[6] Ayesu-Offei, E.N. and Clare, B.G. (1970) Processes in the infection of barley leaves by *Rhynchosporium secalis*. *Aust. J. Biol. Sci.* 23, 299–307.

[7] Baker, R.J. and Larter, E.N. (1963) The inheritance of scald resistance in barley. Canadian Journal of Genetics and Cytology, 5, 445–449.

[8] Baik and Ullrich. Barley for food: Characteristics, improvement, and renewed interest. Journal of Cereal Science. 2008;48:233-242

[9] Beer, W. W. 1991. Leaf blotch of barley (*Rhynchosporium secalis*).Zentralbl.Mikrobiol. 146:339-358.

[10] Beigi S., Zamanizadeh H., Razavi M., Zare R. 2013. Genetic diversity of Iranian isolates of barley scald pathogen (*Rhynchosporiumsecalis*) making use of molecular markers. Journal of Agricultural Science and Technology 15 (4): 843–854.

[11] Backes, G., Graner, A., Foroughi-Wehr, B. et al. 1995.Localization of quantitatives trait loci (QTL) for agronomic important characters by the use of a RFLP map in barley (Hordeum vulgare L.). _/ Theor Appl Genet 90: 294-302.

[12] Bouajila A., Haouas S., Fakhfakh M., Rezgui S., Ahmed M.E., Yahyaoui A. 2006. Pathotypic diversity of Rhynchosporium secalis (Oudem.) in Tunisia. African Journal of Biotechnology 5 (8): 570–579.

[13] Chilot Y, Fekadu A, Woldeyesus S. Barley-based farming systems in the highlands of Ethiopia. Ethiopian Agricultural Research Organization (EARO), Addis Ababa, Ethiopia. 1998.

[14] CSA., 2017. The federal democratic republic of Ethiopia, central statistical agency. Statistical Bulletin No. 584, Addis Ababa, pp: 1-118.

[15] Dahleen LS, 2006. Coordinator's report: chromosome 7H. *Barley Genetics Newsletter* 36, 63–5.

[16] Fowler, A. M and H. Owen, 1971. Studies on leaf blotch of barley (*Rhynchosporium scalis).* Trans, of the Brit. Mycol. Soc. 56: (1) 137-152.

[17] Forgan AH, Knogge W, Anderson PA (2007) Asexual genetic exchange in the barley pathogen Rhynchosporium secalis. Phytopathology 97: 650–654.

[18] Fountaine J, 2005. Epidemiological Studies of*Rhynchosporium commune (*Leaf Blotch of Barley*).* UK: University of Reading. PhD thesis.

[19] Hansen, L.R. and Magnus, H.A.(1969) Leaf spot fungi on barley in Norway.*Forskn.Fors.Landbr.*20, 95–105.

[20] Hanemann, A., Schweizer, G.F., Cossu, R., Wicker, T. and Roder, M.S. (2009) Fine mapping, physical mapping and development of diagnostic markers for the Rrs2 scald resistance gene in barley. Theoretical and Applied Genetics, 119, 1507–1522.

[21] Hansen, L.R. and Magnus, H.A. (1973).Virulence spectrum of *Rhynchosporium secalis* in Norway and sources of resistance in barley. Journal of Phytopathology, 76, 303–313.

[22] Islamovic, E., Bregitzer, P., and Friesen, T. L. 2017. Barley 4H QTL confers NFNB resistance to a global set of P. teres f. teres isolates. Mol. Breed. 37:29.

[23] Hoad SP, Wilson GW, 2006. Influence of plant population density, nitrogen fertiliser rate and variety on *Rhynchosporium secalis* in winter barley. In: *ProceedingsCrop Protection in Northern Britain 2006, Dundee, UK*. 185–90

[24] Home-Grown Cereals Authority (HGCA).(2011). The HGCA barley disease management guide. Stoneleigh Park, Warwickshire, UK: HGCA.

[25] Hunde, B., K. Meles, F. Abebe, A. Tekalign and M. Ayalew et al., 2011. Achievements in Barley Scald research in Ethiopia. In: Barley Research and Development in Ethiopia, Mulatu, B. and S. Grando (Eds.). ICARDA, Aleppo, Syria, pp: 245-255.

[26] Jackson L (1997). Scald. In: Mathre DE (eds), Compendium of barley diseases. The American Phytopathological Society, 33-35.

[27] Jensen, 1965.Multiline superiority in cereals.Crop.Sei. 5: 566-568.

[28] Jensen J, Backes G, Skinnes H, Giese H (2002) Quantitative trait loci for scald resistance in barley localized by a non-interval mapping procedure. Plant Breed 121:124-128

[29] Jones, J.D.G. and Dangl, J.L. (2006).The plant immune system.*Nature*, 444, 323–329.

[30] Karjalainen R, Jokinen K, 1993. Influence of barley scald disease on yield and competition in barley-oat mixtures. *Journal ofAgronomy and Crop Science* 171, 314–20.

[31] Kendall SJ, Hollomon DW, Cooke LR, Jones DR, 1993. Changes in sensitivity to DMI fungicides in *Rhynchosporiumsecalis*. *Crop Protection* 12, 357–362.

[32] Kendall, S. J., Hollomon, D. W., Ishii, H., & Heaney, S. P. (1994). Characterization of benzimidazole resistant strains of Rhynchosporium secalis. Pesticide Science, 40, 175–181.

[33] Kenya Breweries Ltd., 1990.

[34] Marshall, 1977.The advantages and hazards of genetic homogeneity. Ann. N.Y. Acad. Sei. 287: 1-20.

[35] Mayfield, A.H. and Clare, B.G. (1984) Survival over summer of *Rhynchosporium secalis* in host debris in the field. *Aust. J. Agric. Res.* 35, 789–797.

[36] Murray TD, Parry DW, Cattlin ND (1999) Sygdomme i korn - en håndbog i farver. Copenhagen: DSR Forlag.

[37] Nielsen GC, Jensen JP (2001) Markens sygdomme og skadedyr. Frederiksberg C.: Det Kgl. Danske Landhusholdningsselskab.

[38] Newman PL, Owen H (1985) Evidence of asexual recombination in *Rhynchosporium secalis*. Plant Pathol 34: 338–340.

[39] Shipton W.A., Boyd W.J.R., Ali S.M. 1974. Scald of barley. Review of Plant Pathology 53: 839–861.

[40] Sierotzki, H., Wullschleger, J. and Gisi, U.(2000) Point mutation in cytochrome bgene conferring resistance to strobilurin fungicides in *Erysiphe graminis* f. sp. *Tritici*field isolates. *Pestic.Biochem. Physiol.* 68, 107–112.

[41] Skoropad WP, Grinchenko AHH (1957). A new spore form in *Rhynchosporium secalis*.,Phytopathology47, 628-629.

[42] Skoropad W P (1959). Seed and seedling infection of barley by *Rhynchosporium secalis*.*Phytopathology*, 49, 533-622.

[43] Walters, D. R., Avrova, A., Bingham, I. J., Burnett, F. J., Fountaine, J., Havis, N. D., Hoad, S. P., Hughes, G., Looseley, M. & Oxley, S. J. 2012. Control of foliar diseases in barley: towards an integrated approach. *European Journal of Plant Pathology*, 133, 33-73.

[44] Williams, K. J., Lichon, A., Gianquitto, P., Kretschmer, J. M., Karakousis, A., Manning, S., Langridge, P., and Wallwork, H. 1999. Identification and mapping of a gene conferring resistance to the spot form of net blotch (Pyrenophora teres f maculata) in barley. Theor. Appl. Genet. 99:323-327.

[45] Williams, K., Bogacki, P., Scott, L., Karakousis, A. and Wallwork, H. (2001) Mapping of a gene for leaf scald resistance in barley line "B87/14" and validation of microsatellite and RFLP markers for marker-assisted selection. Plant Breeding, 120, 301–304.

[46] Yahyaoui, A.H., 2004. Occurrence of barley leaf blights in central western Asia and North Africa. Proceedings of the 2nd International Workshop on Barley Leaf Blights, April 7-11, 2002, Aleppo, Syria, pp: 13-18.

[47] Zaffarano P.L., McDonald B.A., Linde C.C. 2011. Two new species of *Rhynchosporium*. Mycologia 103 (1): 195–202.

[48] Zantinge, J., Xue, S., Holtz, M., Xi, K. and Juskiw, P. (2019) The identification of multiple SNP markers for scald resistance in spring barley through restriction-site associated sequencing. Euphytica, 215, 8.

[49] Zhang, Z., Henderson, C., Perfect, E., Carver, T., Thomas, B., Skamnioti, P. & Gurr, S. 2005. Of genes and genomes, needles and haystacks: Blumeria graminis and functionality. *Molecular plant pathology*, 6, 561-575.

[50] Zhan J, Stefanato FL, McDonald BA, 2006. Selection for increased cyproconazole tolerance in *Mycosphaerellagraminicola* through local adaptation and in response to host resistance.*Molecular Plant Pathology* 7, 259–68.

[51] Zohary and Hopf (2000) Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. 3rd Edition. Oxford.

Custard Apple Seed Oil as a Pesticide

Vedant Lal[1], Swapnil Bansi[2], Rugved Deshpande[3], Nita Mehta[4*]

[1,2,3]Student, Chemical Engineering, Thadomal Shahani Engineering College, Mumbai, Maharashtra, India
[4]Associate Professor, Chemical Engineering, Thadomal Shahani Engineering College, Mumbai, Maharashtra, India
*Corresponding Author

Received:- 13 August 2021/ Revised:- 19 August 2021/ Accepted:- 26 August 2021/ Published: 31-08-2021

Abstract— *Essential oils are oils extracted from plants. These categories of oils are obtained through distillation or mechanical methods such as cold pressing. Custard Apple Seed Oil is a type of essential oil. This oil can be used as an eco-friendly biopesticide. They are cheap, safe to use also maintains the fertility of the soil. Therefore natural pesticides like custard apple seed oil are given preference over synthetic pesticides. Oil extracted from it can be used as a pesticide against several common pests like the white mealybug, aphid, termite, etc. The oil extracted from custard apple seeds contain acetogenin a group of powerful respiratory inhibiting toxic components, which is responsible to act as a bio-pesticide. Cold pressing, solvent extraction, steam distillation, maceration, percolation, tincture, and infusion are the methods that are used for custard apple seed oil extraction.*

Keywords— Essential oils, distillation, cold pressing, acetogenin, maceration.

I. INTRODUCTION

Maharashtra is the leading state in custard apple production with 92,320 tons. The raw material required for the process; custard apple seeds are available in abundance. There are various methods of custard apple seed oil extraction. The selection of appropriate solvent for extraction is done by taking into account various factors like cost, oil extraction efficiency, solvent recoverability, and environmental impacts.

II. METHODS OF OIL EXTRACTION

2.1 Conventional Methods

Conventionally, the oil for pesticides can be obtained by three methods:

1. Custard apple seeds are boiled in an approximate amount of water until the liquid is reduced to half. Dilute the filtrate with a high quantity of water to obtain the pesticide.
2. Add finely ground custard apple seeds to water. Leave it to stand for a day or two. Strain**.**
3. Grind seeds to extract oil using a grinder. Dilute one part of oil to 20 parts.

2.2 Experimental Methods

2.2.1 Cold Pressing

This method is most preferred for extracting oil from citric rinds such as orange, lemon, and grapefruit. This method involves the simple pressing of the rinds at a temperature of about 120 F to extract the oil. These rinds are first separated from the fruit, ground, or chopped and then are finally pressed. The result obtained is a watery mixture of oil and ethanol. The liquid which we separate given time little alteration from the oil original state occurs this citrus oil retain their bright, fresh, uplifting, aroma like that of smelling wonder cooling ripe fruit. The drawback of this method is the oil extracted using this has a short shelf life.

2.2.2 Solvent Extraction

In the solvent extraction method, the oil recovery and extracting unit is loaded with perforated trays of oil plant material and repeatedly wash with the solvent. A carbon and hydrogen-based solvent are used for extraction. All the extractable material from the plant is dissolved in the solvent. This includes highly volatile aroma molecules as well as non-aroma waxes and pigment. The extract is distilled to recover the solvent for future use. The waxy mass that remains is known as the concrete. The concentrate concrete is further processed to remove the waxy material which diluted pure oil. To prepare the absolute from the concrete the waxy concrete is warm and stirred with alcohol (Ethanol). During the heating and stirring process, the concrete breaks up into min globules. Since the aroma molecule are more soluble in alcohol than the waxes and efficient separation of two results.

2.2.3 Steam Distillation

Steam distillation is a special type of distillation or a separation process for temperature-sensitive materials like oils, resins, and hydrocarbons, etc. which are insoluble in water and may compose at their boiling point. The fundamental nature of steam distillation is that it enables a compound or a mixture of the compound to be distilled at a temperature that contains a substance substantially below that of the boiling point of the individual constituents. Essential oils contain a substance with a boiling point up to 200°C or higher temperature. In the presence of steam or boiling water, however, the substances are volatilizing at a temperature of 10°C very close to atmospheric pressure. Various factors determine the final quality of a steam distilled essential oil. Apart from plant material, the most important are time, temperature and pressure, and quality of the distillation equipment. Essential oils are very complex products. Each is made up of many, sometimes hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties. So of these molecules are fairly delicate structures that can be altered or destroyed by adverse environmental conditions so much like a fine metal is more flavourful that longer distillation times may give more complete oil. It is also possible, however, that longer distillation times may lead to the accumulation of more artifacts than normal. This may have a curious effect of appearing to improve the odor, as sometimes when materials that have a large number of components are sniffed, the perception is often of slightly increased sophisticated, added fullness and character, and possibly, and extra pleasantness.

2.2.4 Maceration

The simple widely used procedure involved leaving the pulverized plant to soak in suitable solvents in a closed container. Simple maceration is performed at room temperature by mixing the ground grub with the solvents and leaving the mixture for several days with occasional shaking or starring. The extract is then repeated from the plant particles by stirring. The process is repeated with a fresh batch of solvent at least two times. Finally, the last residue is pressed out of the plant particles using the mechanical press or centrifuge. Kinetic maceration is different from a simple one by continuous stirring. This method can be used for both initial and bulk extraction.

2.2.5 Percolation

The powdered plant material is socked initially in a solvent in a percolator. Additional solvent is then poured on the top of the plant material and is allowed to percolate slowly out of the bottom percolators. Additional filtration of the extract is not required because there is a filter at the percolator.

2.2.6 Tincture

A tincture is typically an alcoholic extract of plant or animal material or a solution of such or of a low volatility substance. To qualify as an alcoholic tincture extract, the extract should have an ethanol percentage of at least. Sometimes an alcohol concentration higher than 90% is used in tincture. Alcoholic tinctures are made with various ethanol concentrations are the most common.

III. SOLVENT SELECTION

N-hexane is considered to be the most efficient solvent when dealing with oil extraction processes. When n-hexane is used the color obtained is the favorable yellowish-light brown as compared to the dark woody brown color obtained on using methanol as a solvent. Methanol gives the second-best yield after n-hexane. The acid value obtained was 1.683 for n-hexane. The natural pesticide produced from custard apple seed oil proves itself efficient, advantageous, cheap, and safe to handle. Its recovery by using Hexane solvent is 19% than the methanol solvent is 10.5%. This pesticide material can be made easily available for every farmer throughout India without taking much more effort. This raw material will be very cheap which

minimizes the total cost of processing along with solvent recovery. Many factors like oil extraction efficiency, environmental impacts, and the renewability of solvents must be considered while selecting the ideal solvent. Hexane is the preferred solvent for the extraction of oils from plant sources due to its low boiling temperature and easy recovery from the extract. Most oils are soluble in hexane too. The cost of n-hexane in laboratories is Rs.45 per liter.

IV. EXPERIMENTAL PROCEDURE

Seed kernels crushed and grounded to powder. Then the powder which is obtained from crushing is mixed with hexane or methanol solvent to extract oil from seed kernels. While doing the extraction, the solvent is used in the ratio of 15ml/g of seed kernels powder, and extraction time was 3hr, 4hr, 4hr for two hexane, and one methanol solvent respectively. The temperature was maintained near about 65- 70 degrees Celsius by regulating the magnetic cum heater and stirrer. After extraction, the sample is filtered out to remove solid material as residue, and the filtrate is contained with oil extracted. This filtered sample is lead to vacuum distillation for the first sample and simple distillation for the other two samples. Then after distillation solvents were distilled out while the oil extracted was remain in the distillation chamber. Then lastly the oil separated is analyzed for density, percent of oil, and acid value.

V. ANALYSIS AND APPLICATION OF PESTICIDE

The oil obtained above is tested for insecticidal properties by standard methods such as HPLC, Spectrophotometry, Polarography, and FTIR. After carrying out these standard tests, and analyzing the properties, the oil is applied to the target pests. For example, when applying mealybugs on a guava tree, preparation of the blank solution is carried out. The blank solution is formed by mixing 6 parts of labolene soap with 94 parts of water. To this blank solution, the required percentage of custard apple seed oil is added and sprayed on the pest attacked surface with a help of a spray gun.[16][17][18].

VI. PESTICIDE TEST ON WHITE MEALY BUGS

An experiment was carried out to test the effect of the pesticide on white mealybugs by Sikdar D.C et al,2016 [1].Oil solution of (blank 0.0%,0.15%,0.30%, and 0.75% was prepared by mixing with labolene soap solution and sprayed in one shoot on white mealybug present on the guava tree leaves surface affected by white mealy bugs. The numbers of the white mealy bugs left on the leaf's surface after spraying the pesticide solution are counted daily. Based on the data tabulated by them, at 0.75% concentration, the pesticide was most effective on the target pests.

The number of mealy bugs decreases to zero at 0.75% concentration within 2 days. Thus, an oil solution of 0.75% is effective to keep away the pests.

VII. FUTURE SCOPE

The project work is on how a pesticide is prepared from a discarded waste material, custard apple seeds. This pesticide can be used in place of toxic synthetic pesticides.

A study on the overall feasibility and profitability of the process can be carried out. The available cheap raw material required for the process can lead to the development of this industry.

This operation could be carried out on a small scale and could generate employment for skilled as well as unskilled labor.

VIII. CONCLUSION

The various methods of oil extraction were compared and solvent extraction came forward as the best method of oil extraction. The entire process of extracting oil from custard apple seeds takes place in three and half hours including the time required for cleaning. N-hexane is the most preferred solvent for the extraction process as it is available for a price of Rs.45/liter and provides recovery of about 19%. The three conventional methods have a low yield but can be performed at home at a negligible cost. Oil at 0.75% concentration is ideal to keep pests away.

REFERENCES

[1] Sikdar D.C, Sushmita Kushary, Roshnee Das, Vishva Mehta:" Evaluation of Effectiveness of Eco-Friendly Bio-Pesticide Extracted From Custard Apple Seeds On White Mealy Bugs"-International Journal of Technical Research and Applications, Volume 4, Issue 2.

[2] Ajay. V. Gawali, Sapna K. Deotale, Tousf Yunus Shaikh –"Annona Squamosa: A Source of Natural Pesticide": IARJSET, Vol.4, Special Issue 3, January 2017.

[3] Aanchal Moolcuandani, Aftab Sharif, Sohel Sayyed: "Eco-friendly Bio pesticide Extraction", IAETSD journal for advanced research in applied sciences.

[4] Prithusayak Mondal, Sritama Biswas, Kumaresh Pal: "Anmonasquamosa as a potential botanical insecticide for Agriculture Domains: A Review", International Journal of Bioresource Science, Citation: IJBS:5(1):81-89, June 2018.

[5] Physio-Chemical Properties of Oil Extracted From Custard Apple Seeds, by MrBharadwaj.

[6] Y.R.HARAL, B.R.PAWAR: "Economics of custard apple production in Maharashtra", International Research Journal of Agriculture Economics and Statistics, Volume 4, Issue 2, September 2013.

[7] KalpeshP.Borole, Jayprakash R.Sirsath, Mr.Swapnil M. Bhonde, Rushikesh D. Sable – " Extraction of Oil from Custard Apple Seeds": IJFEAT Issue 1 Vol 4.

[8] Dalia H. Eshra, Attia R.Shehata, Abdel-Nabey A. Ahmed, Jehan I. Saber - " Physiochemical Properties of Seed Kernels and the oil of Custard Apple": International Journal Of Food Science And Biotechnology Vol 4, No 4 2019.

[9] https://www.persistencemarketresearch.com/mediarelease/global-n-hexane-market.asp [Last accessed August 21,2021].

[10] https://www.expertmarketresearch.com/reports/acetone-market [Last accessed August 21,2021].

[11] https://niti.gov.in/methanol-economy#:~:text=Methanol%20Economy%20program%20initiated%20by,setting%20up%20Methanol%20Production%20Plants. [Last accessed August 21,2021].

[12] https://mcgroup.co.uk/researches/ethyl-acetate-etac [Last accessed August 21,2021].

[13] https://indianpetrochem.com/report/acetonereport [Last accessed August 21,2021].

[14] https://indianpetrochem.com/advisory/methanoladvisory [Last accessed August 21,2021].

[15] https://www.expertmarketresearch.com/reports/n-hexane-market [Last accessed August 21,2021].

[16] https://chemdictionary.org/gas-chromatography/#:~:text=%20How%20does%20gas%20chromatography%20works%3F%20%201,In%20this%20type%20of%20detector%20organic...%20More%20 [Last accessed August 21,2021].

[17] Spectrophotometry(Procedure):Physical Chemistry Virtual Lab: Chemical Sciences- Amrita VishwaVidyapeetham Virtual Lab [Last accessed August 21,2021].

[18] https://chemdictionary.org/hplc/ [Last accessed August 21,2021].

AD Publications
Sector-3, MP Nagar, Rajasthan, India
www.adpublications.org, www.ijoear.com, info@ijoear.com