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Preface

We would like to present, with great pleasure, the inaugural volume-8, Issue-3, March 2022, 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, Terrestrial 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.



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Socio-Economic Assessment of Fisherfolks in Anaku Community, Ayamelum Local Government Area of Anambra State, Nigeria: A Base for Potential Aquaculture Development

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Abstract— The study was designed to examine the socioeconomic development of the fisher folks as a base for potential aquaculture development in Anaku community, Ayamelum Local Government Area, Anambra State, Nigeria. Eighty (80) structured questionnaires were used to collect relevant information from the fisher folks. Data collected were analyzed using descriptive statistics, simple percentage and Chi square. Results obtained revealed that there is a significant relationship ($P < 0.05$) between the socioeconomic characteristics of the respondents and fishing activities. 82.5% of the respondents were males who engaged in fishing activities while 17.5% were females engaging in fish processing and trading, both of whom were mostly within the age bracket of 24-40 years (40.00%). 86.25% of the respondents were married and family size of 5-8 persons recorded the highest value (51.25%). This study also revealed that all the respondents used natural water bodies for fishing activities, 58.75% of the respondents practiced fishing as their only source of income while 50% of the respondents used local fishing gears. Majority of the fisherfolks had no formal education (55.00%) while 45.00% had one form of education or the other. Although a higher percentage of the fisherfolks (56.25%) were engaged in fishing activities as their primary occupation than crop farming, average monthly income of the fisherfolks in Anaku community showed that a monthly income of N50,000 had the highest frequency (41.25%). Hence, the study postulates that there is high possibility for the development of aquaculture in Anaku fishing community and recommends government support for full harnessing of fishery resources from Anaku community.

Keywords— Aquaculture development, Fishing activities, Fisher folk, Anaku community, Socio-economic parameters.

I. INTRODUCTION

Aquaculture continues to grow rapidly every day, and production is increasing so as to meet the ever-increasing demand for aquatic foods. Understanding the basic part behind aquatic production facilities is of increasing importance for all those working in this industry (FAO, 2010). Aquaculture requires knowledge and skills of the many general aspects of production such as spawning, production of feeds, water quality management etc (Anderson, 2004). Fisheries constitute an important sector in Nigerian agriculture, providing valuable food and employment to millions and also serving as a source of livelihoods mainly for women in coastal communities, in view of the ever-increasing importance of fish as a source of good quality animal protein in Nigeria (Nwuba *et al.*, 2009).

Aquaculture according to Ayinla (2012) is the fastest growing food producing industry in the world which has quadrupled over the past twenty years. Aquaculture production is likely to double in the next fifteen years, as a result of wild fisheries approaching their biological limits and the world demand for cultured fish continuing to increase. Production from world capture fisheries has leveled off and most of the fishing areas have reached their maximum potential yield (FAO, 2010a). It is estimated that in order to maintain the current level of per capita consumption, global aquaculture production will need to reach 80 million tonnes by 2050. Aquaculture has the potential to address the projection of fish demand, but will face enormous challenges to achieve this goal (FAO, 2010a). Currently, domestic fish production is put at 620,000 metric tons as against the present national demand of about 2.66 million metric tons (Ayinla, 2012).

The major animal protein sources in Nigeria include cattle, goats, sheep, poultry birds, pigs and fish. Out of these sources, fish and fish products provide more than 60% of the total protein intake in adults especially in the rural areas Adekoya and Miller, 2004). Therefore, the importance of the fishing industry to the sustainability of animal protein supply in Nigeria

cannot be over-emphasized. Regrettably, the supply of food fish has been on the decline. This is due to consistent decline from the country's major source of food fish (Gupta and Acosta, 2011).

Nigeria as a nation is endowed with good natural resources which can enhance aquaculture development to a greater extent, however aquaculture production in Sub-Saharan Africa is limited despite its natural potential (FAO, 2010a). There is high possibility for the development of aquaculture in the continent, which can be done through intensive use of the abundant inland water and coastal areas. Although aquaculture may be the new frontier for producing fish, wild capture fisheries remain significant for overall supply (Allison *et al.*, 2011). In order to achieve this milestone, Africa continent will need to address the existing constraints, such as: inadequate knowledge on aquaculture among farmers, low allocations for aquaculture development in national budget, poor or slow growth of cultured species; poor broodstock management; and poor species identification (FAO, 2010a).

It has been asserted by Ugwumba (2005) that the only way of boosting fish production is by moving the country towards sufficiency in fish production and embarking on aquaculture production especially catfish farming. This has prompted the Federal Government of Nigeria to package the Presidential Initiative on fisheries and aquaculture development in 2003 to provide financial and technical assistance to government programmes and projects encouraging fish production. According to Adekoya and Miller (2004) an inventory of fish farms has been completed in all the 36 states and the Federal Capital Territory (FCT) with a total of 2,293 fish farms in the country. Therefore, the aim of this study was to determine the potentials of aquaculture development in Anaku fishing community through the socio-economic characteristics of the fisher folks and the fishing activities practiced in the study area.

II. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in fishing community of Anambra state, Nigeria called Anaku which is a fishing community and the administrative headquarters of Ayamelum Local Government Area of Anambra State, South-East Nigeria. The town lies 6°15' North of the Equator and 6°44' East of the Greenwich Meridian. It is bordered by "Omambala," the native name of the Anambra River, which is a tributary of the famous River Niger (North), Ezu River (South), Omor and Umuerum communities (East). It is easily accessible from Onitsha (about 45 km) and Otuocha Aguleri (about 15 km). Anaku is made up of three major villages which include Umuria, Ikenga, Umuezeagu and also several quarters and kindreds.

The fishing activities in the community is of great economic importance as most of the dwellers are crop farmers and fisherfolks who cultivate mainly rice, as well as other crops (yam, cassava, vegetables, and cocoyam) and also engage actively in daily fishing activities from the Omambala river.

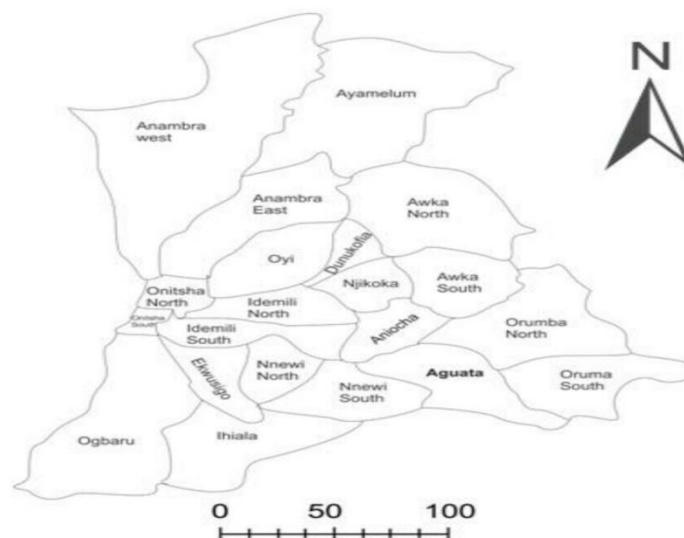


FIGURE 1: Map of Anambra State showing the 21 local government areas (LGAs).

Ayamelum LGA: (*-shows the study area, Anaku town in Ayamelum Local Government Area, Anambra State)

2.2 Methods of Data Collection

The sampling techniques used to select respondents for this study is simple random sampling technique while questionnaires and interviews were employed to obtain the demographic information and socio economic parameters of the respondents

using primary data. The primary data included 80 structured questionnaires obtained from the field through direct observation, the use of questionnaires which were structured based on the specific objectives of the study and interview schedule information from the respondents. The interview schedule was used to collect information from illiterate respondents (44) that could not read nor write while structured questionnaire was used to collect information from literate respondents (36).

Data were collected on the socio-economic characteristics of the respondents such as sex, age, marital status, household size, income, educational attainment, religion and fishing activities practiced in the study area.

2.3 Statistical Analysis

Data were analyzed using descriptive statistics and simple percentage. The relationship between socio-economic characteristics and fishing activities were analysed using Chi-square at 5% level of significance.

III. RESULTS AND DISCUSSION

The Socio-economic attributes of the respondents are presented in Table 1.

TABLE 1
PERCENTAGE DISTRIBUTION OF THE SOCIO-ECONOMIC ATTRIBUTES OF THE RESPONDENTS

Respondents		Frequency	Percentage
Sex	Male	66	82.50
	Female	14	17.50
Total		80	100
Age(Years)	15-25	9	11.25
	26-40	32	40.00
	41-60	20	25.00
	Above 60	19	23.75
Total		80	100
Marital Status	Single	8	10.00
	Married	69	86.25
	Divorced	0	0.00
	Widow	3	3.75
Total		80	100
House-Hold Size	1- 4	8	10.00
	5 – 8	41	51.25
	9 -12	22	27.50
	Above 12	9	11.25
Total		80	100
Education Attainment	No formal education	44	55.00
	Primary education	20	25.00
	Secondary education	16	20.00
	Tertiary education	0	0.00
Total		80	100
Religion	Traditional	62	77.50
	Christianity	18	22.50
	Islamic	0	0.00
	No Religion	0	0.00
Total		80	100
Fishing as primary occupation	Yes	45	56.25
	No	35	43.75
Total		80	100
Average monthly income	10,000	28	35.00
	50,000	33	41.25
	100,000	14	17.50
	above 100,000	5	6.25
Total		80	100

The questionnaire analysis showed that the opinion of 66 males (82.50) which was the majority of the respondents and 14 females (17.50) were sampled in this research. Respondents who were between 15-25years were 9 (11.25%), 26-40years were 32 (40%), 41-60 years were 20 (25%), and 60 years and above were 19 (23.75%).

The number of respondents who were single is 8 (10%), those married were 69 (86.25%), divorced 0 (0%) while widows were 3(3.75%).

The table also showed that 8(10%) of the respondents had a household size between 1 and 4 persons, those with household size of 5-8 were 41 (51.25%), 9-12 were 22 (27.5%) while those above 12 were 9 (11.25%). Size of the household is an important factor that determines how cheap the labour is. Those with higher household size will provide cheapest labour that can be utilized for fishing activities than those with smaller household.

The number of respondents who had no formal education were 44 (55%), while those with primary education were 20 (25%), those with secondary education were 16 (20%) and those with tertiary education were 0 (0%).

The number of respondents that were Christians were 18 (22.5%) Islam 0 (0%), Traditionalists were 62(77.5%) while 0 (0%) indicated none of the religions. It can be seen that the highest percentage of the respondents were traditional worshippers.

Respondents who agreed that fishing is their primary occupation were 45(56.25%) while 35 respondents (43.75) disagreed that fishing was not their primary occupation.

Respondents whose average monthly income fall within the range of N10,000.00 were 28 (35%), those who fall under N50,000.00 were 33 (41.25%) while those who were within N100,000 are 14 (17.5%), and those who fall above N100,000 were 5 (6.25%).

TABLE 2
RESPONDENTS VIEW ON PROSPECT OF FISHERIES AND AQUACULTURE DEVELOPMENT

Respondents		Frequency	Percentage
Improvements in the use/production of fishing gears.	Yes	0	0.00
	No	80	100.00
Total		80	100
Production system(s)	Natural water bodies	80	100.00
	Modern facilities	0	0.00
Total		80	100
part of the river most catches are made	Mid river	31	38.75
	Boat side	13	16.25
	Both	36	45.00
Total		80	100
Basic Aquaculture Skills acquisition and technical know-how	Yes	5	6.25
	No	75	93.75
Total		80	100
Embark on other businesses	Yes	67	83.75
	No	13	16.25
Total		80	100
Extension Visit	Frequently	0	0.00
	Less frequent	0	0.00
	Not at all	80	100.00
Total		80	100
Governmental Support	Yes	0	0.00
	No	80	100.00
Total		80	100

The number of respondents who indicated that there had been improvements in the use of fishing gears was 0 (0%) while 80 (100%) said that there had not been improvements in the use of fishing gears. The number of respondents that use the natural water bodies is 80 (100%) while none uses modern facilities. None of the respondents agreed that extension agents visited their community either frequently or less frequent while 80(100%) of the respondents said that extension agents did not visit at all. The respondents affirm that there has been no government support on the fisheries development in the community. It was also observed that respondents have minimal basic aquaculture skills acquisition and technical know-how as 5(6.25%) of respondents had basic aquaculture skills while 75(93.75%) had none.

TABLE 3
RESPONDENTS VIEW ON THE TYPE OF FISHING GEARS THEY USE FOR FISHING.

Fishing Gears for fishing	Frequency	Percentage
Net and trap	34	42.50
Net and Hook and Line	40	50.00
Motorized boat	0	0.00
Cage and net, Hook and line	6	7.50
Total	80	100

The number of respondents that used net trap was 34 (42.50%), the respondents that used net, hook and line was 40 (50%), while those that used cage, net, hook and line was 6 (7.50) and none of the respondents 0 (0%) used motorized boat.

TABLE 4
FISHING ACTIVITIES CARRIED OUT IN ANAKU COMMUNITY

Fishing activities	Frequency	Percentage
Fishing	27	33.75
Processing	18	22.5
Fishing gear	1	1.25
Fishing and processing	34	42.5
Total	80	100

Table 4 showed fishing activities of Anaku community with 27 (33.75%) involved in fishing, 18 (22.5%) involved in processing, 1 (1.25%) involved in fishing gear making, while 34 (42.5) involved in fishing and processing.

IV. DISCUSSION

Table 1 showed that the opinion of 66 males (82.50%) and 14 females (17.50%) were sampled in this research. This implies that a greater percentage of the fishing population in Anaku community were male which support the work of Adeleke (2013) and Akpoko (2003) who reported that male dominates the artisanal fishery sub – sector. The risk associated with such off – shore fishing activities may be responsible for the low female participation in the activity. By implication it showed that fishing in the water bodies of the study area were energy sapping. It is also evident from that the age categories of the respondents that fall within the age bracket of 26-40years has the highest percentage (40.00%) and were in their active working age. Age is an important variable that influences people's attitude, skill and aspiration. This is in line with the report of Olaoye *et al.* (2013). 86.25% of the respondents were married hence, the highest percentage of married people will provide cheapest labour, as availability of labour will come from their family. There is increase in fishing activity to meet up the family demands unlike when they are single. 51.25% of the respondents had a family size of 5-8 persons. This implies that artisanal fisher folks have large household which constitutes an important labour source for farmers. The study carried out by Nafisat *et al.* (2011) and Oladimeji *et al.* (2013) reported that larger family will provide cheaper source of labour. The majority of the fisherfolks had no formal education (55.00%) while 25.00% had primary education, 20.00% had secondary education and none of the respondents had tertiary education. This implies that artisanal fisherfolks have a low educational background. The low educational status of the respondents may influence their acceptance of improved fishing practices. The

positive influence of education on farmers' acceptance of improved farm practices has been established by several studies (Onemolease *et al.*, 2000; Tshionza *et al.*, 2001). This is contrary to Ikeogu *et al.* (2016) who reported that greater percentage of fisherfolks attained secondary education. 77.50% of fisherfolks in Anaku are traditional worshippers. This is also contrary to Ikeogu *et al.* (2016) which stated in their study that greater percentage of fisherfolks were Christians. Although a higher percentage of the fisherfolks (56.25%) are engaged in fishing as their primary occupation than crop farming. Investigation on the average monthly income of the fisherfolks in Anaku community showed that an income of N50,000 has the highest frequency (41.25%).

This study revealed that there had not been any improvement of fishing gears in the community and they still make use of local fishing gears. Fisher folks carry out fishing activities only in natural water bodies with no tanks, fish cage and other modern facilities. Most catches are made from both mid-river and boat side and they engaged themselves in other businesses such as farming of crops, rearing of livestock, trading etc. Extension services and government support are very poor in Anaku community. Most of the respondents were unaware of the fact that the government brings aid to support fisheries and aquaculture development. This shows that fisheries extension officers are not mobilized into the community to provide solution to the problems of fisherfolks. Education and extension services are essential for farmers to adopt new technologies (Oladimeji *et al.*, 2013). The lack of government aid and support can be held accountable for the progressive decrease in growth and development of fisheries and aquaculture in the community as most of the respondents are not educated to understand the technical know-how involved in aquaculture development (Allison *et al.*, 2011). Therefore, the villagers are afraid to venture into the untapped potential in aquaculture and its management practices.

The study also showed that local types of gear were used for fishing and none of the respondents made use of motorized boats, this may be as a result of high cost of motorized boats in comparison to canoes and inability to understand the structure function, operation and maintenance of the motorized boats. The fisherfolks that used net and hook and line have the greater percentage (50.00%). The fisherfolks although fish all season but engage more in fishing activities during the dry season than in the rainy season because catches during the rainy season is low as the river overflows its banks thereby creating hideout for fishes in the surrounding vegetation.

The fishing activities in Anaku community are mainly artisanal fishing, processing (smoking), marketing and gear making. This study showed the nature and magnitude of the relationship that existed between the respondent's socioeconomic characteristics (gender, age, marital status and household size) and their fishing activities respectively. The result obtained showed that the socioeconomic characteristics (gender, age, marital status, income and household size) and their fishing activities have a significant relationship ($P < 0.05$). The value of age suggests that younger farmers earned significantly more than older farmers and the potentials for more wealth creation would be realizable if the fisherfolks are empowered as recommended in this study.

The basic aquaculture skills acquisition and technical know-how of respondents showed that 5% (6.25) of respondents had basic aquaculture skills while 75% (93.75) had none. This implies that respondents had no access to the basic knowledge on skills necessary to practice aquaculture in Anaku community.

V. CONCLUSION AND RECOMMENDATION

The potential to develop the aquaculture industry and substantially increase fish production and economic growth exists in Anaku community. This study revealed that there is a significant relationship ($P < 0.05$) between the socioeconomic characteristics of the respondents and fishing activities. Anaku fishing community showed a high prospect in artisanal fisheries but was constrained by several factors notably high cost of fishing inputs such as motorized boats, insufficient capital and storage problems. Technology undertaken for cage culture in their water bodies may be adapted to suit local conditions.

In order to develop aquaculture production in Anaku fishing community, it is recommended that fisheries extension officers should be sent to engage the community on several aquaculture training programs so as to enlighten the people on the potentials of aquaculture in their community and similar rural communities. Considering the low catches made by the fisherfolks from the Omambala river during rainy seasons, fishes and other aquatic organisms, through aquaculture can be used to restock the river, as this will increase both the biomass and biodiversity of the river, thereby, increasing the culture and capture fisheries development. This study also recommended that basic amenities and modern fishing facilities such as cold storage facilities, modern fish handling and processing facilities, motorized boats and gears should be provided to the

fisherfolks to increase fish production. Further study should be carried out on suitability of the river in Anaku community for aquaculture purposes.

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Detection of Coronavirus-like Particles in Wild, Exotic and Captive Animals by Transmission Electron Microscopy

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Abstract— *Coronaviruses infect humans and a wide diversity of mammalian and bird species causing respiratory, enteric, neurologic and hepatic disorders. Due to their facility of adapting to new species and establishing spillover events, coronaviruses pose a risk to global public health. Considering the zoonotic risk of coronaviruses, their role in wild species that host wild animals, as well as their ability to adapt to new species, seems to be the fundamental key to understanding their pathophysiology. The objective of this work was to report the presence of coronavirus-like particles in wild, exotic and captive animal species, in fecal or small intestine samples, using negative staining technique for transmission electron microscopy. 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. This report is the first worldwide occurrence of coronaviruses in *Falco peregrinus*, *Tayassu tajacu* and *Tayassu pecari* and the first occurrence in Brazil in *Sus scrofa*, *Nosua nosua*, *Puma concolor* and in *Rhea Americana*.*

Keywords— *Coronavirus, wild, exotic and captive animals, Transmission electron microscopy.*

I. INTRODUCTION

The transmission of pathogens from wild animals to humans is called zoonotic spillover, which indicates that this phenomenon plays an important role in the emergence of new infectious diseases, representing a global burden on public health. Although associated with several outbreaks, it is still a poorly understood phenomenon. Coronaviruses circulate in nature in several animal species. There is a consensus that the possible origin of the virus comes from a wild animal, considering that most of the new viruses that crossed the evolutionary barrier and infected humans throughout the epidemiological history of viral diseases, were of animal origin, such as SARS and MERS that caused worldwide epidemics [1]. While still preliminary, current data suggest bats are the most likely initial source of the current Covid -19 outbreak, which began in December 2019 in Wuhan, China [2; 3].

Activities and processes that increase human interaction with the different animal species and pathogens they host, which include handling, poaching and indiscriminate consumption of wild animal meat and by-products without sanitary measures, are associated with an increased risk of spillover events[4]. These factors associated with habitat degradation and fragmentation, climate change and infectious diseases contribute to the extinction of many species of wild animals [5].

Coronaviruses have crossed these species barriers and during the last two decades, three zoonotic coronaviruses have been identified as the cause of large-scale disease outbreaks, Severe Acute Respiratory Syndrome (SARS), Middle Eastern Respiratory Syndrome (MERS) and Syndrome of Acute Swine Diarrhea (SADS) [6]. Coronavirus infection results in respiratory, enteric, neurological, and liver disorders [7].

All pathogenic human coronaviruses have their origin in animals [8], and studies have been carried out in an attempt to obtain clarification on the capacity of domestic and wild animals to be potential reservoirs [9].

Considering the zoonotic risk of coronaviruses, their role in wild species that host wild animals, as well as their ability to adapt to new species, seems to be the fundamental key to understanding their pathophysiology [8].

Coronaviruses are positive-stranded RNA viruses, belong to the order *Nidovirales*, family *Coronaviridae* and have four genera, *Alphacoronavirus* (human coronavirus NL63 (HCoV-NL63), porcine transmissible gastroenteritis coronavirus (TGEV), PEDV, and porcine respiratory coronavirus - PRCV), *Betacoronavirus* (SARS-CoV, MERS-Cov, bat coronavirus HKU4, mouse hepatitis coronavirus (MHV), bovine coronavirus (BCoV), and human coronavirus OC43, *Gammacoronavirus* (avian (infectious bronchitis coronavirus - IBV) e *Deltacoronavirus* (porcine deltacoronavirus (PdCV) [10].

Coronaviruses infect humans and a wide diversity of mammalian and bird species causing respiratory, enteric, neurologic and hepatic disorders [7]. Most coronaviruses replicate in epithelial cells of the respiratory tract producing respiratory symptoms while others infect epithelial cells of the enteric tract, causing diarrhea severely, sometime fatal, in young farm animals, causing serious damage to livestock [11; 12].

The objective of this work was to report the presence of coronavirus particles in wild, captive and companion animal species, in fecal or small intestine samples sent to the Electron Microscopy Laboratory of the Biological Institute for identification of the viral agent.

II. MATERIAL AND METHODS

Description of cases

Wild boar (*Sus scrofa*) – In 2001, around 8 wild boars, from farms located in Paraguaçu Paulista, Serra Azul and Ribeirão Preto, of São Paulo State, Brazil, suddenly presented yellowish and watery diarrhea, loss of appetite, dehydration and death. One of the animals was found dead.

Collared peccary (*Tayassu tajacu*) e White-lipped peccary (*Tayassu pecari*) – In 1995, feces were collected from 2 collared peccaries and 2 white-lipped peccaries from CIZBAS (ESLQ/USP Animal Science and Wildlife Research Committee ESALQ/USP), located in Piracicaba, SP, Brazil, where the animals were kept in the picket sector for experimental studies to determine levels of energy and protein for growth and weight gain. The animals had diarrhea with pasty and yellowish stools, loss of appetite and dehydration. Animals after supportive treatment recovered. In this place, a group of rheas (*Rhea americana*) was also kept for experiments related to the assessment of the relationship between nutrition and bone deformities of the legs during the growth period. Two of these animals suddenly developed profuse, yellowish diarrhea, followed by anorexia, prostration, dehydration and death.

Brocket deer (*Mazama gouazoubira*) – In 1995, a young female brocket deer was rescued and sent to the Serra do Itapety Municipal Park, Mogi das Cruzes, SP. After identification, marking and routine examinations, it was observed that the animal presented watery, yellowish diarrhea, anorexia, apathy and dehydration.

Coati (*Nosua nosua*) – In 2003, a free-living male coati, coming from the Tietê Ecological Park, SP, was found seriously prostrate and with dyspnea in the surroundings of the Park. The animal was attended at the CRAS (Wild Animal Recovery Center), where it was medicated, but after 3 days it died. During necropsy it was possible to observe the presence of severe pyothorax associated with pneumonia, pericarditis and intestines containing yellowish stools.

Peregrine falcon (*Falco peregrinus*) – In 2004, during a survey of viral agents among the birds in the Tietê Ecological Park, feces samples were collected from a rescued peregrine falcon victim of an illegal bird trade. The animal presented watery and greenish stools.

Mountain lion (*Puma concolor*) During a survey of viral agents carried out among animals kept in captivity in a breeding site, located in the municipality of Carapicuíba, SP, in 2004, fecal samples from 2 puppies of Mountain lion (*Felis concolor*) were collected, among others. The animals, with approximately 10 months of age, were kept in a cage together with their parents, from birth. From three months, the puppies were suddenly separated from their parents, being weaned with the purpose of adapting them to human coexistence. Soon after, they presented watery, yellowish diarrhea and signs of dehydration. Samples of feces from adult animals were also collected.

Golden-faced lion tamarin (*Leontopithecus chrysomelas*) – During a study carried out among primates from an Ecological Park located in Americana, SP, in 2004, fecal samples were collected from 6 animals (3 males and 3 females), adults, natives of the south of Bahia, belonging to a group that were kept in enclosures, for research related to captive reproduction. Pasty stools had a yellowish color.

Ferret (*Mustela putorius furo*) - Stool samples from 86 ferrets (offspring aged 20 days to 6 months and adults aged 2 to 7 years) were referred from clinical cases for investigation of the viral agent, between 2006 and 2018. The main clinical symptoms and signs presented by the animals were, coryza, hyporexia, weight loss, periodic colitis, emesis, dehydration, abdominal distension, and yellowish watery to pasty diarrhea, sometimes with mucus or blood, lasting from 2 days to 1 month. One animal died.

Chinchilla (*Chinchilla lanigera*) – A domestic chinchilla suddenly showed clinical signs of anorexia, diarrhea, prostration and sudden death, in 2010. During necropsy, it was observed that the intestines were dilated, containing watery and yellowish stools.

Buffaloes (*Bubalus bubalis*) – Fecal samples from two 60-day-old male buffaloes (*Bubalus bubalis*) from a property in Pilar do Sul, São Paulo State, Brazil, were sent to search for viral agents in 2009. The animals had acute enteritis for 20 days, with yellowish stools.

III. METHODS

Negative staining technique (rapid preparation). Samples of feces and small intestine fragments from the animals 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 carbon 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 [13].

IV. RESULTS

Using a Philips EM 208 transmission electron microscope all the samples were analyzed by negative staining technique and a great number of coronavirus-like particles, pleomorphic, rounded or elongated shaped, with characteristic radial projections forming a corona and measuring 80 -140nm in diameter, were observed in all samples (figures 1-12)

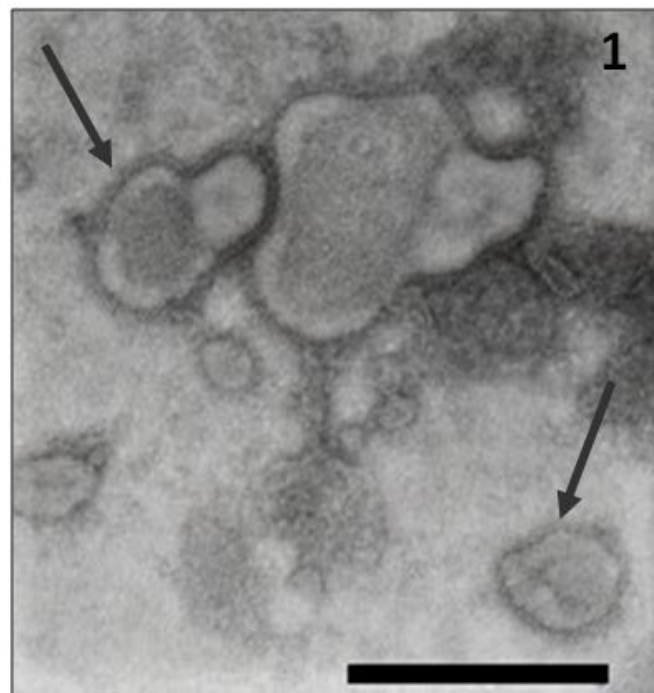


FIGURE 1: Pleomorphic coronavirus-like particles in small intestine suspension of the wild boar (*Sus scrofa*), showing radial projections of the envelope (arrow). Bar: 160 nm.

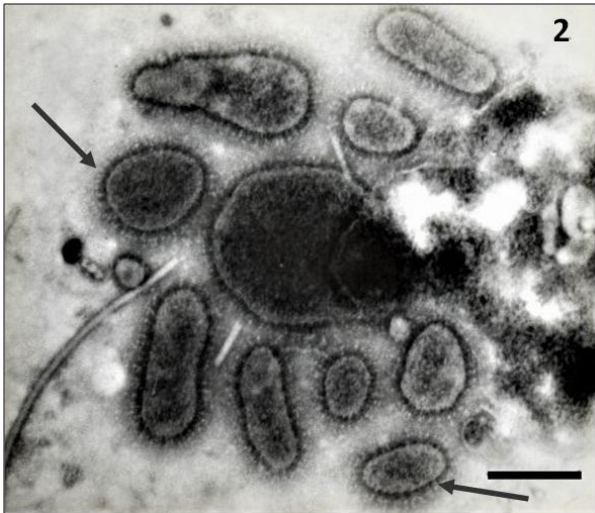


FIGURE 2: Grouping of elongated coronavirus-like particles in feces of collared peccaries (*Tayassu tajacu*), showing characteristic radial projections forming a solar corona (arrow). Bar: 70 nm

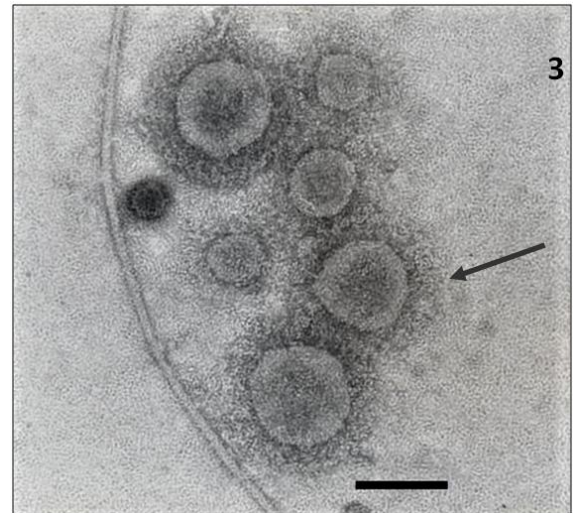


FIGURE 3: White-lipped peccary (*Tayassu pecari*) stool suspension with compact distinctive projections that extend in a corona over the particle surface (arrow). Bar: 90 nm

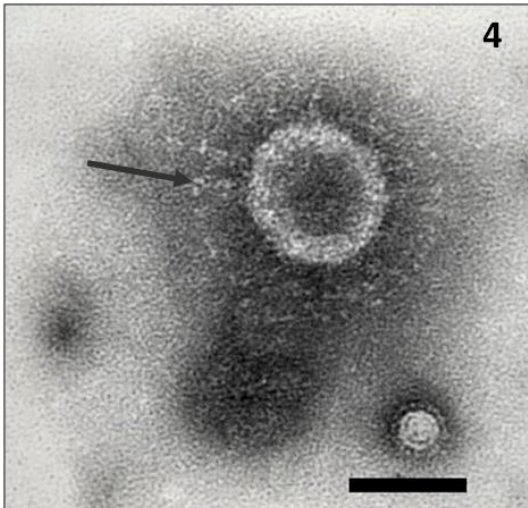


FIGURE 4: Coronavirus-like particle showing a characteristic envelope in the form of a solar corona or goblet (arrows), in feces of *Rhea americana*. Bar: 64 nm.



FIGURE 5: Coronavirus-like particles showing spaced and petal-shaped spikes (arrows) in feces of *Mazama gouazoubira*. Bar: 120 nm

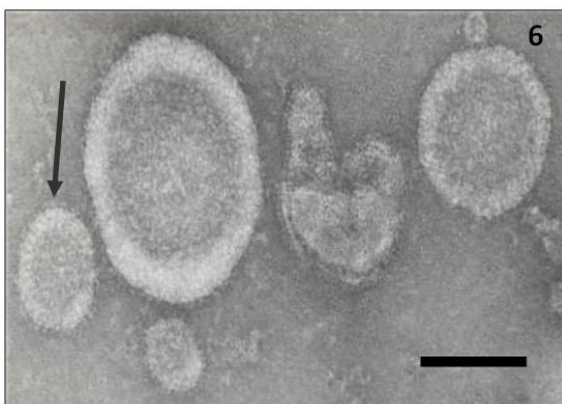


FIGURE 6: Oval coronavirus-like particles showing a characteristic electron dense central corion in the small intestine of *Nosua nosua* (arrow). Bar: 120 nm

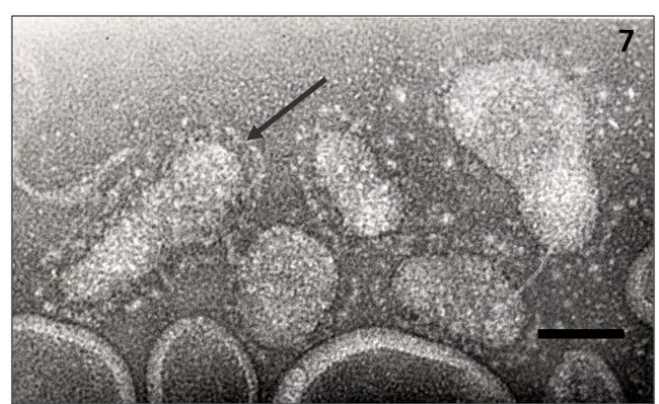


FIGURE 7: coronavirus-like particles exhibiting thin, wispy, and widely spaced spikes in feces of Falcon peregrine (arrow). Bar: 54 nm.

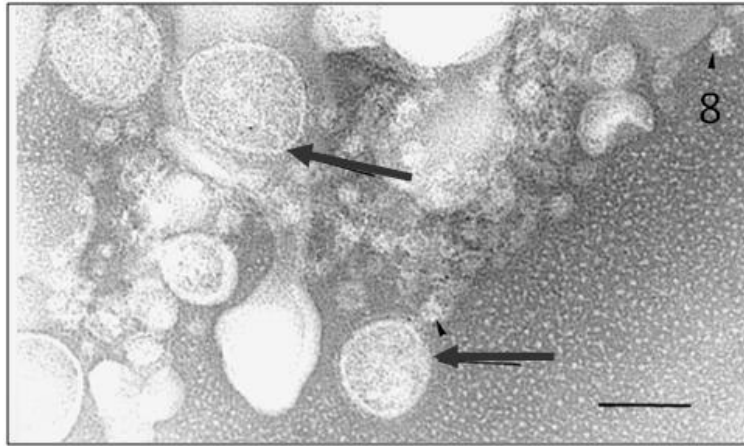


FIGURE 8: Coronavirus-like particles showing a fringe of shorter spikes (arrow) and astrovirus (arrow head) in feces of *Felis concolor*. Bar: 70 nm.

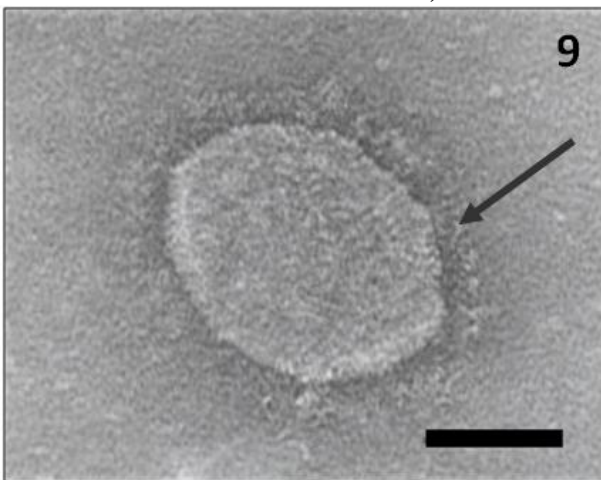


FIGURE 9: Coronavirus-like particles showing delicate spikes that make up the envelope in *Leontopithecus chrysomelas* (arrow). Bar: 60 nm.

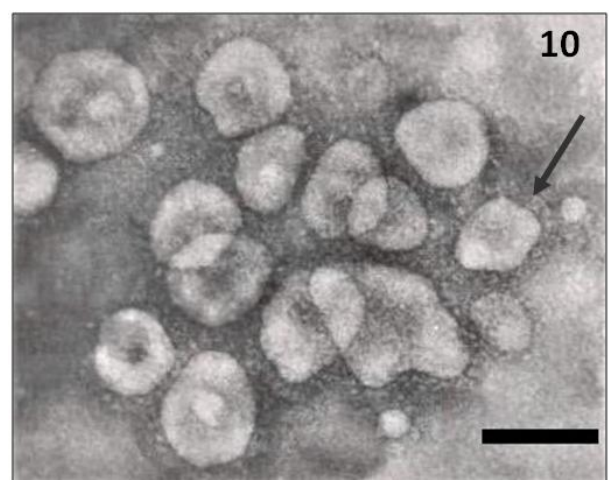


FIGURE 10: Large numbers of rounded coronavirus-like particles showing a characteristic envelope and an electron-dense central corion in feces of *Mustela putorius furo* (arrow). Bar: 130 nm.

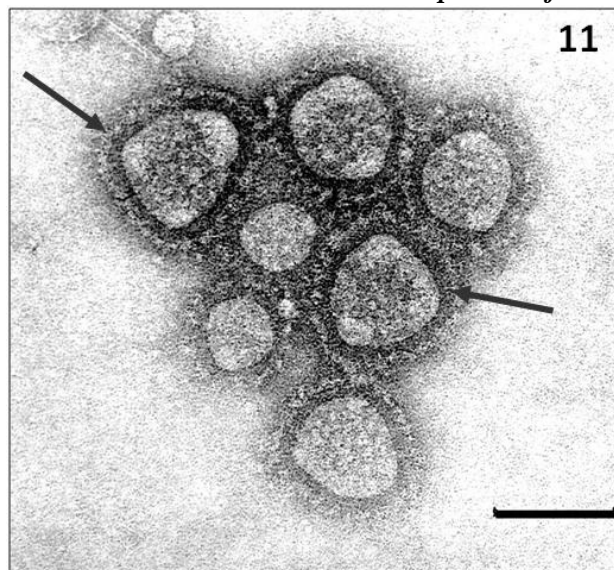


FIGURE 11: Coronavirus-like particles displaying petal or globet-shaped spikes on the surface of the virion in small intestine suspension of *Chinchilla lanigera* (arrow). Bar: 160 nm.

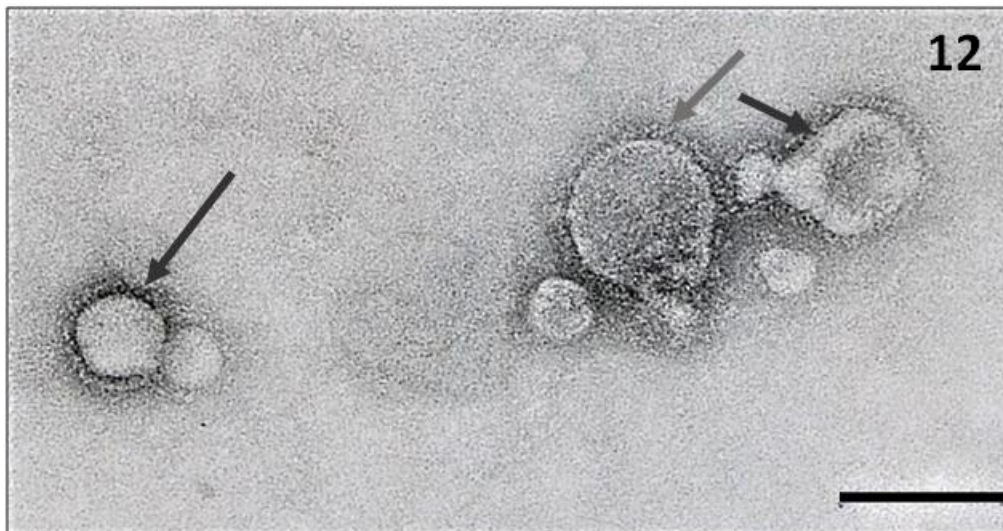


FIGURE 12: Rounded (big arrow) and pleomorphic (minor arrow) coronavirus-like particles with typical envelope (gray arrow), in feces of *Bubalus bubalis*. Bar: 140 nm.

V. DISCUSSION

Anthropogenic and natural actions are closely linked to ecological changes that can influence the epidemiology of zoonoses, affecting wild animals that can serve as reservoirs [14].

The knowledge accumulated so far shows that their occurrence may not result from the direct presence of animals, and even less from high levels of biodiversity, but from human action and the way we interact with the natural environment. In fact, the reduction in the number of species and their habitats appears to be a much more direct cause for the spread of wild-borne diseases [15; 16].

In our study we found coronavirus particles in several species of wild animals. The particles were pleomorphic, rounded or elongated, containing radial projections or spikes that emerge from the envelope forming a corona and measuring 80-140 nm in diameter. The morphological characteristics of these particles correspond to those described by other authors in other species of wild animals such as capybaras [17], Giant Anteater [18]; ferrets [19]; doves [20], owls [21], buffaloes [22]; [23], frogs [24], and, minks [25].

The presence of watery to pasty diarrhea with yellowish coloration was the main clinical sign observed in all animals. Other signs, such as, anorexia, apathy, prostration, dehydration and death, were also found in the animals in our research, also reported in other wild animal species [17, 18, 19, 20, 21].

Coronaviruses have been associated with important enteric processes in several animal species, which can cause high mortality in breeding, causing damage to livestock [11].

The Mountain lion puppies of the our study were suddenly separated from their parents, a procedure recommended for adapting to human life, and were suddenly subjected to a stress factor. Since the parents maintained a position of healthy carriers, favoring the contagion of the offspring, probably after weaning, when there is a decrease in immunity. According to Pedersen et al. (2004) [26] factors such as confinement, mating, feeding and handling practices constitute important procedures that cooperate in favor of the release of viral processes, especially in carriers animals. Animals were co-infected with astroviruses. These viruses, however, have been associated with mild diarrhea in cattle [27], sheep [28], swine [29], dogs [30] and birds [31]. In cats, however, they are responsible for severe diarrhea (Aydin &Timurkan, 2018) [32]. In most cases, astroviruses have been isolated in association with other viruses, also present in the feces of diseased animals [11].

Studies have shown that cats can be naturally infected with SARS-CoV-2. During a survey conducted among felines in Spain, a lion tested positive for SARS-CoV-2 RNA and a Zoo puma located in South Africa had a confirmed diagnosis for the viral RNA after coming into contact with an infected handler. Another investigation showed that a snow leopard was positive for viral RNA at a Zoo in Kentucky, United States [33].

The brocket deer (*Mazama gouazoubira*) and the golden-faced lion tamarin (*Leontopithecus crysomelas*) in our work were also subjected to stressful conditions. The first one was rescued and sent to a Wildlife Recovery Center, a foreign place to the

animal and the monkeys were kept in enclosures for research related to captive reproduction, due to its status as an endangered species, according to the International Union for the Nature Conservation (IUCN) and the Chico Mendes Institute for Biodiversity Conservation (ICMbio) [34]; [35]. In addition, their habitat is increasingly fragmented, which raises concerns about the species' survival [36, 37]. The phylogenetic proximity between non-human primates and humans favors the cross-transmission of infectious agents and poses a threat. It must be considered, therefore, that unlike most mammal species, non-human primates are sociable species which exponentially expands the spread of shared intraspecific diseases [38], highlighting the yellow fever that caused an outbreak in 2016 and 2019 [39] and Zika cases [40]. According to Guimarães et al. (2020) [41] although the natural cross-transmission of SARS-CoV-2 between humans and non-human primates has not been demonstrated, the global spread of the virus represents a potential threat. Thus, establishing preventive surveillance, and control measures for viruses of the family *Coronaviridae* in Neotropical non-human primates populations is crucial for their conservation. White-tailed deer and some monkey species are susceptible to SARS-CoV-2 infection [42].

The Long-tailed Chinchilla (*Chinchilla lanigera*), included in our work, is a species currently threatened with extinction, as the animal is widely used as a pet and in fur production in the early 2000s. Because the chinchilla is an exotic species in Brazil, there are few reports of viral infections in this species [43]. It is likely that chinchillas are carriers of coronaviruses. According to the CDC (Center Disease Control) [44] about 75% of infectious diseases that affect humans originate in other animals. Similar to the chinchilla, the mink, a mustelid species also threatened, has been bred for years focused on the production of skins [45]. Mink, chinchillas, raccoons, foxes and rabbits are bred for fur production in several countries [46]. According to a report by PETA - People for the Ethical Treatment of Animals (2021) [47], these animals are constantly exposed to various conditions of exhaustion, such as extreme confinement in breeding sites with dubious sanitary conditions, fear, poor nutrition, diseases, physical and psychological disorders and death inhuman. These conjunctions can trigger several diseases whose viral agents are housed in many of these species. The World Health Organization has reported the emergence of a mink-associated SARS-CoV-2 variant [48]. People in Denmark and the Netherlands are already infected with a mutant SARS-CoV-2 (Y453F) originated from minks [49]. The susceptibility of mink to SARS-CoV-2 was not a surprise, as they are closely related to ferrets that have already been found to be susceptible in experimental inoculation [50,51, 52]. During an outbreak of epizootic mink catarrhal gastroenteritis, coronavirus particles were detected by transmission electron microscopy [25].

All ferrets in our survey presented yellowish diarrhea. Two types of coronaviruses affect ferrets, enteric coronavirus (FREVC) and systemic coronavirus (FRSCV), an emerging disease of ferrets. FREVC is known to cause gastroenteric disorders (epizootic catarrhal enteritis) and FRSCV for its pyogranulomatous similarity to Feline Infectious Peritonitis, in cats. In epizootic catarrhal enteritis the stools are greenish, but in the hypersecretion phase there may be a period of poor digestion or malabsorption and the stools become yellowish containing a granular material [53, 54], where coronavirus particles can be easily identified by electron microscopy, during the acute phase of the disease process [53]. Two animals had bloody diarrhea and one of them died. The severity of clinical signs varies and the presence of blood in the stool can occur in the presence of secondary infections, causing ulcerations of the intestinal wall [53]. The presence of coronavirus in feces of ferrets with diarrhea has been reported in Brazil [19]. The color of the feces, together with clinical signs and symptoms, suggests that the animals were affected by epizootic catarrhal enteritis. In Europe, Asia and the United States, *Mustela putorius furo* was used for commercial fur farming and is currently considered a pet [55]. The results of experimental inoculation of SARS-CoV-2 indicate that cats and ferrets are highly susceptible [52]. Currently in Brazil, the ferret is considered a pet, and its commercialization is monitored by IBAMA in order to control pathogens and avoid ecological instability [55, 56]. Natural infections of SARS-CoV-2 have been reported in various animal species, including ferrets [57]. Both mink and ferrets belong to the Family Mustelidae. Therefore, the pattern of infection suggests that other members of the family Mustelidae may be susceptible to SARS-CoV-2 and therefore require further investigation [42].

The collared peccaries (*Tayassu tajacu*) and white-lipped peccaries (*Tayassu pecari*) in our survey are species currently raised in captivity for meat consumption. The breeding of these species in captivity has been widespread in several countries in recent decades, potentially because the meat has quality standards close to those required in the consumer market and due to the demand for exotic meats, mainly in specialized restaurants. Captive breeding of collared peccaries and white-lipped peccaries can minimize the effects of predatory hunting, habitat fragmentation, trafficking and species extinction, which could result in ecological instability. The free life or captivity Tayassuids are susceptible to the main infectious agents that also affect domestic pigs, including the swine transmissible gastroenteritis (TGEV) and pathogens with zoonotic potential [58, 59].

Wild boar meat (*Sus scrofa*) is highly appreciated and sought after all over the world due to peculiar flavor and nutritional content, however, its consumption is not recommended due to health risks [60]. Wild boar meat is consumed, mainly in the form of barbecue, in the South and Southeast regions (<https://revistapesquisa.fapesp.br/carne-de-javaporco-pode-transmitir-doencas/>) [61]. Several coronaviruses (PEDV, PDCV, SADS-CoV and TGEV), which originated from interspecies, infect the pig (*Sus scrofa*) and cause acute gastroenteritis in neonatal piglets and death of animals, leading to economically relevant problems to the pig industry [12, 62]. Frequent contact between humans and swine could lead to a higher risk of cross-species transmission or virus recombination [63]. Zhou et al. (2018) [62] reported that SARS-CoV-2 could use ACE2 from four animal species including the porcine ACE2 as the receptor to enter the cell in vitro, showing that pigs might be potentially susceptible to SARS-CoV-2 infection and could be a potential intermediate host. Crossbred pigs (*Sus scrofa domesticus*) with 8-week-old were found to be susceptible to SARS-CoV-2 infection following oronasal inoculation. Although SARS-CoV-2 RNA was detected in the oral fluids and nasal wash collected from two pigs, live virus was isolated only from one animal [64]. Based on the phylogenetic tree and recombination detection program, Hu et al. (2020) [63] stated that swine and mice could be probable reservoirs for the Sars-CoV-2.

The buffaloes in our work came from beef breeding. In Brazil, the exploitation of buffaloes is primarily intended for meat production, however, from the 1980s onwards, there was a growing interest in their dairy or dual purpose (beef and milk) exploitation [65]. The presence of coronaviruses has been reported in feces of young buffaloes with diarrhea on properties in the State of São Paulo, Brazil [66] and in Italy [67]. The productivity of bovine and buffalo herds is closely related to success in raising calves. Among the main causes of failure in buffalo and bovine breeding, neonatal diarrhea stands out, responsible for the highest rates of morbidity and mortality in these breeding [68]. Bovine coronavirus (BCoV) is the etiological agent responsible for acting and/or aggravating infections associated with diarrhea and respiratory diseases in cattle of all ages, being an extremely contagious virus that affects the health of the herd and the world economic sector due to its high morbidity and significant mortality rate among calves [69]. Bovine CoVs have been reported to infect children and thus possess zoonotic potential. Spillover is managed by the interaction of viral-specific proteins like S protein and host ACE2 receptor [70].

The coati (*Nosua nosua*) included in our research presented severe pyothorax associated with pneumonia and pericarditis. Coronaviruses cause enteric and respiratory problems in cattle (Franzo et al., 2020) [71] and bovine-like CoVs have been identified in wild species such as elks [72], minks [73], raccoons [74] and in alpaca [75, 76]. In mountains lions vascular necrosis and necrotizing myocardial changes have also been observed [77]. SARS-CoV-2 was recently detected in a coati mundi at an Illinois Zoo, in the United States. Samples of several zoo species, including coatimundi, were collected and tested after a zoo tiger showed signs of virus infection [78]. The coati (*Nosua nosua*) is host to several etiological agents that can be harmful to its populations and others with zoonotic potential. In urban areas, coatis can be found in close association with humans and domestic animals [79].

The rheas and the peregrine falcon from our work suddenly developed profuse diarrhea. In birds, the disease caused by the coronavirus is infectious bronchitis, whose main clinical signs are respiratory, including sneezing, nasal discharge, tracheobronchiolar rales, conjunctivitis and sinusitis. Strains of IBV that affect the kidney, but can result in diarrhea, severe dehydration and mortality due to kidney failure [80]. The chickens and pheasants are natural hosts of IBV [81], although IBV isolates have also been reported in peafowl, turkey, teal, geeze, quill, duck, parrot, quail, penguin and guinea fowl [82]. Coronavirus particles have already been found in free-living animals such as doves, owls and in passerines such as the Lined Seedeater (*Sporophila lineola*) [20, 21, 83]. Probably the rheas acquired the disease through contact with infected chickens present in the creation, or through the feces of wild birds that carry the virus. A similar occurrence was reported in a breeding of rheas in the United States, where the bird showed weakness, ataxia, enteritis and death, after introducing new birds into the breeding. Coronavirus particles were detected in small intestine fragments by transmission electron microscopy [84]. The peregrine falcon could also be acting as an IBV host and suddenly develop diarrhea after being removed from the wild, considering that the bird was a victim of illegal trade. The presence of the IBV in wild and exotic birds can be assigned by the interaction between species or by its close proximity to commercial poultry farms [85]. The variation in host range, tissue tropism, and pathogenesis of CoVs is mainly due to variations in the glycoprotein and lead to different antigenic forms, serotypes, or variant strains [86, 87]. Although the presence of the coronavirus in wild birds might suggest the possibility that they are reservoirs of COVID-19 with transmission to humans, infection of COVID-19 in poultry would be unlikely, mainly due to the incompatibility of cell receptors that would not recognize the same type of virus [88].

People with COVID-19 can spread the virus to animals during close contact. It is important for people with suspected or confirmed COVID-19 to avoid contact with pets and other animals to protect them from possible infection [78].

It is likely that the new coronavirus is circulating in wild reservoir animals and that these have the potential to emerge very quickly and cause a new epidemic due to human consumption habits and their interrelationship with nature [88].

Crucially, efforts to reduce poaching and the indiscriminate consumption of wild animals must be conducted to change the normative values of consumers through education, raising awareness not only for public health, but also for protection and welfare animal living and global biodiversity [89].

VI. CONCLUSION

Studies aimed at determining the presence of the coronavirus in wild animals should be continued to better characterize the interspecies barrier transmission between these viruses and different animal species in order to avoid possible losses, mainly due to the fact that many species are included in the list endangered species official. Attention should be given to public health and veterinary surveillance programs, including monitoring of wild animals in order to contribute information to assist prevent future emergencies, outbreaks and pandemics [90].

This report is the first worldwide occurrence of coronaviruses in *Falco peregrinus*, *Tayassu tajacu* and *Tayassu pecari* and the first occurrence in Brazil in *Sus scrofa*, *Nosua nosua*, *Puma concolor* and in *Rhea Americana*.

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Evaluation of Suitability of Omambala River in Anaku Community for Aquaculture Production

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Abstract—

Background and Objectives: Water quality is the most important factor affecting fish health and performance in aquaculture production. This research was therefore carried out to evaluate the suitability of Omambala river in Anaku community for aquaculture purposes.

Materials and Methods: Physicochemical properties, heavy metals and biological parameters were assessed to determine the suitability of the river water for aquaculture production using three different sampling stations. Data obtained were compared with the permissible limit of World Health Organization. Analytical tools used were T-test and ANOVA with SPSS Version 20.

Results: The study revealed that the physicochemical parameters and heavy metal concentration were in the range of temperature, $27.33\pm 0.57^{\circ}\text{C}$, turbidity, $49.80\pm 5.70\text{cm}$, nitrate, $1.06.00\pm 0.52\text{mg/l}$, Ammonia, $0.53\pm 0.03\text{mg/l}$, pH, 6.77 ± 0.06 , Dissolved oxygen, $9.33\pm 0.57\text{mg/l}$, lead, $0.123\pm 0.015\text{ppm}$, cadmium, $0.133\pm 0.003\text{ppm}$, mercury, $0.861\pm 0.101\text{ppm}$, zinc, $0.518\pm 0.03\text{ppm}$ and iron, $7.436\pm 0.58\text{ppm}$. This study revealed that the physicochemical parameters were within tolerable limit except nitrate while the heavy metals were above the tolerable limits except zinc. There were abundance of phytoplankton and zooplankton in Omambala river.

Conclusion: Omambala river in Anaku fishing community is suitable for aquaculture purposes when properly treated to reduce high concentrations of heavy metals and it is recommended that proper environmental impact assessment of industrial, domestic and anthropogenic effluents into Omambala river be carried out to establish adequate detoxification measures.

Keywords: Physicochemical parameters, heavy metals, Aquaculture suitability, plankton productivity and tolerable limits.

I. INTRODUCTION

The importance of water as a resource that supports growth, survival and production of fish cannot be over emphasized. Nigeria is blessed with numerous water bodies ranging from the marine (Atlantic Ocean), through the brackish waters (deltaic rivers and estuaries) to inland freshwaters (rivers, streams, springs and lakes). Despite these available resources, recent trends have shown decline in the Nigerian capture fisheries and this calls for development of aquaculture especially in fishing communities as they possess the potentials¹.

Aquaculture is said to be the rational rearing of aquatic organisms (fish, shrimp, prawns, water snails) in an enclosed and fairly shallow body of water where all its life processes can be controlled². It is an important sector for the nation's economic development, at a time when government is seeking ways to diversify the economy, from being purely oil based. This can contribute to alleviation of food insecurity, malnutrition and poverty through the provision of food of high nutritional value, income, and employment generation, decreased risk of monoculture production failure, improved access to water, enhanced aquatic resource management and increased farm sustainability³. Aquaculture is the fastest growing food producing industry in the world and global aquaculture production is likely to double in the next fifteen years, as a result of wild fisheries approaching their biological limits and the world demand for cultured fish continuing to increase⁴.

Water pollution is the introduction of substances by man directly or indirectly which can cause harm to living resources, hazard to human health, hindrance to aquatic activities and impairment of water quality with respect to its use in agriculture, industrial and other economic activities⁵. Natural (erosion) and anthropogenic (agricultural discharge) activities affect the quality of regional surface water⁶. These activities have undesirable, effects on the natural environment. Also, uncontrolled land use, urbanization, deforestation, accidental (or unauthorized) release of chemical substances and discharge of untreated wastes or leaching of noxious liquids from solid waste deposits have impacted negatively on the quality of water resources⁷.

Water quality evaluation is considered as a critical issue in recent years, especially when freshwater is becoming a scarce resource in the future⁵. Water quality evaluation aims to identify the sources of water pollution and develop a strategy for sustainable water source management, maintaining and promoting human health and other social and economic growth⁸.

The fishing activities in the Anaku community is of great importance since fishing is an important income source, contributing to the economy of fisher folks living around Omambala river. There has been a recent report on the number of fisher folks in Anaku fishing community who intend to increase fish production by engaging in fish culture but there is inadequate information on the quality of Omambala River for aquaculture production. Thus, this work was conducted to evaluate the suitability of Omambala river for aquaculture purposes in Anaku fishing community.

II. MATERIALS AND METHODS

2.1 Study Area

This research work was conducted in Omambala river which is located in Anaku fishing community in Anambra State, Nigeria in October, 2019. Omambala is the native name of the Anambra River, which is a tributary of the famous River Niger (North), Ezu River (South), Omor and Umuerum communities (East). Anaku is a fishing community and the administrative head quarters of Ayamelum Local Government Area of Anambra State, South-East Nigeria.

The study area lies 6°15' North of the Equator and 6°44' East of the Greenwich Meridian. The area falls within the tropical climate which accounts for the prevailing moist rainforest vegetation. Climate of the area is characterized by dry season during November to March and rainy season from April to October with mean annual rainfall of about 1805 mm. The river is the most important feeder of the River Niger which flows 210 kilometres (130 miles) into the Niger River before finally being released into the Atlantic ocean through various outlets. The crop farming and fishing activities in the community are of great economic importance as most of the dwellers are crop farmers and fisherfolks who cultivate mainly rice, as well as other crops (yam, cassava, vegetables, and cocoyam) and also engage actively in daily fishing activities.

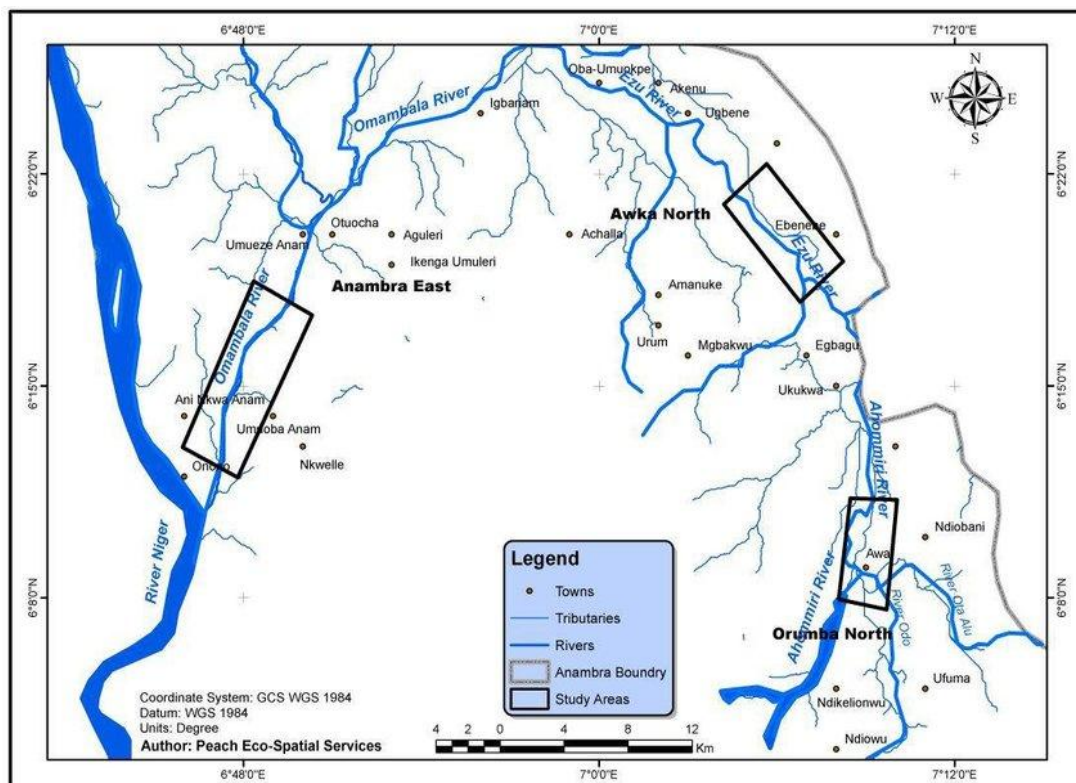


FIGURE 1: Map showing Omambala river

2.2 Water sampling and Method of Water Sample Collection

Three sampling stations were selected along the length of the river and labelled Station 1, Station 2 and Station 3 respectively. These sampling stations were chosen based on the hydrodynamics and characteristic features of the area. Sterilized and sealed bottles were used for sample collection. The marked sterilized bottles were dipped into the river, sealed and covered with black sack with water to avoid solar radiation effect. Then, the water samples from the three different stations in Omambala River was analysed for physico-chemical (temperature, turbidity, pH, ammonia, dissolved oxygen and nitrate), biological parameters (planktons) and heavy metals such as Iron(Fe), lead(Pb), Mercury(Hg), Cadmium(Cd) and Zinc(Zn) in order to determine the suitability of Omambala River for Aquaculture purposes.

2.3 Analysis of Physico-chemical parameters in the Water Samples

2.3.1 Temperature

The temperature was determined *in situ* using Mercury-in-glass thermometer. It was lowered into the water and allowed for three to five minutes for the thermometer to assume the temperature of the water and the reading was taken while the thermometer was still in the water to avoid interference with ambient temperature.

2.3.2 pH

The portable Combo HANNA instrument water check (model HI 98129) was used to determine the water pH. The instrument was dipped in a water sample until a stable pH value was displayed.

2.3.3 Transparency

The Sec chi disk attached to a graduated rope was lowered at the sampling station and the point of disappearance and appearance were noted and recorded as the value of transparency.

2.3.4 Odour and Colour

The odour and colour of the river was determined by perception and sight.

2.3.5 Ammonia

1ml of the water sample was introduced into a 30ml of Kjehdal flask and 1ml of the kjehdal catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30minutes and allowed to cool. After cooling, about 100ml of distilled water was added to avoid caking and then transferred to the kjedahl distillation apparatus. A 500ml receiver flask containing 5ml of boric acid was placed under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution. The 10ml of 40% sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until distillation reached the 35ml mark of the receiver flask, after which it was titrated to pink colour using using 0.01N hydrochloric acid adding necessary indicators.

2.4 Dissolved Oxygen in Water determination

Dissolved oxygen (DO) was measured using Winkler's method. This was carried out by adding 1cm³ of Manganese sulphate solution followed by 1cm³ of alkaline iodide solution to the water samples. The contents were thoroughly mixed by inversion and rotation until a clear supernatant water was obtained. Then, 1cm³ of concentrated sulphuric acid was added with the tip of the pipette below the level of solution and the stopper was replaced. The mixture was shaken until the precipitation was completely dissolved. Then, it was titrated against standard sodium thiosulphate (0.025 moldm⁻³) using freshly prepared starch solution as the indicator (added when solution became yellow). The titration was carried out in duplicate.

2.5 Nitrate Determination Method

Nitrate was determined using PD303 UV spectrophotometer⁹. 50ml of the water samples was measured into a porcelain dish and evaporated to dryness on a hot water bath. Then, 2ml of phenol disulphonic acid was added to dissolve the residue followed by the addition of concentrated solution of sodium hydroxide and distilled water in order to make it alkaline. This was filtered into a Nessler's tube and made up of 50ml with distilled water, the absorbance was read at 410mm using a

spectrophotometer after the development of colour. The value of nitrate was found by comparing absorbance of sample with the standard curve and expressed in mg/l.

$$\text{Cone of sample} = \frac{\text{Abs of sample} \times \text{conc. of std}}{\text{Abs of std}}$$

2.6 Methods for the Heavy Metal Analysis

Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer (AAS) according to the method of APHA9 (American Public Health Association). For the heavy metal analysis of iron, cadmium, mercury, zinc and lead, the water sample was aspirated into the flame and atomized when the AAS's light beam was directed through the flame into the monochromator and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelengths, a source lamp composed of that element was used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample. The standard solution for each tested element was prepared according to its concentration and the calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

2.7 Plankton sampling and analysis

The biological properties were determined by collecting water samples from the three stations. The water samples were sieved with 1mm mesh plankton net. The sieved materials were collected in three plastic containers containing 500ml of the river water each. They were left to stand for an hour after which 480ml of the water was decanted. Then, 20ml of the filtrate was centrifuged at 3000rpm for 5minutes after which the deposit was viewed under a light microscope at X100 oil immersion and the planktons were identified using the guide to fresh water algae.

2.8 Statistical Analysis

Data from physicochemical and heavy metal analyses were subjected to T-test while data from biological parameters was analysed using analysis of variance (ANOVA) with Statistical Package for Social Science (SPSS) version 20 to obtain their mean differences.

III. RESULTS

TABLE 1
FISH SPECIES AVAILABLE IN OMAMBALA RIVER

Common name	Native name	Species
Electric catfish	<i>Elulu</i>	<i>Malapterus electricus</i>
Trunk fish	<i>Asa mkipete</i>	<i>Gymnarchus niloticus</i>
Nile tilapia	<i>Ikpokpo</i>	<i>Oreochromis niloticus</i>
African Bony Tongue	<i>Okpo</i>	<i>Heterotis niloticus</i>
Mud catfish	<i>Asu isi/ikere</i>	<i>Clarias gariepinus</i>
Moonfish	<i>Orowo</i>	<i>Citharinus citharus</i>
Snakehead	<i>Efi</i>	<i>Parachanna obscura</i>
African Lungfish	<i>Ekwumu</i>	<i>Propterus annectens</i>
Elephant snout	<i>Uzeh</i>	<i>Mormyrus rume</i>
Upside down catfish	<i>okpor</i>	<i>Synodontis membranaceus</i>
Squanga whitefish	<i>Azu ocha</i>	<i>Coregonus sp.</i>

Table 1 showed the various fish species available in Omambala river which includes; Electric catfish, Trunk fish, Nile tilapia, African Bony Tongue, Mud catfish, Moonfish, Snakehead, African Lungfish, Elephant snout, Upside down catfish, Squanga whitefish. However, fish species that are mostly demanded by consumers in the study area are Mud catfish, Trunkfish and Nile tilapia.

TABLE 2
PHYSICO-CHEMICAL PROPERTIES OF THE WATER SAMPLES COLLECTED FROM THREE DIFFERENT STATIONS IN OMAMBALA RIVER.

Physicochemical Properties	Water Bodies Sampled			Mean \pm SD	WHO limits
	Station 1	Station 2	Station 3		
Turbidity	44	50	55.4	49.80 \pm 5.702	5-50
Temperature	27	28	27	27.33 \pm 0.577	15-32
pH	6.7	6.8	6.8	6.77 \pm 0.057	6.5-8.5
Nitrate	1.45	0.459	1.28	1.06 \pm 0.529	0.5
Ammonia	0.50	0.56	0.53	0.53 \pm 0.033	0.50
Dissolved oxygen	9	10	9	9.33 \pm 0.57	>5.0
Odour	Odourless	Odourless	Odourless		Odourless
Colour	Light brown	Light brown	Light brown		-

Table 2 showed that the mean value of the physiochemical parameters were in the range of temperature, 27.33 \pm 0.577⁰C, turbidity, 49.80 \pm 5.702cm, nitrate, 1.06.00 \pm 0.529mg/l, Ammonia, 0.53 \pm 0.033mg/l, pH, 6.77 \pm 0.057 and Dissolved oxygen, 9.33 \pm 0.57mg/l. This study revealed that the physicochemical parameters were within tolerable limit except nitrate.

TABLE 3
HEAVY METAL CONCENTRATIONS OF THE WATER SAMPLES COLLECTED FROM THREE DIFFERENT STATIONS IN OMAMBALA RIVER

Heavy metals	Water Samples			Mean \pm SD	WHO limits	USEPA limits
	Station 1	Station 2	Station 3			
Lead	0.120	0.140	0.110	0.123 \pm 0.015	-	0.015
Cadmium	0.130	0.136	0.132	0.133 \pm 0.003	0.005	0.01
Mercury	0.76	0.861	0.961	0.861 \pm 0.101	0.01	-
Zinc	0.491	0.519	0.543	0.518 \pm 0.026	5.0	5.0
Iron	6.989	7.216	8.102	7.436 \pm 0.588	0.30	-

Table 3 showed that the heavy metal concentrations were in the range of cadmium, 0.133 \pm 0.003ppm, mercury, 0.861 \pm 0.101ppm, zinc, 0.518 \pm 0.026ppm and iron, 7.436 \pm 0.588ppm. This study revealed that the heavy metals were above the tolerable limit except zinc. The Fe>Hg>Zn>Cd>Pb order of occurrence of the heavy metal investigated in this study means that Fe had the highest concentration while lead had the least.

TABLE 4
PHYTOPLANKTONS ABUNDANCE AT THE THREE SAMPLING STATIONS IN OMAMBALA RIVER

	Phyoplanktons			
	Chlorophyta	Cyanophyta	Rhodophyta	Phaeophyta
Station 1	23	33	41	29
Station 2	34	62	18	17
Station 3	27	31	31	18
Mean \pm SD	28.00 \pm 5.57	42.00 \pm 17.35	30.00 \pm 11.53	21.33 \pm 6.66

From the result above, the taxon Cyanophyta had the greatest population mean of 42.00 \pm 17.35. The second most abundant taxon was rhodophyta with a mean of 30.00 \pm 11.53 followed by chlorophyta with a mean of 28.00 \pm 5.57 while the lowest being phaeophyta with a mean of 21.33 \pm 6.66.

TABLE 5
ZOOPLANKTONS AT THE THREE SAMPLING STATIONS IN OMAMBALA RIVER

Zooplanktons	Station 1	Station 2	Station 3	Mean abundance± SD
Nematode (Adenophorea)	6	7	9	7.33±1.53 ^{ab}
Rotifier- <i>Branchionus quadridentatus</i>	2	9	3	8.00±5.57 ^{ab}
Protozoa- <i>Vorticella convallaria</i>	4	13	7	8.00±4.58 ^{ab}
Rotifier- <i>Keratella cochlearis</i>	7	12	15	11.33±4.04 ^{ab}
Copepods- <i>Valdiviella insignis</i>	18	27	3	16.00±12.12 ^b
Tadpole	1	3	1	1.67±1.16 ^a
Cladocerans- <i>Bosmina</i> spp	2	4	3	3.00±1.00 ^a
Cladocerans- <i>Viaplinia</i> spp	2	4	3	3.00±1.00

Columns sharing similar superscripts are not significantly different (P>0.05)

The zooplankton community of Omambala River is dominated by Copepods with a mean of 16.12±12.12 of the total population. Rotifera was next to Copepods (mean of 11.33±4.04), followed by Protozoa (8.00±4.58) and then Cladocerans with a mean of 3.00±1.00 and the least was tadpole with a mean of (1.67±1.16).

IV. DISCUSSIONS

The physicochemical parameters, heavy metals, phytoplankton, and zooplankton in Omambala river were evaluated to determine its suitability for Aquaculture production. The results obtained from the physicochemical parameters of Omambala river in Anaku community showed that there was a significant difference in all the physicochemical parameters of the three stations in the Anaku water bodies ($P<0.05$) except nitrate ($P>0.05$). The analyses showed that the physicochemical parameters of the river water were within the standard requirement for fish culture which is an encouragement for aquaculture production in the community. The turbidity values as observed from this result are within the WHO permissible limit showing that the water contained adequate nutrients since fairly turbid water allows light penetration which support photosynthesis and oxygen production in the river¹⁰. The surface water temperature of the Omambala River recorded during this study (27.33±0.577) was optimum for normal growth and survival of aquatic organisms. The results from these findings are in agreement with the findings of Abowei¹¹ who reported that surface water temperature varied from 27°C – 31 °C in Nkoro River, Niger Delta, Nigeria. Temperature controls the rate of nutrient cycling therefore affects the availability of food and thus rate of productivity. Temperature influences water chemistry, e.g. Dissolved Oxygen, solubility, density, pH, and conductivity. Water holds less oxygen at higher temperatures¹². At elevated temperatures, metabolic activity of organism increases, requiring more oxygen but at the same time the solubility of oxygen decreases, thus aggravating the stress¹³.

The mean pH value of water samples analysed from the three stations in Omambala River in Anaku community river was 6.77± 0.057 which was significantly ($P< 0.05$) close to neutrality of the river. The pH values obtained from this result supports the work of Bellingham¹⁴ who reported that the pH of natural water usually lies in the range of 4.4 to 8.5. Based on the WHO guidelines, the pH of Omambala River in Anaku community was within permissible limit. The pH of water determines the solubility and biological availability of certain chemical nutrients such as phosphorus, nitrogen, carbon and heavy metals like lead, copper, cadmium, etc¹⁴.

The ammonia value was within the permissible limits of 0.5 while the nitrate value was found to be higher than the permissible tolerance value of 0.50mg/l as suggested by WHO¹⁵. This may be due to inflow of sewage and runoff in the rainy season and leaching of fertilizers from farmlands into the river.

The concentration of dissolved oxygen (9.33±0.57) recorded in this study is apparently within the maximum allowable limit of >5 recommended by WHO¹⁵. The high level of dissolved oxygen recorded across the stations can be linked to the agitation of the water body which is caused by the activities of canoes that constantly convey passengers across the river to their various farms, school and other locations. DO concentrations of below 5 mg/L may adversely affect the functioning and survival of biological communities while below 2 mg/L may lead to fish mortality¹⁶.

The Fe>Hg>Zn>Cd>Pb order of occurrence in the heavy metals investigated in this study means that Fe had the highest concentration in Omambala River of Anaku community during the period of study. This agrees with the report of Asonye *et al*¹⁷ who also reported iron as the most dominant metals in different rivers. The Iron level in the river at the time of this study exceeded the recommended standard of WHO¹⁵ for surface water. This supports the work of Ikeogu *et al.*,¹⁸ who reported that

iron is a major element in various minerals and rocks types and reaches natural water from sources including leaching and flaking rust from Ferro metals pipes. Mercury as seen from the result is above the standard set by WHO¹⁵ (0.01ppm) for water. Anthropogenic sources have been known as one of the causes of accumulation of mercury in the form of methyl mercury in water and fish as reported by Alfred *et al*¹⁹. The heavy human activities in the stream might have contributed to this high level of mercury in the three sampling stations. From the results of heavy metal analyses, zinc was the only heavy metal below the recommended limit of WHO¹⁵ while cadmium and lead concentrations were above the recommended limit of 0.01 mg/l and 0.015 mg/l respectively adopted by WHO¹⁵. According to Oguzie and Ogubere²⁰, the presence of heavy metals in the environment is of major concern because of their toxicity, bioaccumulation tendencies and threat to the environment and human life.

Phytoplankton productivity of Omambala River in Anaku community was composed of four taxa which included chlorophyta, cyanophyta, rhodophyta and phaeophyta. There was no significant ($P > 0.05$) difference between the abundance of phytoplanktons sampled in the three stations in the Anaku water bodies. The presence of phytoplanktons is an indication of high nutrient content available for aquatic life. The taxon Cyanophyta had the greatest number of total mean population of 42.00 ± 17.35 followed by Rhodophytes with total mean population of 30.00 ± 11.53 . This indicates that the water has high nutrient because Rhodophytes are rarely found in rivers. The high percentage composition of Cyanophyceae indicates higher productivity of Omambala River water due to nutrient enrichment. The same observation was made by Pramila *et al.*²¹ on Nagpur city Lakes. There was no significant difference ($P > 0.05$) between the abundance of zooplanktons sampled in the three stations in the Anaku water bodies. This rich zooplankton community is an indicative of good water quality with the presence of some organic pollution as suggested by Pramila *et al.*²¹ who reported that the presence of pollution indicator species such as *Branchionus* along with clean water indicates a good water quality of the water body with presence of some organic pollution.

V. CONCLUSION

Water quality analysis for physicochemical parameters, heavy metals concentrations and plankton productivity are very important in order to determine the suitability of Omambala River for Aquaculture purposes. The results of water quality parameter analysis from this study was found suitable for aquaculture purposes in Anaku community. This study revealed that the physicochemical parameters were within the permissible limit of World Health Organization standard except nitrate while the heavy metals were above the tolerable limit except zinc. However, this result showed that heavy metal concentrations could be a limitation to aquaculture production in Anaku fishing community. There were also abundance of fish, phytoplankton and zooplankton in Omambala River. This study revealed that the concentration of heavy metals in Omambala River present extremely serious risk for Aquaculture production. This study, therefore recommends the implementation of proper and relevant treatment techniques for treating the water before use for aquaculture production. The villagers should also be sensitized on the danger of dumping refuse inside the river. Further research on Omambala river should include the determination of source and possible solutions of heavy metal contents of the river as well as fish species harvested from this river in order to ascertain their safety for human consumption.

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Isolation and Identification of Pathogenic Bacteria from Table Egg (Eggshells) Sold in Benghazi Market, Libya

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Abstract— This study aimed to conduct a microbial survey of the table eggs (eggshells) sold in the markets of Benghazi city and to identify some pathogenic bacteria. Samples collected from the markets of Benghazi city, which were divided into five sectors (Bouhdima (A), Al-Laithi (B), Hay-Al-Salam region (C), Twenty Street (D), Suburbs of Benghazi (E)). The eggs were bought from several stores in each sector randomly, where twenty samples were collected (four from each sector) with three random replications each. The results showed significant differences ($P < 0.05$) between the sectors. According to this study, 80 % of pollutants are from the Enterobacteriaceae family, which includes *E. coli*, *Enterobacter sp.*, *Klebsiella sp.*, *Proteus sp.*, and *Citrobacter sp.*, and 20 % are from the genera (*Streptococcus sp.*, *Pseudomonas sp.* and *Salmonella Sp.*).our results showed that the mean total number of aerobic bacteria, coliform bacteria, and *Staphylococcus aureus* bacteria were 4.2×10^4 , 3.9×10^5 , 4.4×10^5 CFU / egg respectively. *Salmonella* bacteria were isolated from eggshell samples at the Benghazi suburbs markets (sector E) (7.0 % from isolated samples). From farm to consumer, the study underlines the importance of encouraging eggshell bacterial contamination prevention strategies.

Keywords— Contamination, Enterobacteriaceae, microbial content, *Staphylococcus aureus*, table eggs.

I. INTRODUCTION

In the previous two decades, the production and consumption of global chicken eggs have demonstrated amazing, broad, and comprehensive dynamics. [1]. Every year, more than 50 billion chickens are farmed for food, both for meat and for eggs. [1]. In the same vein, in 2012, Asia, America, Europe, Africa, and Oceania had roughly 21.2 billion chickens (90 percent of global egg output). (12.0, 5.28, 2.01, 1.79, 0.13, and 0.13, respectively)[2].

Eggs have a very stable composition in terms of total protein, essential amino acids, total lipid, phospholipids, phosphorus, and iron [1]. Egg proteins, at about 6.5 g per egg, appear to contain an adequate quantity of the 9 essential amino acids for human health: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine [3]. Furthermore, eggs contain around 70 mg of omega-3 (n-3) fatty acids and 200 mg of cholesterol. [1]. Also, according to Zaheer, egg contains both water-soluble and fat-soluble vitamins, such as (B vitamins; thiamine (B1), riboflavin (B2), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9), cobalamin (B12), choline, and vitamins A, D, E, and K,) in relatively high amounts; levels of B5, B9, A, and D are moderate [1].

There are two possible routes for germs to invade eggshells: vertically or horizontally. [4]. Vertical transmission or ascending transmission infection from contaminated cloaca into the vagina and lower oviduct occurs in the reproductive organs of infected hens from infection of the ovaries by systemic infection. [5,6]. When eggs are exposed to a contaminated environment and bacteria breach the eggshell, horizontal transmission occurs [4]. *Staphylococcus aureus*, *Salmonella spp.*, *Streptococcus spp.*, *Escherichia coli*, *Bacillus spp.*, and *Listeria monocytogenes* were among the bacteria detected on the eggshell [7]. According to Indhu et al., 2014, *Salmonella spp.*, *E.coli.*, *Campylobacter*, *Staphylococcus aureus*, and Coliform were detected on eggshells based on biochemical data and microscopic examination [8]. In the same context, the researchers reported that microbial species isolated from eggshells were *Enterobacter aerogenes*, *Escherichia coli*, *Citrobacter freundii*,

Bacillus cereus, *Enterococcus faecalis* and *Proteus mirabilis* for the bacterial isolates [9]. The goal of this study is to look for harmful bacteria on an eggshell from table eggs sold in Benghazi and estimate the bacterial contamination of eggshells.

II. MATERIALS AND METHODS

2.1 Sample collection

Egg samples were collected from the markets of Benghazi city, which were divided into five sectors (Bouhdima (A), Al-Laithi (B), Hay-Al-Salam region (C), Street twenty (D), suburbs of Benghazi (E)). The eggs were bought from several stores in each sector randomly, where twenty samples were collected (four from each sector) with three random replications each. Egg samples were taken from exposed cardboard packaging and transported to the Faculty of Agriculture Laboratory in Benghazi in sterile closed plastic bags, where microbiological testing was performed immediately.

2.2 Sample preparation:

The microbial content on the eggshells was estimated according to the method AOAC [10], prepared sterilized bottles each containing 100 ml Peptone water. The content of each bottle poured into a polyethylene bags containing the repeater of each sample (egg) in sterile conditions and shake carefully at the corner of the bag for five minutes with rubbing by hand [11]. The liquid medium returned to the bottles and the first dilution was considered 10^{-1} . The remaining decimal point dilutions were completed to the 10^{-6} dilution [10]. The culture media required for microbial counting and examination, biochemical testing, and differential media were prepared and sterilized according to the manufacturer's instructions OXOID / UK.

Colonies were tested on selective and differential media using gram stain, morphological and several biochemical tests (motility test, catalase test, oxidase test, citrate test, Indole test, MRVP tests, triple sugar iron (TSI) agar, Brilliant green broth, lactose broth test) [12-14]. Bacterial species were identified according to the Bergy's manual of determinative bacteriology 9th ed. [15].

2.3 Statistical analysis:

The experiment was performed a Completely Randomized Design with three replications. Statistical analysis was conducted using Statistical Package for Social Science version 23 (SPSS/ IBM). ANOVA and LSD test ($P \leq 0.05$) were applied to compare (mean) between sectors.

III. RESULTS

The results show (Table 1) that the mean total number of aerobic bacteria on the shells of table eggs tested was 7.7×10^6 CFU/egg in samples from the second sector B, with the maximum amount (the upper limit of the total number of microbes) being 7.7×10^6 CFU/egg. The mean total number of bacteria was 4.2×10^5 CFU / egg. The number of aerobic bacteria per egg ranged from 7.4×10^2 to 7.6×10^6 CFU/egg, with a total mean of 4.2×10^5 CFU/egg.

TABLE 1
THE MEAN AND LIMITS OF THE TOTAL NUMBER OF BACTERIA POLLUTION ON THE EGG SHELLS TO EACH SECTOR

Means*	Upper limits	Lower limits	Sectors
2.59×10^4 b	5.7×10^4	1.5×10^4	Sector A
1.81×10^6 a	7.7×10^6	7.4×10^2	Sector B
5.87×10^4 b	3.2×10^5	1.5×10^3	Sector C
1.03×10^5 b	3.3×10^5	1.5×10^3	Sector D
7.65×10^4 b	2.8×10^5	7.5×10^2	Sector E
4.2×10^5	7.6×10^6	7.4×10^2	Means**

* Means counts with the different letters are significantly different at $p \leq 0.05$.

** Means for all Sectors

Figure 1 depicts, the frequency distribution of total bacteria counts on the eggshell revealed that the majority of the samples had a total bacterial count in the range of 10^3 to 10^4 (38.3% of the samples), followed by 10^4 to 10^5 (28.3% of the samples).

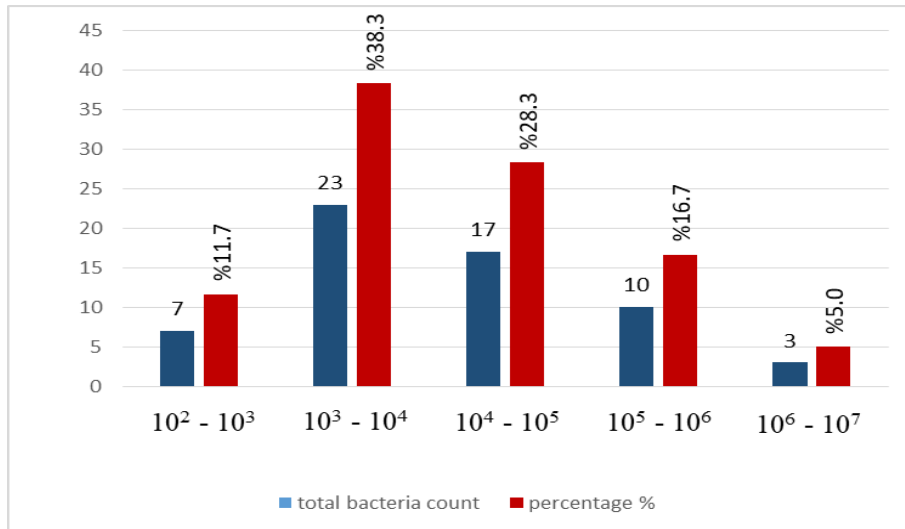


FIGURE 1: Frequency distribution of total bacteria counts on the eggshell

The mean number of coliform bacteria per egg (all sectors) was 3.85×10^4 CFU/egg, with a range of 6.5×10^2 to 4.0×10^5 CFU/egg (Table 2). Although there were no significant differences ($P < 0.05$) across all sectors in the coliform bacteria data (table 2).

**TABLE 2
AVERAGES AND LIMITS OF THE TOTAL NUMBER OF COLIFORM POLLUTION ON EGGSHELLS TO EACH SECTOR**

Means*	Upper limits	Lower limits	Sectors
2.05×10^4	5.6×10^4	6.5×10^2	Sector A
8.16×10^4	4.0×10^5	1.0×10^3	Sector B
2.56×10^4	5.0×10^4	9.0×10^2	Sector C
3.37×10^4	7.7×10^4	7.5×10^3	Sector D
3.13×10^4	7.7×10^4	7.5×10^3	Sector E
3.85×10^4	4.0×10^5	6.5×10^2	Means**

*Means counts with the different letters are significantly different at $p < 0.05$.

** Means for all Sectors.

The frequency distribution of the total number of coliform bacteria on the eggshells is shown in Figure (2), where it was discovered that 61.7 % of the samples had numbers of coliform bacteria at a level of $10^4 - 10^5$, which is a high percentage when compared to total bacteria counts.

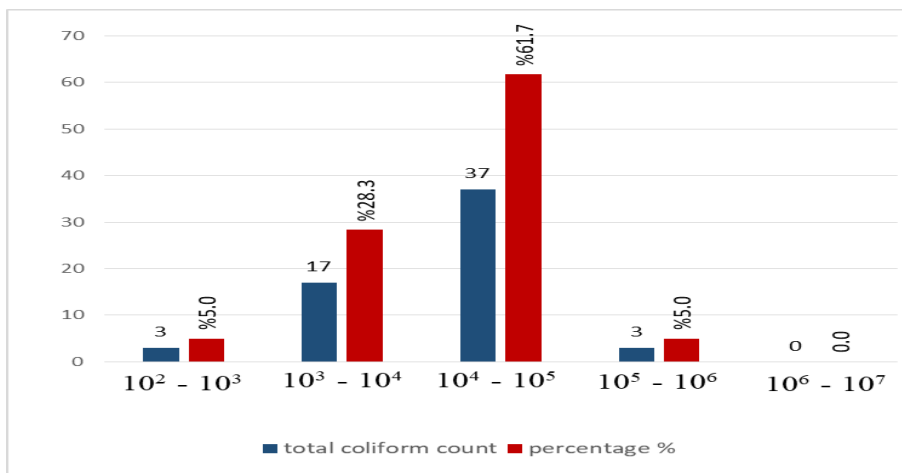


FIGURE 2: Frequency distribution of total Coliform number on the eggshell

Table 3 shows significant differences ($P < 0.05$) in the overall number of *Staphylococcus aureus* between the five areas. The average total number of *Staphylococcus aureus* CFU/egg was 4.4×10^5 CFU/egg, with the lowest and highest limits of the total number of *Staphylococcus aureus* CFU/egg being 1.0×10^4 and 2.5×10^6 CFU/egg, respectively.

TABLE 3
AVERAGES AND LIMITS OF STAPHYLOCOCCUS AUREUS BACTERIA POLLUTION ON EGGSHELLS TO EACH SECTOR.

Means*	Upper limits	Lower limits	Sectors
9.42×10^4 b	2.2×10^5	1.0×10^4	Sector A
7.52×10^5 a	2.5×10^6	2.0×10^4	Sector B
2.55×10^5 b	4.25×10^5	2.0×10^4	Sector C
5.60×10^5 ab	1.7×10^6	9.0×10^4	Sector D
5.96×10^5 ab	2.3×10^6	9.0×10^4	Sector E
4.41×10^5	2.5×10^6	1.0×10^4	Means**

* Means counts with the different letters are significantly different at $p < 0.05$.

**Means for all Sectors.

The frequency distribution of the total *Staphylococcus* number on the eggshell is shown in Figure (3). When compared to coliform bacteria, 60 percent of the samples were contaminated with *staphylococcus* bacteria at a level of 10^5 - 10^6 , it is a high percentage.

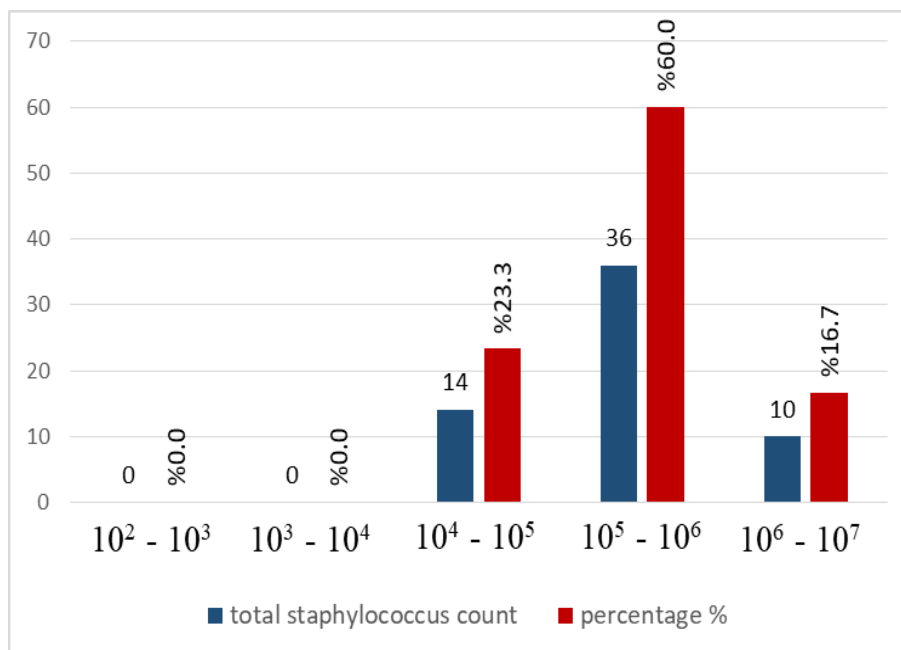


FIGURE 3: Frequency distribution of total *Staphylococcus* number on the eggshell

Table (4) displays the several types of Gram bacteria (positive and negative) that were isolated from eggshell samples sold in Benghazi marketplaces, as well as the percentage of each species. *E. coli* was found in 18 isolates, accounting for 36% of the total. *Escherichia coli* was found to be the most common species among the isolates. It was isolated from all sectors in various degrees. *Citrobacter* sp. was found in variable quantities and at 20% in ten isolates from various sectors. In the experiment, *Enterobacter* sp., *Proteus* sp., and *Streptococcus* sp. were found in 10% of the isolates. *Klebsiella* sp. and *Pseudomonas* sp. were the bacteria with the lowest number of isolates. They were discovered in two isolates at 4% each, while *Salmonella* sp. was found in three isolates at 6% each.

TABLE 4
PERCENTAGE AND TYPE OF ISOLATED BACTERIA OF EGG SAMPLES SOLD IN BENGHAZI MARKETS.

Identification	Sector A Isolates (%)	Sector B Isolates (%)	Sector C Isolates (%)	Sector D Isolates (%)	Sector E Isolates (%)	Number of isolation	Percentage of isolation
E. coli	6	3	2	4	3	18	36%
Enterobacter spp.	0	2	0	1	2	5	10%
Citrobacter spp.	1	3	3	2	1	10	20%
Klebsiella spp.	0	1	0	1	0	2	4%
Proteus spp.	2	0	1	1	1	5	10%
Pseudomonas spp.	1	0	1	0	0	2	4%
Salmonella spp	0	0	0	0	3	3	6%
Streptococcus spp	1	2	0	0	2	5	10%
Total	11(22 %)	11(22 %)	7(14 %)	9 (18 %)	12(24 %)	50	100%

IV. DISCUSSION

The results shown in (Table1) showed the range number of aerobic bacteria was 7.4×10^2 to 7.6×10^6 CFU/ egg, the total mean 4.2×10^5 CFU/egg. These results in agreement with several studies. The total aerobic count range of bacteria on eggshells was 2.9 to 6.2 \log_{10} CFU/ml (7.94×10^2 to 1.58×10^6 CFU/ml) in the market layer, according to Chaemsanit et al. [16]. In the same context, researchers stated the significantly ($P < 0.01$) higher average eggshell contamination by aerobic bacteria in eggs coming from alternative housing systems as compared to those coming from conventional ones, in particular, 5.46 (2.88×10^5) against 5.08 (1.20×10^5) \log_{10} CFU/ eggshell [17].

At the probability level of $p < 0.05$, statistical analysis of these results revealed that sector B has substantial differences in microbial contamination from the other sectors, but no significant differences between the other sectors. This demonstrates that despite considerable disparities between the components of each sector separately, there is no difference in the application of health conditions in the supply of eggs in all five sector stores. Enterobacteriaceae, our results are slightly higher than Folorunsho & Charles [18] results, they scored 0.9 to 1.8×10^2 CFU/ml in fresh, unwashed egg samples, while the number decreased to 1×10^2 CFU/ml in the washed eggs. Similarly, Yazji & Azizieh [19] found 1×10^3 CFU/ml.

The mean number of coliform bacteria per egg in our study ranged from 6.5×10^2 to 4.0×10^5 CFU/egg, with the mean being 3.85×10^4 CFU/egg. The number of coliform bacteria in the analyzed samples varied from 10^4 to 10^5 CFU/egg, suggesting significant levels of bacterial contamination. This suggests that hygienic requirements are not being followed in the farm or markets to keep eggs clean and free of contamination.

Stępień-Pyśniak (2010) found an elevated level of *Enterobacteriaceae* on table eggs and isolated 12 types [20]. Moreover, Abdel-Latif & Saad, (2015) reported that the upper limit of the population of *Staphylococcus aureus* was 8×10^8 and an average was 1.94×10^7 CFU /g. [21]. El- kholy reported that *Staphylococcus aureus* in 13% of positive samples [22].

Contamination of poultry sheds, water used for cleaning, and risky behavior of laying hen farm employees are the primary factors behind the occurrence and existence of E. coli, which contaminate eggshells. The bacteria Enterobacteriaceae, which included E. coli, Enterobacter spp., Klebsiella spp., Proteus sp., and Citrobacter spp., were the most infected eggshell isolates in the five sectors, accounting for 80 percent of the overall isolated and identified samples. The remaining three genera (isolated and identified) *Streptococcus sp.*, *Pseudomonas sp.*, *Salmonella sp.* is not from the Enterobacteriaceae family and has been isolated in other studies[20–28].

V. CONCLUSIONS

The findings of the study reveal that egg samples sold in Benghazi markets were contaminated with salmonella and that the presence of salmonella in the sector isolated bacteria (E), which is considered one of the most serious effects on community health in other areas of the world. The experiment discovered that in the city of Benghazi, customer-accessible table eggs are contaminated with a variety of germs, many of which are classified as foodborne diseases and are harmful to public health.

Employees' dangerous actions in poultry farms, during storage, and the surrounding environment all contribute to microbial contamination, putting the customer at risk of disease infection. One of the common tasks that healthcare institutions should implement is the development of effective and consistent safety measures in the city's marketplaces and businesses. In

addition, the producer and seller must store the eggs in special refrigerators at appropriate temperatures to prevent microbial contamination of the table eggs sold in markets. In a future study, we will investigate the role of microbial content on table eggshells in transmitting diseases to foods processed using it, the relationship between content density and the rate of food contamination, and the severity of public health.

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Climatic Variability and Agricultural Season: Analysis of the Evolution of Essential Agroclimatic Parameters in Central East Cote D'ivoire

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Abstract— *The relationships between rainfall indices and the increase in agricultural production in Côte d'Ivoire over the period 1980-2010 do not show significant results of the current climatic trends. The study of the beginnings of the agricultural season of the "old cocoa-loop" highlights the rainfall variations that have persisted since the 1970s, and exposes rainfed agriculture to climatic risks. In addition, both daily and seasonal rainfall data collected from 1961 to 2010 were analyzed to reveal the current rainfall trends. It is worth to note that the simple study of the chronological series of the effective installation of precipitations shows both a delay and sudden fluctuation of the agricultural seasons throughout the region under investigation, at the end of the 1960s and the beginning of the 1970s. Other variables such as "the duration and the end" of the season, allow a more "qualitative" characterization of the investigated phenomenon. They provide additional information regarding both the manifestations of the rainfall variability and the confirmation of the importance of the phenomenon. The risks to which rainfed agriculture is exposed in relation to the current rainfall conditions are: disruption of the agricultural cycle, loss of seeds, and reduction in yield.*

Keywords— *climatic variability, agricultural season, Central-East of Côte d'Ivoire.*

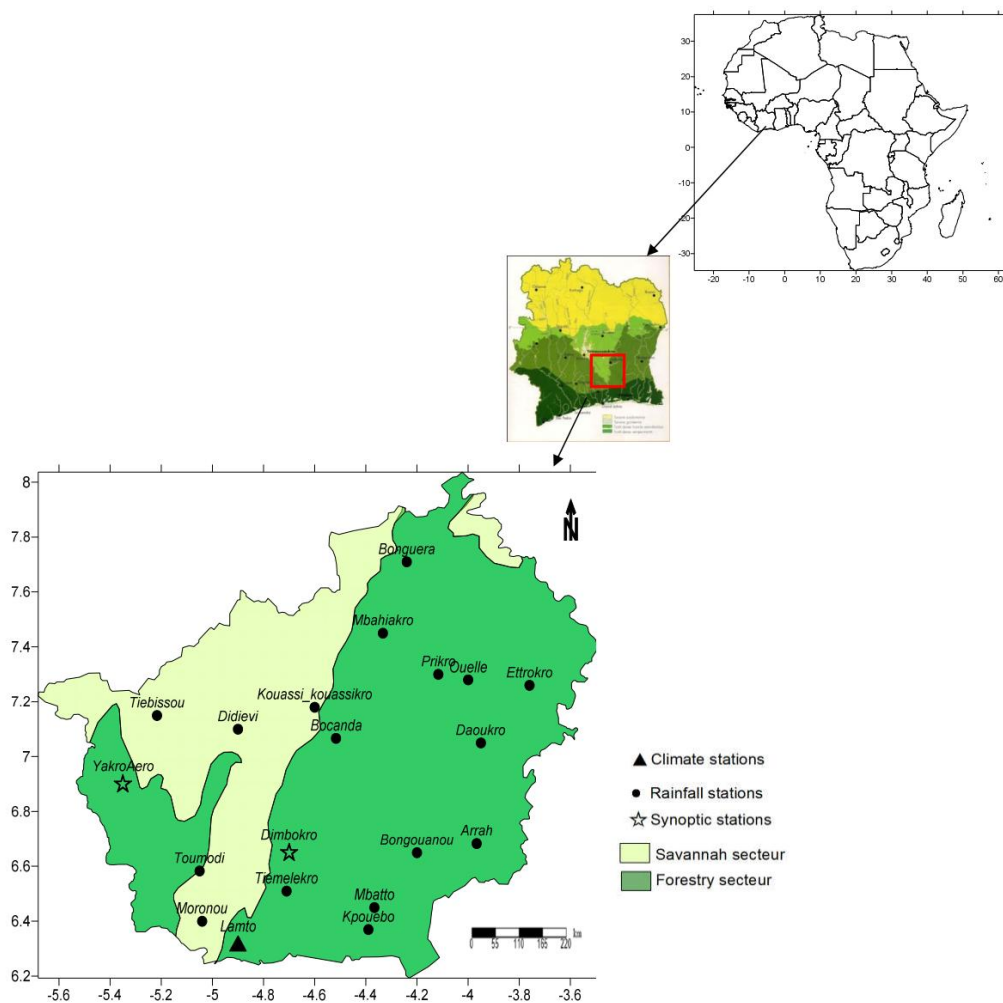
I. INTRODUCTION

Agriculture is no longer the main source of money income in many rural areas of West Africa owing to the low agricultural productivity due to climatic hazards. Even with the gradual return of rainfall, it is probable that this trend will continue. Climatic hazards are a real concern for all West African countries owing to their adverse socio-economic and environmental effects. Interannual fluctuations in rainfall represent the main climatic hazard for West African countries such as Côte d'Ivoire, whose economy is principally based on agriculture [1]. This is of the pluvial type, i.e. dependent on rain, which is the most variable climatic element. The decline in rainfall that began in the Gulf of Guinea countries at the end of the 1970s, became more intense in Côte d'Ivoire during the 1980s and 1990s before experiencing a certain remission during the 2000s [2,10]. Thus, these rainfall recessions induce variability in agricultural calendars through the variation of start and end dates, as well as the duration of the seasons [3,9]. Therefore, the fundamental question that emanates from these observations is the following: what are the variations of the major agroclimatic parameters in the Center-East of Côte d'Ivoire? Thus, this study aims to show the modifications of the major agroclimatic parameters of the agricultural calendars in the Center-East of Côte d'Ivoire between 1971 and 2020. Once prosperous and a major center of rural immigration, the east-central part of Côte d'Ivoire has experienced an inversion of the situation in recent years, which has made it an important center of emigration. The decline in planting activity can be observed both in terms of areas and in terms of production [4,9]. In recent decades, the Center-East of Côte d'Ivoire has suffered a drop in rainfall marked by droughts [5,10]. The economic consequences of such climatic and ecological changes are dramatic in this region mainly oriented towards farming [6]. The irregularity of the temporal distributions of precipitations and the heterogeneity of their spatial distribution constitute today the essential characteristics of the pluviometry of this region. The analysis of rainfall over the three sub-periods 1971-2000, 1981-2010 and 1991-2020 shows that agriculture has been going through a rainfall crisis for five decades now. The typical aspects of rainfall variability are its persistence and amplitude [7]. The impact of this phenomenon has been observed through the drying up of the majority of water points, the rotting of seedlings, a reduction in arable land, lower yields and the resurgence

of diseases (human, plant and animal). Thus, entire regions have become more vulnerable than in the past, thus weakening their agricultural future and accentuating the process of deforestation [8]. In addition, this has imposed on them transformations in their lifestyles, regardless of the economic sector considered [9,7]. These characteristics give the climate a determining role in the development of the agricultural season and in agricultural production. Thus, given their immediate and lasting repercussions on agriculture and on humans. This variability leads to great uncertainty on the date of sowing which, on the same site, can occur over a period of nearly two months depending on the onset of the rains [10]. Our interest as a geographer aims to assess the extent of the evolution of rainfall in the Center-East in order to reveal its influence on the agricultural season. In agriculture, it has been shown that farmers who are convinced of the existence of climate variability have better capacities to manage risk and change. They are likely to anticipate it, learn and organize themselves and they are more willing to modify their systems [8,9]. Hence, an understanding of this climatic phenomenon could at least help to program interventions aimed at stabilizing and increasing yields [11].

II. AREA OF STUDY

The study is located in the ecological zone of the forest-savannah contact or "V Baoulé". It is located between longitudes 3° and 6° West and latitudes 6° and 9° North. It includes the administrative regions of Iffou, N'Zi, Moronou and the northern part of the Bélier region (Figure 1). Agro-economically, this area has been known as the "cocoa loop". With a climate combining the humid tropical (two wet and two dry seasons) and the dry tropical (one wet and one dry season), the studied area records temperatures oscillating between 14 and 33°C, humidity varying between 40 and 70% and an average annual rainfall of 1100 mm. It is a vast ensemble (the altitudes vary little from 80 m to 120 m) whose monotony is interrupted to the east by the hills of Bongouanou whose highest peak culminates at 615 m. The study area is characterized by an environment of both savannah and forest and is part of the mesophilic sector of the Guinean domain in Côte d'Ivoire [12].



Source : SODEXAM, 2019

FIGURE 1: Geographical location of the study region and characteristics of rainfall stations (Central-East of Côte d'Ivoire).

III. MATERIALS AND METHODS

3.1 Data collection

In order to characterize the essential agroclimatic parameters, daily rainfall records from 11 observation posts in the center-east of Côte d'Ivoire over the period 1971-2020 were used (SODEXAM, 2020). These positions were chosen because of the regularity of the observations. Potential Evapotranspiration (ETP) and soil water retention capacities or Useful Reserve (RU) as defined by Perraud (1971) were used. The RU values should be considered as indicative orders of magnitude for a study across Côte d'Ivoire. In addition, the average ETP of 4 mm per day was considered. Missing values were filled in using the average of neighboring stations in order to have complete, homogeneous and long series.

3.2 Analysis method

The start and end dates of the agricultural season were determined by taking into account the amount of rain, the value of potential evaporation (ETP) and the useful reserve of the soil. The various parameters (start dates, end dates and duration) of the growing season were calculated over the period from 1971 to 2020 with the reference period 1971-2000. On this basis, to characterize the climatic variability in the Center-East of Côte d'Ivoire, the rainfall normals for the periods 1981-2000 and 1991-2020 are analyzed and compared with the reference period. These time scales are retained in order to understand the evolution of the agricultural calendar of the study period (1971-2020). The duration of the agricultural season is calculated by deduction between the start date and the end of the season. The date of the beginning of the agricultural campaign is determined as soon as an accumulation of rainfall of 20 mm is recorded in one or two successive days and provided that no sequence of 10 days without rain is observed in the 30 days which follow this accumulation. The choice of the 30-day period makes it possible to prevent false starts to the agricultural campaign which are a constraint to the installation and development of crops [13,7]. The average start date of the 1971-2000 reference period in our study area is the 83rd day of the year, i.e. March 23. Due to the food characteristic of the Center-East, the last useful rain in our study region should above all allow the harvest of the yam in good conditions and, possibly, the plowing at the end of the cycle. Hence, the average end of the agricultural season is located from the 320th day of the year, i.e. November 15, when the soil water reserve reaches 0.05 mm [13]. The determination of dry and wet rainfall sequences makes it possible to assess the quality of the agricultural season, in terms of good spatial and temporal distribution of rainfall and sufficient quantity to positively impact the progress of agricultural activities. A start is considered early when it occurs two weeks before the average date. It is also considered late when it occurs two weeks after the average date. Similarly, an end is considered early when it occurs two weeks before the average date and is considered late when it occurs two weeks after the average date [14]. The cumulative rainfall heights recorded during the season made it possible to obtain the seasonal rainfall height by calculating the cumulative frequency using the equation (1) [15]:

$$F(x) = \frac{i-0,5}{N} \quad (1)$$

With, i = rank of the observation and n = number of observations (number of years retained). This formula made it possible to better determine the level of instability at the start and end of the agricultural season in the area under investigation.

IV. RESULTS

4.1 Differences in agroclimatic parameters and statistical significance of Averages

4.1.1 Rainy season start dates

The analyzes of the start dates of the big season evolve irregularly without any apparent periodicity. Thus, the beginnings of the late and early seasons alternate at unequal and unpredictable intervals (Table 1).

TABLE 1
AVERAGE DISTRIBUTION OF START DATES AND DIFFERENCE IN AGRICULTURAL SEASON (1971-2000; 1981-2010; 1991-2020) BY LOCALITY YAKRO AERO=YAMOUSSOUKRO

Start of the season	1971-2000	1981-2010	gap (days)	1981-2010	1991-2020	gap (days)	1971-2000	1991-2020	Gap (days)
Arrah	18 mars	21 mars	3	21 mars	29 mars	8	18 mars	29 mars	11
Bocanda	25 mars	30 mars	5	30 mars	4 avril	5	25 mars	4 avril	10
Bongouanou	20 mars	26 mars	6	26 mars	6 avril	11	20 mars	6 avril	17*
Didiévi	27 mars	30 mars	3	30 mars	6 avril	7	27 mars	6 avril	10
Dimbokro	23 mars	26 mars	3	26 mars	2 avril	7	23 mars	2 avril	11
Mbahiakro	25 mars	1 ^{er} avril	7	1 avril	4 avril	3	25 mars	4 avril	10
Mbatto	24 mars	29 mars	5	29 mars	5 avril	7	24 mars	5 avril	12
Prikro	25 mars	27 mars	2	27 mars	1 ^{er} avril	5	25 mars	1 ^{er} avril	7
Tiébissou	22 mars	4 avril	13	4 avril	7 avril	3	22 mars	7 avril	16*
Toumodi	24 mars	28 mars	4	28 mars	6 avril	9	24 mars	6 avril	13
YakroAero	23 mars	27 mars	4	27 mars	5 avril	9	23 mars	5 avril	13

*Source : SODEXAM, 2019; *Écarts statistiquement significatifs*

The installation of the date of the average start of the rainy season varies according to the time periods of analysis. Based on the thirty-year periods, the average starts of the rainy season (March 23 between 1971 and 2000) experiences a shift in space and time in the study region. . This shift reflects a clear and generalized decrease in annual rainfall and the late start of agricultural activities in the region (April 1 to April 7 between 1991 and 2020). The month of March was the most affected in all the stations in the region? The onset of the rainy season went from March 23 (1971-2000) to April 4 (1981-2010) and April 7 (1991-2020) respectively on average. On average, the start date of the rainy season between 1971 and 2020 is 15 days late. The analysis of the dates of the beginning of the rainy season over a long series shows strong temporal and spatial variability in the Center-East of Côte d'Ivoire. This phenomenon is more marked in the savanna region (Mbahiakro, Tiébissou, Toumodi and Yamoussoukro). Such a development renders the cropping calendars determined on the basis of empirical knowledge ineffective. There is, in fact, a persistence of the water deficit around 1990 in the two cases of analysis, which is confirmed in the analysis of the evolution of the third sequence (1991-2020). The first two normals are marked by a west-east direction shift in the study area. This means the increasingly late start of the normal season (1971-2000) and a renewal of the rainfall for the decade (2000-2010); this could be accompanied by a good condition for plant productivity. The month of March (beginning of the long rainy season) seems to be very affected by the rainfall recession (Table 1). June remains the wettest month. The wettest months remain March, April, May and June, which saw a regression in the amount of precipitation. Such characteristics jeopardize the smooth running of the agricultural campaign, the calendar of which is set according to average values. This instability and decline in the trend indicate a late start to the agricultural season. In 90% of cases, that is to say one year out of 10, the start date is prior to or equal to March 15 for all stations in the region. In other words, four out of five years, the start date of the season is after March 15.

4.1.2 Rainy season end dates

Like the beginning of the seasons, the analysis of the dates of the end of the rainy season show a change and a precocity during the period considered from 1971 to 2020 (Table 2):

TABLE 2
AVERAGE DISTRIBUTION OF END DATES AND DIFFERENCE IN AGRICULTURAL SEASON (1971-2000; 1981-2010; 1991-2020) BY LOCALITY YAKROAERO=YAMOOUSSOUKRO

End of the season	1971-2000	1981-2010	gap (day)	1981-2010	1991-2020	gap (day)	1971-2000	1991-2020	gap (day)
Arrah	20 nov	10 nov	- 10	10 nov	5 nov	-5	20 nov	5 nov	- 15*
Bocanda	30 oct	14 nov	+15	14 nov	2 nov	-12	30 oct	2 nov	+3
Bongouanou	20 nov	13nov	-7	13 nov	29 oct	-15	20 nov	29 oct	-22*
Didiévi	16 nov	5 nov	-11	5 nov	14 nov	-11	16 nov	14 nov	-22*
Dimbokro	19 nov	8 nov	-11	8 nov	9 nov	1	19 nov	9 nov	-10
Mbahiakro	14 nov	9 nov	-5	9 nov	22 oct	-18	14 nov	22 oct	-23*
Mbatto	13 nov	4 nov	-9	4 nov	15 nov	+11	13 nov	15 nov	+2
Prikro	22 nov	8 nov	-14	8 nov	7 nov	- 1	22 nov	7 nov	-15*
Tiébissou	16 nov	1 nov	-15	1 nov	18 oct	-14	16 nov	18 oct	-29*
Toumodi	20 nov	18 nov	-2	18 nov	17 nov	- 1	20 nov	17 nov	-3
YakroAero	20 nov	6 nov	-14	6 nov	16 nov	+10	20 nov	16 nov	-4

Source : SODEXAM, 2019.oct.= octobre ; nov. =novembre; Note : * Écarts statistiquement significatifs

There is a fluctuation of the end of season date throughout the study region. Considering the evolution of the date of November 15, it turns out that there is a variation in the end of the season date and that it would also be earlier and earlier. The analysis of the average values (1971-2020) shows an inequality in the spatial distribution of rainfall in Central Côte d'Ivoire. Indeed, at equal latitude, the savannah sectors (Tiébissou, Prikro, Didiévi, Ouéllé and Kouassi-Kouassikro) experience a sudden end-of-season shutdown (310th day of the year, i.e. October 30). In the two major ecological regions, forest and savannah, the end of the season is more spread out (i.e. between October 22 and November 16). For Anhuf (1993), the northern limit of the dense humid forest is reached at an annual rainfall level of about 1000 mm. Similarly, the distribution of the most important daily rainfall events follows this same north-west/south-east gradient. In the Center-East region, the rainfall regimes have undergone significant changes that result in decreases in precipitous annual heights that can reach 20 to 25%. This drop in rainfall affects each month whether it is dry or wet. There is also a trend in many savannah areas to move from a more humid climatic regime called "Guinean" to a drier regime [17,18]. The end of the agricultural season is generally less spread out over time (1st decade of October).

4.1.3 Rainy season durations

Analysis of the duration of the rainy season shows that it varies from one time scale to another (Table 3):

TABLE 3
AVERAGE DISTRIBUTION OF DURATION DATES AND DIFFERENCE IN AVERAGE DAYS OF DURATION OF THE AGRICULTURAL SEASON (1971-2000; 1981-2010; 1991-2020) BY LOCALITY YAKROAERO=YAMOOUSSOUKRO

duration of the season	1971-2000	1981-2010	gap (day)	1981-2010	1991-2020	gap (day)	1971-2000	1991-2020	gap (day)
Arrah	171	170	-1	170	171	+1	171	171	0
Bocanda	142	160	-18	160	161	+1	142	161	-17*
Didiévi	159	164	-5	164	161	-3	159	161	-8
Bongouanou	169	168	-1	168	149	-19	169	149	-20*
Dimbokro	165	156	-9	156	153	-3	165	153	-11
Mbatto	160	165	+5	165	165	0	160	165	+5
Tiébissou	159	141	-18	141	147	+6	159	147	-12
Prikro	154	148	-6	148	141	-7	154	141	-13
YakroAero	165	145	-20	145	141	-4	165	141	-24*
Toumodi	165	163	-2	163	162	-1	165	162	-3
Mbahiakro	163	146	-17	146	141	-5	163	141	- 22*

Source : SODEXAM, 2019 ; Note : * Écarts statistiquement significatifs

The examination of the interannual variability according to the 3 sub-periods 1971-2000, 1981-2010 and 1991-2020 helps to better appreciate the trend of the average duration in the Center-East of Côte d'Ivoire. The average length of the rainy season is 164 days, or 16 and a half decades in 1971-2000 throughout the study region. However, this average hides disparities in each ecological zone.

The ends of season dates are early for 98% of positions after the break years, i.e. the entire study region. The longest gaps (greater than 20 days) were observed in Mbahiakro, Bongouanou and Yamoussoukro. These results agree with those of Dekoula et al. (2018) who studied the variability of intra-seasonal rainfall descriptors with agricultural impact in the cotton basin of Côte d'Ivoire. However, these authors used time series from 1950 to 2000. Goula Bi et al. (2010) also obtained at similar discrepancies for the start dates, end dates and lengths of agricultural seasons in Côte d'Ivoire between the periods 1951-1980 and 1971-2000. Thus, this study is an update of the variability of agricultural calendars in the Center-East of Côte d'Ivoire. These updated results also show the persistence of the reduction in season lengths until 2020. During the period (1971-2000), in the savannah sector (Mbahiakro, Prikro, Didiévi, Tiébioussou), the rainy season lasted an average of 160 days in decades. Sometimes, it covers only 147 days on average between 1991 and 2020. In the two ecological zones (forest and savannah), the average duration of the rainy season between 1971 and 2000 which is 158 and 152 days increases respectively to between 1991 and 2020 at 167 and 162 days of rain. Indeed, the impact of this early end seems unremarkable in the localities of Arrah, Didiévi and Toumodi, in the other localities. It negatively impacts the length of the rainy season with an average reduction of 20 days in Bongouanou, 22 days in Mbahiakro, 24 days in Yamoussoukro, 17 days in Bocanda and 13 days in Prikro. A season length of less than 150 days appears in the forest region and extends to the vicinity of Bongouanou and Mbatto. On the other hand, the relative improvement in annual rainfall totals observed since the 1990s has not yet led to an improvement in the length of the agricultural season, which has still remained low. This new trend reflects the manifestation of migration from the subequatorial bimodal regime to a Sudanese monomodal regime.

V. DISCUSSION

The analysis of the evolution of climatic parameters in the Center-East of Côte d'Ivoire are in agreement with the conclusions of the work carried out in West Africa in general [17,18,19] and in Côte d'Ivoire. Ivory in particular [20,21]. The disturbances of the agricultural season determined during this study are generally after 1970 and confirm previous studies [10,15,22]. In forestry, the month of July generally marks the end of the growing season. However, in the savannah region the rains seem to stop late during the period 1970-2010. This apparently late end is in fact due to the transfer of the rains from the short rainy season to the months of July and August and reflects global changes in the distribution of precipitation in this region. We are thus witnessing a real change in the rainfall regime characterized by the progression of the tropical climate to this zone classified as a Subequatorial climate. As such, we share the opinion of Brou taken up by Noufé who specifies that: the precipitation deficit is accompanied by a modification of the distribution in the direction of a transition to a tropical climate [28,6]. To measure the extent of the temporal disturbances of the parameters of the agricultural season, a farmer says: "The yam harvest, which used to be done all year round, now only takes place between October and January due to poor production. The early cessation of the small rainy season rains reduces the possibility of maize bearing cobs, and if it does, those cobs are grainless." It also results from these balances that the agricultural campaign is badly affected insofar as the average length of the season is less than the duration of the vegetative cycle of the crop species (yam).

In Benin, the work of certain authors has also shown that the quality of the agricultural seasons has seriously deteriorated over the past few decades [23,25]. The length of agricultural seasons is getting shorter and shorter. Similarly, false starts and rain interruptions at the heart of the season are increasing. The analysis of the length of the season confirms a very high variability from one normal (30 years) to another and a reduction in the agricultural season throughout the study area. The significant reduction in precipitation appears in the form of episodes of strong deficits in 1972-1973, 1982-1984 and 1997 also seen elsewhere in the Sudano-Sahelian zones [24,26,30]. Regarding the end of the season, the results of this study coincide with those of Sivakumar and Paturel, the great intra-annual irregularity of rainfall along the Gulf of Guinea [24,26]. Thus, there is a disturbance in the end and the period of the seasons which reveals a shortening of the rainy season in favor of the dry season which is lengthening [7,20], in phase with the drop in precipitation which has intensified. in Côte d'Ivoire as in the other countries of the Gulf of Guinea during the 1980s and 1990s [28]. This observation was also made in a similar study carried out by Ourega and Biemi in the N'zi-Comoé region [29]. For these authors, since the 1970s, because of the droughts that marked the sub-region, the rainy season presents an accentuated variability which compromises the implementation of agricultural activities whose calendars are modeled on the average rainfall conditions. This finding confirms the fact that in northern Benin, agriculture and livestock are the two human systems most exposed to climate risks [15,24,25]. The probabilities of the end of season date also show a late end of the season in the Sahel [32]. Similarly, the analysis of the

evolution of the number of rainy days in August reveals a disappearance for all stations during the same period. This drop in rainfall, accompanied in West Africa by one of the worst droughts during the 1970s-1990s, is a sign of climate variability, the frequency and intensity of which have increased over the past 30 years [30,26]. Although these stations are few and scattered, the results reflect the regional behavior of the agricultural season in the area studied, from one locality to another, thus confirming the poor annual production recorded for three decades [31,33].

VI. CONCLUSION

The study of the evolution of the rainfall regime in the former Cacao loop in Côte d'Ivoire shows that seasonal variations are associated with a modification of agroclimatic parameters such as the beginning, the end and the duration of the agricultural season. There is a decrease in the length of the season, which has gone from 4 months to 3 months, a delay in the effective onset of the rains and a virtual disappearance of the short rainy season. End of season dates vary little in space and time. While it was believed for a long time to be confined to the savannah region, this analysis showed that the rainfall deficit also affected the forest regions and, more generally, the so-called "wet" region. This fall in rainfall has, of course, consequences on the regimes of watercourses and therefore on the availability of water resources, key to the success of many development projects.

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Effectiveness of Some Chemical and Biological Pesticides against *Sitophilus zeamais* (Motschulsky)

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Abstract— The study on “Effectiveness of some chemical and biological pesticides against *S. zeamais*” was carried out at National Entomology Research Center, NARC, Khumaltar, and Lalitpur. This study was carried out to find the residual effect of the pesticides on *S. zeamais* mortality. Each pesticide was applied in 3 concentrations.: Emamectin Benzoate @ 0.3ml/ltr, @0.1ml/ltr and @0.6ml/ltr, Neem @ 5ml/ltr, @2.5ml/ltr and @10ml/ltr, Chloropyrifos (50%) + Cypermethrin (5%) @ 1.5ml/ltr, @0.75ml/ltr and @3ml/ltr; and Malathion @ 2ml/ltr, @1ml/ltr and 4ml/ltr. The residue of pesticide on weevil mortality was seen the highest on Chloropyrifos (50%) + Cypermethrin (5%) till the 87th Day and was least on Neem even on the 1st day of observation. The mortality % was highest (100%) on Chloropyrifos (50%) + Cypermethrin (5%) and Malathion and was lowest (0%) on Neem. The maximum weight loss was observed on Neem @2.5ml/ltr which was 9.4% whereas, minimum wt. loss was observed on Chloropyrifos (50%) + Cypermethrin (5%) @ 3ml/ltr which was 0.25% of the total grain weight. The maximum percent of damaged grain was observed on Neem which was 100% while the minimum percent of damaged grain was observed on Chloropyrifos (50%) + Cypermethrin (5%) @1.5ml/ltr which was 11.21% of the total grain. No weevil progeny emerged from Chloropyrifos (50%) + Cypermethrin (5%) @1.5ml/ltr treated seeds whereas the maximum number of progeny emerged from Neem @2.5ml/ltr treated seeds which were 149.67. Out of the 4 pesticides tested on the adult of *Sitophilus Zeamais*, Chloropyrifos (50%) + Cypermethrin (5%) was found to be most effective while Neem was the least effective. Since the residual of the chemical pesticides are long lasting, it is not recommend to use pesticides for consumption but can be used to store for seed purpose.

Keywords— *S. zeamais*, mortality, biological pesticide, weevil, maize.

I. INTRODUCTION

Maize development is a lifestyle for most farmers in the slopes of Nepal. It is a traditional crop which is cultivated as food, feed, and fodder on inclining land which is rain-fed upland in the hills. It is developed under downpour took care of conditions throughout the mid-year (April-August) as a solitary yield or transferred with millet later in the season. In the terai, internal terai, valleys, and low-lying river basin regions, maize is likewise grown in the winter and spring with irrigation system (Paudyal et al., 2001). Different cereal harvests have assumed significant parts intending to food security issues in Nepal. Lately, there have been vacillations in crop production and demand situations because of different reasons (Gairhe et al. 2018). Maize is the second most significant yield after rice as far as region and production in Nepal. It is a lifestyle for the farmers of the hilly area in Nepal. It is a traditional yield developed for food, feed, and fodder. Maize demand has been continually developing by about 5% yearly in last decades. Per capita, maize consumption in Nepal was 98 g/individual/day. The stored maize is attacked and harmed by a few pests that lead to quality fading driving farmers to sell at scaled down costs and underneath the production cost. Insect-Pests are frequently viewed as the main reason for maize grain

losses. The main pests that cause harm to maize in the field and capacity are Lepidopterist stalk borers and Coleopterans weevils, respectively. In excess of 37 types of arthropod pests are related with maize grain in storage. During the storage time frame, insects -pests and diseases assume a huge part in diminishing production and productivity combined with germination potential (Alam et al., 2019). Among pests, maize weevil (*Sitophilus zeamais*) and Angoumois grain moth (*Sitotroga cerealla*) were the main pests found in stored maize in Nepal. This happens on the grounds that the majority of the maize produced by farmers stays on the open floor of their room without keeping up appropriate storage standards. The primary reason for this is farmer's absence of sufficient information in regards to the situation with insect pests in a stored condition. (Alam et al., 2019).

S. zeamais Motsch stays quite possibly the most serious and internal feeding pests of maize in storage (Pameru et al., 1997). It falls among the most dangerous pests in stored grain, particularly maize in tropical areas. Grown-up female of weevils causes harm by drilling into the kernel and laying eggs (ovipositing) (Longstaff., 1981). The larvae and pupae eat the inward pieces of the kernel, which brings about a harmed kernel and diminished grain weight. The pervasion boosts temperature and dampness content in the stored grain mass, which can prompt fungal growth, including toxigenic species, for example, *Aspergillus favus* Link. *S. zeamais* can cause an extensive loss in quality and amount of the grain on the field just as in the storage. (Bhusal and Khanal., 2019).

There have been different sorts of insecticides that have been suggested for the control of storage pests in Nepal (Neupane, 2000). In any case, direct utilization of such insecticides is neither relevant nor doable (Mallah et al., 2018.). The chemical control is compelling, quick, secure, and conservative yet it has some significant downsides: such as adverse consequence on products and surrounding environment; the steady peril of intoxication for people and animals; the presence of residue in various pieces of the plants; (RÖMBKE J et al., 2000). Disposal of these downsides should be possible by utilizing some fewer contaminating insecticides, from the IIIrd and IVth groups of toxicity, and by utilizing efficient dosages, as least as possible (Porca et al., 2003). Some storage gain can be protected with chemical and biological pesticides for seed and feed purposes. Therefore, this study has been devised to study the effective pesticides and their residual effects on mortality of maize weevil along with the grain damage assessment.

II. MATERIALS AND METHODS

The following experiment was conducted in the laboratory of the National Entomology Research Center of NARC, Khumaltar, and Lalitpur, Nepal. Rearing of *S. zeamais* was performed in a laboratory setting by maintaining appropriate temperature, and sanitary conditions. Firstly, about 1 kg of healthy, dry and pest-free maize of mixed variety was selected. For rearing *Sitophilus zeamais* (Maize weevil), a total of 5 cylindrical glass jar 16 cm × 8 cm were used. These cylindrical glasses were filled with 300gm of a mixed variety of maize in each vessel. Fifty *S. zeamais* each (without separating male and female) were kept in each vessel for mating. Black muslin cloth of suitable length was used to cover the open end of the cylindrical glass jar. After a week the fifty *S. zeamais* which were kept for mating were removed from each of the vessels and the rearing of *S. zeamais* was started. After 30-35days, adult *S. zeamais* started to emerge. The age of the weevil used in the experiments was of 1-7 days of age.

For the implementation of the experiment, 60 small, clean cylindrical plastic container was taken and labelled Manakamana-4 maize variety. Fifty gram each of maize grain was placed in those 60 containers and was covered with perforated lids. Chemical pesticides and bio-pesticides were selected and prepared in appropriate quantity according to the requirement for the experiment. Altogether, there were 4 treatments with 3 concentrations with 4 replications for each experiment. One treatment was assigned as control (Table 1). Each container with maize grain was treated with a particular dose of treatment and wait until the inoculation of test insect. On the 5th day, 10 weevils each was placed into each container and mortality was observed at the interval of 48hrs. Ten new weevil were added to each container after 5 days interval discarding the previously added dead/ alive until 25th Day. In the later stage, 10 weevils were added to each container at the interval of 10 days. During this process, the weevils were discarded after each observation. This experiment was conducted for 87 Days.

After the completion of an experiment to find the residual effect of pesticide on weevil mortality, the data to observe the weight loss, damaged grains and no of weevil progeny were taken which was a week after the final observation for weevil mortality of treatment 3 and 4 and 67 days after the final observation for weevil mortality of treatment 1 and 2.

TABLE 1
DESCRIPTION OF DIFFERENT INSECTICIDE USED IN THE EXPERIMENT AGAINST *SITOPHILUS ZEAMAI*S

SN	Trade name	Active Component	Formulation type	Dose1 (ml/ltr)	Dose2 (ml/ltr)	Dose3 (ml/ltr)
1	Top Killer	Emmamectin Benzoate 5.7% WDG	Water dispersible granule	0.3	0.1	0.6
2	Neem pro	Neem oil	Thick oil	5	2.5	10
3	G-Sunami	Chloropyrifos 50%+ Cypermethrin 5%EC	Liquid	1.5	0.75	3
4	Plant Malathion	Malathion 50% EC	Liquid	2	1	4
5	Control	Water	Liquid	5		

The weight loss percentage was determined by the following formula:

$$\text{Weight loss \%} = \frac{W_1 - W_2}{W_1} * 100\% \quad (1)$$

(Ngatia and Kimondo, 2011)

Similarly, the percentage of damaged grains was determined by the following formula:

$$\% \text{ damaged grain} = \frac{\text{No. of damaged grains}}{\text{Total no of grains}} * 100\% \quad (2)$$

The data was managed in the MS. EXCEL file. Later, two-way ANOVA was used to compare the mortality caused by different treatments and concentrations of different pesticide. Weight loss, damage % and the number of weevil progeny were also subjected to two-way ANOVA. The means were compared using Turkey HSD Test at 0.05 significance level (SPSS Inc., Chicago, II, USA).

III. RESULTS AND DISCUSSION

The residual effect of different concentration of pesticides was found significantly different after twelve days to sixty-seven days for weevil mortality (Table). Among the tested chemicals, the residue of pesticide on weevil mortality was seen the highest on Chloropyrifos (50%) + Cypermethrin (5%) till the 87th Day. The residue of pesticide on weevil mortality was least which was (0%) with no mortality of any weevil population for all three concentrations of Neem even on the 1st day of observation. i.e., on the 7th Day. The maximum mortality for control treatments was observed on the 22nd day which was 17.5% whereas the minimum mortality was 0% for all other days. Mortality percent of different concentrations was highly significant for the 12th, 17th, 22nd, 27th, 37th, 47th and 67th day among the concentrations ($P < 0.01$) (Table 2). Similarly, the mortality percent were not significant for the 7th, 57th, 77th and 87th Day among the concentrations $P (> 0.01)$. The mortality percent of different treatments were highly significant for all the observations made on the 7th Day till the 87th Day among the treatments ($P < 0.01$). Interaction of concentration and pesticides was highly significant for the 12th day, 37th day, 47th day and 67th day of observation among the different conc \times trt ($P < 0.01$). The residual effect for emamectin benzoate was evident upto 27 days (Figure 1), for chloropyrifos+cypermethrin and malathion was 87 days.

TABLE 2

MEAN MORTALITY PERCENTAGE (\pm SE) OF *SITOPHILUS ZEAMAI* DUE TO THE RESIDUAL EFFECT OF VARIOUS CHEMICAL AND BIOLOGICAL PESTICIDES TREATMENTS ON DIFFERENT DAYS

Pesticide	Seven	Twelve	Seventeen	Twenty two	Twenty seven	Thirty seven	Forty seven	Fifty seven	Sixty seven	Seventy seven	Eighty seven
Emmamectin Benzoate	12.5c \pm (4.01)	1.67c \pm (1.12)	2.5c \pm (1.30)	3.33d \pm (1.88)	0d	0d	0c	0c	0c	0c	0c
Neem	0d	0d	0d	0e	0d	0d	0c	0c	0c	0c	0c
Chloropyrifos (50%) + Cypermethrin (5%)	99.17b \pm (0.83)	83.33a \pm (2.24)	64.17a \pm (4.51)	56.67b \pm (4.97)	47.5b \pm (5.09)	70a \pm (4.08)	66.67a \pm (3.55)	5ab \pm (1.94)	50.83a \pm (4.16)	7.5b \pm (2.17)	4.17a \pm (1.48)
Malathion	100a	68.33b \pm (3.85)	61.67b \pm (5.05)	65.83a \pm (6.45)	51.67a \pm (7.86)	40b \pm (8.70)	38.33b \pm (9.03)	8.33a \pm (2.70)	30.83b \pm (9.49)	8.33a \pm (4.05)	1.67ab \pm (1.12)
Control	0d	0d	0d	5.83c \pm (3.98)	2.5b \pm (1.79)	2.5c \pm (1.79)	0c	0c	0c	0c	0c
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.005
Concentration	0.33	<0.01	0.009	<0.01	0.006	<0.01	<0.01	0.028	<0.01	0.299	0.499
(Treatment * Concentration)	0.359	<0.01	0.124	0.271	0.021	<0.01	<0.01	0.057	<0.01	0.028	0.974

TABLE 3
MEAN PERCENTAGE (\pm SE) OF WEIGHT LOSS OF MAIZE GRAINS, PERCENTAGE OF DAMAGED GRAIN (\pm SE) AND MEAN NUMBER (\pm SE) OF WEEVIL PROGENY IN DIFFERENT TREATMENTS OF CHEMICAL AND BIOLOGICAL PESTICIDES.

Treatment	weight loss	% Damaged grains	Weevil Progeny
Emmamectin Benzoate	1.5 \pm (0.34)	47.98 \pm (4.44)	3.90 \pm (1.20)
Neem	7.25 \pm (0.80)	91.47 \pm (0.00)	104.2 \pm (18.65)
Chloropyrifos (50%) + Cypermethrin (5%)	1.25 \pm (0.39)	12.84 \pm (1.13)	0.25 \pm (0.17)
Malathion	2.08 \pm (0.47)	70.21 \pm (2.69)	3.33 \pm (1.32)
Control	3.23 \pm (0.56)	66.37 \pm (4.42)	57.25 \pm (7.02)
Concentration	0.01	0.008	0.171
Treatment	0.01	0.01	0.01
Concentration* Treatment	0.096	0.01	0.17

Compared to Cypermethrin (5%) and Emmamectin benzoate based treatments, the percent weight loss was very high in Neem based treatment and control. The percent weight loss of three different concentrations of Malathion was 2.97% for Malathion @1ml/ltr, 1.91% for Malathion @2ml/ltr and 0.35 % for Malathion @4ml/ltr. The percent weight loss of three different concentrations of Chloropyrifos (50%) + Cypermethrin (5%) was 3.16% for @ 0.75ml/ltr, 0.73% for @ 1.5ml/ltr and 0.25% for @ 3ml/ltr. Similarly, the percent weight loss of three different concentrations of Emmamectin benzoate was 2.41% for @0.1ml/ltr, 0.9% for @0.3ml/ltr and 1.46% for @ 0.6ml/ltr. The percent weight loss was highest for Neem among all the other treatments which were 9.4% for @ 2.5ml/ltr Neem, 3.74 for @ 5ml/ltr Neem and 7.63% for @10ml/ltr Neem. The weight loss percentage was seen highest in the control treatment after Neem which was, 3.41% of the total weight.

The maximum percentage of damaged grain was observed on all three concentrations of Neem which were 100% for all the three concentrations. The minimum percentage of damaged grain was observed on Chloropyrifos (50%) + Cypermethrin (5%) @1.5ml/ltr which was 11.21% of the total grain. The percentage of the damaged grain of three different concentrations of Malathion was 79.23% @1ml/ltr, 68.3% for @2ml/ltr and 71.34 % for @4ml/ltr. The percentage of the damaged grain of three different concentrations of Chloropyrifos (50%) + Cypermethrin (5%) was 14.51% for @ 0.75ml/ltr, 11.21% for @ 1.5ml/ltr and 14.35% for @ 3ml/ltr. Similarly, the percent damaged grain of three different concentrations of Emmamectin benzoate was 39.2% for @0.1ml/ltr, 69.9% for @0.3ml/ltr and 38.73% for @ 0.6ml/ltr. The percent weight loss was highest for Neem among all the other treatments which were 100% for @ 2.5ml/ltr Neem, 100% for @ 5ml/ltr Neem and 100% for @10ml/ltr Neem. 61.63% of damaged grain was observed on the control treatment.

The movement of adults of *S. zeamais* was significantly affected by insecticide formulations, particularly due to the insecticides themselves as the sole biologically active ingredients of the formulations tested (velez et al., 2018). The F1 progeny emergence in different observations was significant among different management practices. The lowest number of weevil progeny emerged from Chloropyrifos (50%) + Cypermethrin (5%) @1.5ml/ltr treated seeds was 0 whereas the maximum number of weevil emerged from the Neem @2.5ml/ltr treated seeds was 149.67 (Table 3). Result of the present study show that the ingredient of neem caused no effect in the mortality of *S. zeamais*. In contrast to our finding, Neem was reported to be highly effective against *S. zeamais* and found that within 14 days of exposure maximum mortality of 99% and 100% reduction in F1 progeny (Nukenine et al., 2013). The number of weevil progeny that emerged from three different concentrations of Emmamectin benzoate was 2 for @0.1ml/ltr, 9.33for @0.3ml/ltr and 1for @ 0.6ml/ltr. (Parilama and Maheswori., 2011) also showed that the emamectin benzoate was effective in controlling maize weevil. The number of weevil progeny that emerged was highest for Neem among all the other treatments was 149.67for @ 2.5ml/ltr Neem, 81.5 for @ 5ml/ltr Neem and 96 for @10ml/ltr Neem. The number of weevil progeny emerged from three different concentrations of Chloropyrifos (50%) + Cypermethrin (5%) was 0.67 for @ 0.75ml/ltr, 0 for @ 1.5ml/ltr and 0.33for @ 3ml/ltr. Similarly, the number of weevil progeny that emerged from three different concentrations of Malathion was 7.33for Malathion @1ml/ltr, 5.33for Malathion @2ml/ltr and 0.33 for Malathion @4ml/ltr. Malathion was the best chemical in reducing *S. zeamais* population. This report corroborate the previous report wherein malathion was more toxic 4.913 ppm against *S. zeamais* that exhibited superior toxicity (Pathak and Jha, 1999).

The number of weevil progeny that emerged in the control treatment was $49.67 \pm (3.51)$. The weight loss ($p=0.01$) and percent damaged grains ($p=0.008$) were found to be significantly different among concentrations whereas there was no significant difference in the number of weevil progeny $P (>0.171)$. Similarly, all the three dependent variables; weight loss, Percent damaged grains and no of weevil progeny were found to be highly significant $P (=0.01)$ among the different treatments. As for the source (Concentrations \times treatment), the percent damaged grain was found to be highly significant ($P<0.01$) while weight loss ($p=0.096$) and no of weevil progeny ($p=0.017$) were not significant (Table 3).

IV. CONCLUSION

Insects are often considered the principal cause of maize grain losses. Pests are one of the major constraints that limit the potentiality of maize in Nepal. They attack the maize plants directly from the seeds sown in the field during maturity and feed on all parts of the plants. The chemical control is effective, quick, secure and economical but it has some major drawbacks: negative impact on products and environment; the constant danger of intoxication for humans and animals; the presence of residues in different parts of the plants; appearance, at the pest species, of resistance to pesticide. Out of the 4 pesticides tested on the adult of *Sitophilus zeamais*, Chloropyrifos (50%) + Cypermethrin (5%) was most effective followed by Malathion, Emamectin Benzoate and at last Neem. Neem treatment had a 0% mortality rate which showed no reduction of the weevil population, rather resulted in the highest no of progeny during the last stage of the data observation. This means these 3 pesticides except Neem can be recommended for control of *Sitophilus zeamais*. Chloropyrifos (50%) + Cypermethrin (5%) has a longer residual effect so that it could prevent damage from *S. zeamais* for a longer period which could be used to preserve maize seed. Chemical pesticide treated grains should not be used for consumption purpose.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Analysis of Factors Influencing the Adoption of Charcoal-Powered Processing Kiln by Fish Farmers: Evidence from Nigeria

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Abstract— This study assessed the factors influencing the adoption of charcoal-powered kiln in Akure Metropolis, Ondo State. The specific objectives of the study were analysed using descriptive and inferential statistics. Primary data were sourced from 30 fish processors through a snowball sampling technique using a well-structured questionnaire. Data collected for the study were analyzed using descriptive statistics, budgeting techniques and logit model. Results revealed that 76.7% of the fish processors were below 60 years. Most (80.0%) of the sampled farmers were male, 83.3% were married, having a household size of 6-10 persons while more than half (56.7%) of the respondents were educated. The benefit-cost analysis showed that charcoal-powered smoking kiln was found to be profitable. The marginal effects estimated from the logit coefficients showed that years of schooling, household size, income level, shelf life and consumer's taste and preference for the product were positively related to the adoption of charcoal-powered kiln technology. Based on the findings, the study concluded that charcoal-powered smoking kiln is reliable and profitable for fish smoking. It is, therefore, recommended that policy that will enhance and facilitate its adoption should be promoted. Extensive awareness and further training of fish processor through mass media should be carried out and sponsored by both government and non-governmental agencies across the country.

Keywords— Charcoal-powered kiln, Logit model, smoked fish, Nigeria.

I. INTRODUCTION

Fish occupies a strategic position in Nigerian food basket, contributing immensely to the food security of the country. Fisheries provide employment to over 8.6 million people directly while about 19.6 million people are also indirectly depended on this subsector as their means of livelihoods (World Fish, 2018). Nigerian fishing activities are made up of three sources such as artisanal, industrial and aquaculture. Fish is an important source of protein to a large extent and thus provides 40% of the dietary intake of animal protein for average Nigerian.

About 2.7 million metric tons of fish annually is required to satisfy the dietary needs of Nigerian citizens. However, the aggregate total domestic fish supply from all sources, both from capture and culture fisheries, is about 0.8 million metric tons per annum thereby creating a deficit of 1.9 million tons to fill the demand-supply gap (Emefiele, 2019) cited in Johnson et al. (2020). The gap between demand and supply has raised Nigeria import bill on frozen fish to over \$1.2 billion annually (FAO, 2007).

According to Adekoya (2004), fish and fish products constitute more than 60% of the total protein intake in adults especially in rural areas. Fish as food is known to be superior to all terrestrial meats (beef, pork and chicken, etc), rich in animal protein and highly digestible energy.

Fish is suitable for supplementing diets of high carbohydrates contents, good source of thiamine and rich in Omega-3 poly-saturated fatty acids, fat and water soluble vitamins and minerals. It is highly rich in Polyunsaturated Omega III fatty acids,

which are important in lowering blood cholesterol level and high blood pressure. It is able to mitigate and alleviate platelet of cholesterol aggregation and various arteriosclerosis conditions in adult populations.

Fish value chain covers production, processing, storage and distribution to the final consumers. Fish processing is an important stage in the handling of fish product. One of the major activities in fishery is post-harvest handling of the fish. This stage requires efficient management and when it is not efficiently handled, it results in losses and low returns to the efforts of fish processors. This has been the situation in Nigeria over the years.

Despite abundance of on-shelf technologies in our various institutions, fish processors are still making use of traditional methods (drums) for fish smoking which produces fish characterized with poor quality and high content of Polycyclic aromatic hydrocarbon (PAH) knowing to be dangerous to public health. Smoking of fresh fish is primarily done to preserve it from spoilage and also to prolong the shelf life of fish products. There are four major ways of smoking fresh fish namely sun drying, salting, smoking, and refrigeration. In Nigeria, traditional smoking is often done in the open air using fuelwood of different kinds. The sun drying and smoking in the open fire are often characterized with dirt, sand and dust (Bolaji, 2005). Open fire smoking makes smoked fish prone to external contamination and thus extends the length of time spends on fish smoking and sometimes it is destroyed by rain and strong wind (Bomfeh, et al., 2019). This method is not only laborious but also constitutes environmental hazards to the society. The use of traditional techniques take longer drying time and product-quality is difficult to control. Traditional methods of smoking fish can cause eye and skin irritations to fish processors.

There are several improved fish processing technologies suggested in the literature, among them include charcoal and gas powered kilns. Both technologies are reported to have very close time range for drying fish and low PAH (Okusanya et al. 2021). However, in the literature, charcoal-powered kiln technique for smoking fish is found to be more likely cost-effective compared to the cost of gas-powered kiln in the study area.

Scientifically, findings showed that several smoking methods have been designed and suggested to be used in order to bring down the content of polycyclic aromatic hydrocarbon on smoked fish. PAH recommended limits by European Union are yet to be met in developing countries, Nigeria inclusive. However, the adoption of improved smoking techniques has a lot of challenges due to several perceptions towards these technologies. Perception of cost and profitability associated with the technology, compatibility, and some other socio-cultural constrains preventing fish farmers from using the technologies.

Most available studies are related to technical aspects of smoking techniques, but factors constraining the utilization of improved technologies on fish smoking are still grossly inadequate if not lacking, thus this study is conceptualized to fill the knowledge gap. This study therefore seeks to provide answers to the following questions. What are the socio-economic characteristics of the respondents ?; and What are the factors affecting the adoption of charcoal-powered fish smoking? Therefore, the findings from this study would assist government to make the necessary polices that will help accelerate the utilization of modern method of smoking fish.

II. LITERATURE REVIEW

Traditional smoking and sun drying of fish are common methods of fish preservation in Nigeria. In the process, moisture content present in the fish is extracted through heating, thereby inhibiting the action of micro-organisms and prolong their shelf life (Amoo *et al.*, 2007; Singh and Heldman, 2013; Pigott, 2015). These methods are employed probably due to irregular supply of or lack of access to electricity to preserve and or process their products. Bolaji (2005) reported that despite the rudimentary nature of traditional processing methods, the lack of control over the drying rate, sometimes results to under- or over-drying and expose fish to wind, dust, dirt, insect infestation and contaminants such as flies. The quality of fish declines if not properly processed after harvesting and thus affecting consumer's preference (Sen, 2005). Different processing techniques of fish produce different output that suits consumers taste.

However, different processing techniques produce different physical and chemical properties that may or may not produce the qualities consumers are actually looking for in smoked fish. Limited studies exist in the economic literature on adoption of fish smoking technologies. Some of the few include, Bolorunduro et al. (2005) who investigated adoption of improved fish preservative technologies in North-western, Nigeria. They found years of experience, extension contact, access to credit, age, education, and acquisition cost as factors that are positively affecting adoption of chokor, Burkinabe and Altona kiln in their study area. From technical perspective, Okusanya et al. (2021) also investigated the impact of improved smoking kiln design on hygiene and timeliness of drying of smoked fish in Nigeria. They reported that both charcoal and gas-powered kiln

produced smoked fish that were hygienic and not likely to exceed maximum limits of PAH allowed by the United States Environmental Protection Agency.

Ajang et al. (2010) used cost-benefit analysis to evaluate the performance of chorkor smoker in smoking fresh fish in Nigeria. The study found that chorkor smoker was proved to have superior qualities over traditional methods of smoking fish product. Chorkor smoker also gives higher economic returns compared to traditional methods. Study by Akinola et al. (2006) compared traditional and solar fish drying systems towards enhancing storage and preservation in Nigeria. The study concluded that solar drying system eliminates the contact between fish and flies.

Odediran and Ojebiyi, 2017 assessed the awareness and adoption of improved fish processing technologies among fish processors in Lagos State. The study found that drum and mud oven were common techniques employed by the people in their study area. Nti (2002) used participatory appraisal approach to evaluate the determinants for effective adoption of an improved fish-processing technology in Ghana. They found that 88% of the processors completely adopted improved technology and reduced drudgery of fish-smoking activity, however, the key limiting factors were reported as lack of finance, high cost and non-availability of input and inability for group formation. Hassan et al. (2020) used logit regression and propensity score matching to evaluate the impact of adoption of modified drum-oven technology on income of fish processors in Niger State. The study found that age, sex, processed output and affordability had positive influence on the adoption of modified drum-oven technology (MDOT). Result revealed that adoption of MDOT increased average output by 1.008kg.

III. MATERIALS AND METHODS

3.1 Study area

The study was conducted in Ondo State, Nigeria. The state is bounded in the west by Osun and Ogun States and in the north by Ekiti and Kogi States. Ondo State shares boundaries with Edo and Delta States in the east and in the south by the Atlantic Ocean. The State lies between latitudes 5° 45' and 8° 15' north of the equator and longitude 4° 30' and 6° 60' east of the Greenwich Meridian. Ondo State has eighteen (18) Local Government Areas with an estimated population of about 3.4 million inhabitants (National Population Commission [NPC], 2006). There are two distinct seasons which are the rainy season (April to October) and the dry season (November to March). Though, there is usually an August break for a period of two weeks. The annual rainfall varies from 1,150mm to 2,000mm. Ondo State riverine area is a veritable ground for fishing.

A multi stage sampling procedure was used to select fish processors. At a start, dual smoking kiln (using both charcoal and gas) was fabricated with locally sourced materials such as aluminum plates to coat both the interior and exterior of the kiln. The second stage involved snowball sampling of thirty (30) fish farmers who are into production only.

In the third stage, 50 kg of fresh fish (*Clarias gariepinus*) was purchased from local markets. The fishes were slaughtered and non- edible parts such as gills were removed and thoroughly washed to remove sand as well in order to achieve good hygiene. Little quantity of table salt and seasonings were added to enhance its taste. In the fourth stage, we placed the fishes in the oven for drying using both charcoal and gas as sources of energy. In the final stage, group method of teaching and result demonstrations were employed to train the farmers using these improved methods of smoking.

3.2 Analytical Techniques

Data collected were analyzed using descriptive and inferential statistics. The descriptive statistics used were frequency count, tables, percentage distribution. Budgeting analysis was used to evaluate the profitability of using charcoal-powered kiln to smoke fresh fish while binary logistic regression was used to identify factors influencing the adoption of charcoal-powered smoking kiln by the respondents.

3.2.1 Budgeting Analysis

Budgeting analysis is a technique used to compute cost and returns of enterprises. In this case, we used gross margin to evaluate the profitability of using charcoal and charcoal-powered smoking technique. Mathematically, the gross margins of using both techniques are expressed as follows:

$$GM_i = TR_i - TVC_i \quad (1)$$

$$NP_i = GM_i - D_i \quad (2)$$

Where;

GM_i is the gross margin realised by farmer (₦).

TR_j = Total Revenue realized from charcoal powered kiln by jth farmer (₦)

TVC_j = Total variable cost incurred from charcoal-powered kiln by jth farmer (₦)

NP_i = Net profit accrued to i^{th} farmers (₦)

D = Depreciation cost on fixed items

Depreciation for the fixed variables used by fish processor was calculated using equation 3.

$$\text{Depreciation} = \frac{\text{Original cost} - \text{Salvage value}}{\text{useful life}} \quad (3)$$

Profitability Ratios

The profitability of investing on charcoal-powered kiln is evaluated by using following ratios

$$\text{Benefit - Cost ratio (BCR)} = \frac{\text{Total Revenue}}{\text{Total Cost}} \quad (4)$$

$$\text{Gross ratio (GR)} = \frac{\text{Total cost}}{\text{Total revenue}} \quad (5)$$

$$\text{Rate of Return (ROR)} = \frac{\text{Total cost}}{\text{Total revenue}} \quad (6)$$

3.2.2 Binary Logistic Regression

Binary logit model is a popular econometric tool in adoption related studies. Several studies have used this model to identify factors affecting adoption when the dependent variable is dichotomous (i.e adopter or non-adopter).

Following Gujarati (2003), binary logistic regression model can be expressed as

$$\ln\left(\frac{P_i}{1-P_i}\right) = Z_i^* = \frac{1}{(1 + \exp^{-(\beta_0 + \beta_i X_i)})} \quad (7)$$

Z_i^* = Latent dependent variable

$\ln(P_i/1-P_i)$ = Log of odds ratio of adopter/non-adopter.

P_i = Probability of utilising charcoal-powered kiln by i^{th} fish processor

$1-P_i$ = Probability of not utilising charcoal-powered kiln by i^{th} fish processor

X_i = Row vector of fish processor and technology characteristics

β_i = Column vector of unknown parameters to be estimated,

β_0 =constant,

Linearizing equation 7, the reduced equation becomes:

$$Z_i^* = \beta_0 + \sum_{i=1}^J \beta_i X_i + \varepsilon_i \begin{cases} \text{if } J > 0, Z_i^* = 1 \\ \text{otherwise } Z_i^* = 0 \end{cases} \quad (8)$$

ε_i = Vector of unobserved random effect.

TABLE 1
VARIABLES USED IN THE MODEL

Variable	Description	Measurement	Hypothesized sign
Z_i	Dependent variable	Charcoal-powered smoking kiln = 1, 0 otherwise	
	Explanatory variables		
	Age category		
X_1	31-40	Dummy, If yes-1, 0 otherwise	-
X_2	41-50	Dummy, If yes =1, 0 otherwise	-
X_3	51-60	Dummy, If yes =1, 0 otherwise	-
X_4	>60	Dummy, If yes =1, 0 otherwise	-
X_5	Years of schooling	Continuous, Years spent in schooling	+
	Income category	Dummy, If yes-1, 0 otherwise	
X_6	30001-40,000	Dummy, If yes-1, 0 otherwise	+
X_7	40,001-50,000	Dummy, If yes-1, 0 otherwise	+
X_8	50,001-60,000	Dummy, If yes-1, 0 otherwise	+
X_9	>60,000	Dummy, If yes-1, 0 otherwise	+
X_{10}	Average cost of material	Continuous, measured in ₦	-
X_{11}	Family size	Number of family members	+
X_{12}	Average cost of fish	Continuous, measured in ₦	-
X_{13}	Shelf life	Lasting = 0, 0 otherwise.	+
X_{14}	Taste preference	Satisfactory =1, 0 otherwise	+/-

IV. RESULTS AND DISCUSSION

4.1 Socio-economic characteristics of respondents

The socioeconomic characteristics of the farmers that were analyzed included age, gender, level of education, marital status, pond size, household size and income.

The distribution of respondents' socioeconomic characteristics is presented in Table 2. Majority of the farmers (80.0%) were males while 20.0% were females, suggesting that both male and females are involved in fish processing in the study area. This negates a similar study by Akangbe (2012) who stated in his report that majority of fish processors are women and that the dominance of women is attributed to the fact that women bear primary responsibilities for household sustenance and well-being.

The age structure showed that 40% were between the age of 31 and 40 years, and 40% of the sample farmers were within 50 years of age. This implies that the fish processors in the study area are young and active to cope with the stress of fish processing. Result also showed that many (83.3%) of the processors were married which implies that most of the respondents were mature and responsible to cater for their households. About 43.3% of the fish processors had no formal school education, 20.0% had primary education, 16.7% had secondary school education while 20.0% acquired tertiary education. Okunlola *et al.* (2011) and Agbam (2006) stated that educational level is one of the factors that influence adoption of new technology by farmers. Educational level of the respondent is an important factor to be considered in adoption of innovation. It was also revealed that half (50%) of the farmers have household sizes of 6-10

TABLE 2
SUMMARY OF SOCIO-ECONOMIC CHARACTERISTICS

Characteristics	Frequency	Percentage (%)
Sex		
Male	24	80.0
Female	06	20.0
Age category		
31-40	02	33.3
41-50	10	6.7
51-60	11	36.7
>60	07	23.3
Marital status		
Single	05	16.7
Married	25	83.3
Educational status		
No formal education	13	43.3
Primary education	06	20.0
Secondary education	05	16.7
Tertiary education	06	20.0
Household size		
1-5	13	43.3
6-10	15	50.0
>11	02	6.7

Source: Field survey, 2021

4.2 Cost and Returns of 50kg of Smoked Fish/Month

As revealed from Table 3, the cost of purchase of fish (50kg) was ₦212,500.00 per 5cycles/month. Thus, this cost represents about 86.8% of the total variable cost incurred on 50kg of fish smoked for the period. The cost of salt and labor incurred for processing was ₦1000 and ₦7,500, respectively and thus accounted for about 3.5% of the total variable cost (TVC).

Similarly, the cost of charcoal, *Potash alum* and 50 litre of water used accounted for about 8.5% of the total variable cost of processing 50kg of smoked fish by the respondents. The result shows that variable cost for the smoking of fish gulps as much as ₦243, 250 representing about 99.4% of the total cost required to process fresh fish into smoked fish. The depreciation cost on kiln acquired was just N1, 500 with 0.6% of total cost (TC).

From the cost and returns Table, the revenue accrued to a processor was about N375,000 per 5 cycles in a month with a gross margin being ₦133,250 and net profit of ₦131,750. These values indicate that smoked fish business is profitable in the study area.

The profitability ratio conducted on the cost and returns of smoked fish using charcoal show that benefit-cost ratio 1.54. This value implies that for every ₦1 invested in the business, a gain of 54kobo would be accrued. This value shows that the processing technique is profitable and viable. This result agrees with the finding of Olutumise (2020) who reported a high profitability of catfish processors using improved processing techniques in the study area.

TABLE 3
COST AND RETURNS OF 50KG OF SMOKED FISH/MONTH

Charcoal-Powered Kiln		
Variables	Mean/month (N)	%
Cost of fish (50Kg)	212,500	86.8
Cost of salt	1,000	0.4
Cost of labor	7,500	3.1
Cost of Charcoal (1 bag)	20,000	8.2
Cost of kerosene	1,500	0.6
Cost of alum	250	0.1
Cost of water (50 litre)	500	0.2
Total Variable Cost	243,250	99.4
(TFC) Depreciation on kiln	1,500	0.6
TC= TFC+TVC	244,750	100
Total Revenue = Unit price x quantity sold (5cycles/month)	375,000	
Gross Margin (GM)= TR-TVC	131,750	
NP=TR-TC	130,250	
Benefit-Cost ratio = TR/TC	1.54	
Gross ratio = TC/TR	0.65	

Source; Field survey, 2021

4.3 Factors influencing the adoption of charcoal-powered smoking technique

The coefficients and odd ratios estimated from binary logistic regression are presented in Table 4. The explanatory power of the model was diagnosed using the log likelihood ratio, F-statistics value and Pseudo R². The log likelihood ratio was valid, F-statistic of 40.7 showed that all explanatory variables were significant at probability level of 1%. The discussion of the results was based on the marginal effect estimated from logit coefficients because the parameter estimates of logit model cannot be interpreted directly (Gujarati, 2003). As shown in Table 4, out of seventeen (17) variables postulated, fourteen were statistically significantly different from zero at 1%, 5% and 10%, respectively.

4.3.1 Age of respondent

The respondent whose age ranged between 41 and 50years had a negative but significant influence on the adoption of charcoal-powered kiln at the 1% level. The corresponding odd ratio (0.06) is less than one which implies that a one year increase in the age of the adopter, the odds of adopting a charcoal-powered kiln for fish smoking will be lesser than the odds of a non-adopter, *ceteris paribus*. Similarly, a farmer who is between the ages of 51-60years had a negative influence on the adoption charcoal-powered smoking technique, but statistically significant at the 1% level. This result implies that farmers who are between the ages of 51-60years will be less likely to adopt charcoal-powered compared to a non-adopter. Farmer whose age is greater than 60 years will be less likely to adopt. The finding here negates the findings of Hassan et al. (2020) that age of the processor increases the probability of adoption.

4.3.2 Education of respondents

Education of respondents matters a lot in adoption of technologies because it creates awareness and takes away ignorance (Adetarami, et al. 2020). Ability to read and write makes it easy to scrutinize and weigh future benefits associated with charcoal-powered smoking kiln. Education of the farmers was measured as continuous variable here and the result showed that education had a positive and significant impact at 10% probability level. The corresponding odd ratio of 12.94 is greater than one and thereby suggesting that a one year increase in schooling, *ceteris paribus*, the odds of adopting charcoal-powered kiln for smoking fresh fish will increase by 94% compared to the odds of a non-adopter.

4.3.3 Level of Income of respondents

Income level of the farmer was positively significant at 5% level. The corresponding odd ratios were 3.74 and 17.81 respectively. The results imply that a unit increase in farmer' income, the odds of using charcoal-powered kiln will increase by 3.74 and 17.81 times compared to the odd of a non-user. The finding is in consonant with Hassan et al. (2020) that income of the processor increases the probability of adoption.

4.3.4 Household size

Household size is positively and significantly related to the adoption of charcoal-powered kiln as expected. This implies that an additional member in the family, farmer will be more likely to adopt charcoal-powered kiln compared to a non-adopter of this technology. The coefficient of household size is 0.19 and the corresponding odds ratio of 1.29 is greater than one. The positive sign for this variable has theoretical agreement with the study hypothesized sign. Thus, it implies that a farmer with large family is more likely to adopt charcoal-powered kiln compared to the base category. The result indicates that increasing household size by a unit, *ceteris paribus*, the odds of adopting charcoal-powered kiln will increase by 29%.

4.3.5 Average cost of materials, Fish and Labour

Also significant are average cost of materials, fish and labour. The coefficients for these variables are negative as expected and the corresponding odd ratios are less than one which indicates that a 1% increase in these variables will cause the odds of using charcoal-powered kiln to reduce by 6%, 48%, and 18%, respectively, compared to the odds of a non-user.

4.3.6 Taste preference

Consumer's taste and preference is positively related to the adoption of charcoal-powered kiln. The corresponding odd ratio is also greater than one meaning that the odds of using charcoal-powered kiln will increase by 34% compared to the odds of a non-adopter.

4.3.7 Shelf life

A long Shelf life for fish is expected to enhance its attractiveness and thereby increase the demand for smoked fish. The results show that the variable is positive and significantly related to adoption of technology at 5% level of probability. The positive association with the adoption of charcoal-powered kiln is expected because fish smoking promotes market value of the product. Therefore, a reduction in the water content of fish prolong the shelf life of the fish, Similarly, consumer demand for the smoked fish using a charcoal-powered kiln will lead to higher sales compared to sales of non-adopter.

TABLE 4
RESULTS OF LOGIT MODEL FOR THE ADOPTION OF CHARCOAL-POWERED SMOKING KILN

Variable	Description	Coeff.(sd.err)	Marginal effect
	Age category		
X ₁	31-40	-0.23(0.60)	0.79
X ₂	41-50	-2.85** (0.98)	0.06
X ₃	51-60	-2.96**(1.22)	0.05
X ₄	>60	-2.96**(1.46)	0.05
X ₅	Years spent in schooling	2.56*1.51)	12.94
	Income category		
X ₆	30001-40,000	1.37**(0.60)	3.94
X ₇	40,001-50,000	0.93(0.57)	2.53
X ₈	50,001-60,000	1.47**(0.73)	4.35
X ₉	>60,000	2.88*(1.49)	17.81
X ₁₀	Average cost of material	-2.09*** (0.58)	0.06
X ₁₁	Household size	0.19**(0.09)	1.29
X ₁₂	Average cost of fish	-0.74** (0.33)	0.48
X ₁₄	Texture	1.38*** (0.43)	3.97
X ₁₅	Shelf life	1.08*** (0.06)	2.94
X ₁₆	Taste preference	0.85** (0.34)	2.34

Log likelihood ratio= 58.81, LRCH= 40.67, Prob>chi₂ = 0.00, R² = 0.2

Source: Field survey, 2021.

V. CONCLUSION AND RECOMMENDATIONS

The study, specifically, evaluated profitability of smoked fish using a charcoal-powered smoking kiln as well as identified the determinants of adoption of charcoal-powered kiln in the study area. Primary data were sourced from 60 fish processors through a snowball sampling technique using a well-structured questionnaire. The study concluded 76.7% of the fish processors were below 60 years. Majority (80.0%) of the farmers were male-headed households dominated fish processor, 83.3% were married with more than half of them (56.7%) were educated. Charcoal-powered smoking kiln was found to be profitable. The marginal effects estimated from the logit coefficients showed that years of schooling, household size, income level and shelf life of the smoked fish were positively related to the adoption of charcoal-powered kiln compared to a non-adopter.

Based on the findings, the study concluded that charcoal-powered smoking kiln technology is profitable for fish smoking. It is therefore, recommended that policy that will enhance and facilitate its adoption should be encouraged among the farmers. Extensive awareness through mass media should be carried out and sponsored by both government and non-governmental agencies across the country.

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Impact of Insect Pollination on Fruit Set, Fruit Size and Yield of Three Sweet Cherry Cultivars

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Abstract— In the present research project, we compare three different pollination types (viz., with insects, without insects, and solely with honeybees) of three widespread sweet cherry cultivars, with the aim of conducting measurements and drawing conclusions pertaining to the impact of pollinators on fruit set, yield, and fruit quality in commercial orchards. It included a total of three treatments: Isolation with anti-insect nets (IS), Isolation with cages and Honeybee Pollination (HB), and Open Pollination (OP). Recent research focuses mostly on the role that different types of pollinator have in sweet cherry pollination; however, they do not provide measurements of final yields. The applied experimental method, was fast, simple, of low labor cost, and yielded robust and valid results based upon statistical analysis that are easily comprehensible by the sweet cherry growers. From the results of the experiment, it is evident that the absence of pollinators is a restrictive factor in fruit production, not only in cross-pollinated but also in self-fertile varieties. The value of open pollination, during which cherry flowers are visited by native pollinators and honeybees is beyond doubt. Wind is not a means of transporting pollen to sweet cherry trees. Honeybees have proved to be effective managed pollinators and thus they represent an efficient approach that can ensure increased yields and large size fruits, when colonies placed in sweet cherry orchards.

Keywords— Sweet cherry pollination, insect pollination, honeybees, pollination services.

I. INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a valuable fruit tree species of the Rosaceae family, grown worldwide for its exceptionally high-quality fresh fruit. According to their pollination mode, cultivated cherry varieties are classified into self-pollinating, partially self-pollinating and self-incompatible. The genotype (S-alleles, S_aS_b) of a given cultivar determines whether this particular variety is compatible with another so that pollination can be achieved. If the two varieties have the same S-alleles, then they are incompatible with each other, irrespective of whether they bloom at the same time [27], [32].

The range of factors that influence fruit set and quality in different sweet cherry cultivars have been examined and analyzed in the literature, as for example in the paper by Montiel et al. [31]. Such factors are pollen availability and viability, stigmatic receptivity, ovule longevity, pollen vectors such as bees, as well as high and low temperatures during bloom time [5], [34], [41]. Of critical importance also are susceptibility to frost, rootstock type, rate of pollen germination [1], [40] and age of flower at the time of pollen germination [41]. Inadequate insect pollination, low pollen germination, low viable pollen, low pollen tube growth, and rapid ovule senescence, have negative effects on fruit set [35].

Insect pollination of cherry flowers is a core issue as it impacts both fruit set and yields [26], [29]. Both *Apis mellifera* L. (Hymenoptera: Apidae) species and non-apis species are acknowledged as major crop pollinators worldwide [16]. Recent work has demonstrated that pollination services by wild bees in cherry are superior to those offered by honeybees [21]. In fact, the semi-natural habitats that support their diversity and abundance enhance cherry fruit set [12]. Solitary bees such as

mason bees (*Osmia cornuta*) achieve high pollination rates after a single visit to cherry flowers [13]. Honeybees, on the other hand, that are bred and maintained by beekeepers in hives, have the great advantage that they can be transported over long distances and offer their precious pollination services in a variety of places. Worldwide, the transportation of honeybee colonies to provide supplemental pollination services remains the defacto approach, for the time being [16]. Honeybees are regarded as exceptionally productive pollinators [38]; as a matter of fact, they are the most economically valuable pollinators of crop monocultures around the world, especially when other pollinators do not visit agricultural fields [26], as for example when agricultural land is cut off from natural or semi-natural areas.

The number of honeybee hives recommended for pollination in commercial cherry orchards is 2 to 5 hives per hectare. Sweet cherry fruits drop in three waves. The first happens 2-2.5 weeks after full bloom, the second 1 week after the first, and the third 3 weeks after the second [7]. The natural formation of pedicel-fruit abscission zone varies by cultivar, and the general molecular basis for its activation is not well characterized [20]. Poor pollen quality gives rise to fruitlet abscission [33]. Several factors, such as variety/rootstock selection, type of pruning, number of fruits, leaf/fruit balance, tree vigor, water adequacy at critical times, and heat stress affect fruit size [30].

The aim of the current research paper was to examine the influence of insect pollination on fruit set, fruit size and yield of three commercial sweet cherry cultivars via a field simulation method. Joint isolation of the cross-pollinated sweet cherry cultivars under examination constitutes a method which has never been applied before in pollination experiments with insects. It is adopted for the first time in the current research paper, with the expectation that it may yield comprehensive results for both farmers and beekeepers on the usefulness of pollinators as a valuable input to sweet cherry production.

II. MATERIALS AND METHODS

2.1 Research area

The current experimental project was implemented between 2020 and 2021 at the premises of the Department of Deciduous Fruit Growing based in the city of Naousa, Greece, (40°37'15"N; 22°07'00"E, altitude: 119 m), which belongs to the Institute of Plant Breeding and Genetic Resources of the Hellenic Agricultural Organization-DIMITRA. The climate in this area is Mediterranean, with a mean annual rainfall of 690 mm. The central farm of the Institute, covering an area of 20 hectares, combines a wide variety of cultivated tree species, several of which bloom at the same period, thus acting competitively in their effort to attract bees and other pollinators. Meteorological data was provided during the experiment by the local meteorological station.

2.2 Plant material

The plant material used consisted of: (i) three open-bowl shaped cherry trees of the 'Ferrovia' variety aged 7 years old, grafted on a MaxMa 14 Delbard rootstock; (ii) three open-bowl shaped cherry trees of the 'Regina' variety aged 7 years old, grafted on a MaxMa 14 Delbard rootstock (*P. mahaleb* × *P. avium*); and (iii) three low-bowl shaped cherry trees of the 'Lapins' variety aged 7 years old, grafted on a Mazzard rootstock. The cultivars under investigation as well as the pollinizer varieties planted in the experimental farm are presented in Table 1.

TABLE 1
SWEET CHERRY VARIETIES INCLUDED IN THE POLLINATION EXPERIMENT

Variety	Bloom* (days from B. Burlat)	S-alleles	Pollination group	Pollinizers	Fruit set (OP %)
Ferrovia	+ 5	S ₃ S ₁₂ Incompatible	XXII	* 'Sunburst', 'Lapins', 'Van', 'Kordia', Regina', ** 'Canada Giant', 'Giorgia', 'Hedelfinger'	*14.30
Regina	+ 7	S ₁ S ₃ Incompatible	II	* 'Ferrovia', 'Cristalina', 'Tragana Edessas', 'Kordia', 'Summit'	*48.00
Lapins	- 2	S ₁ S ₄ Self-fertile*	SC	-	*39.20

*Data from Kazantzis [25] **Data from Grandi & Lugli [17]

Tree planting distances were 4.0 m x 5.0 m. Pruning, fertigation, and plant protection practices were carried out in accordance with the principles of integrated management that are widely adopted by cherry growers.

2.3 Experimental treatments

In the present project, we followed the methodology described by Delaplane et al. (2013) concerning fruit set experiments at field level. One target was to exclude all insect pollinators and introduce honey bee colonies in plots, in order to study the influence of honeybee pollination on cross- and self-pollinated cultivars. Another target was to investigate the possibility of wind pollination - without visitors. Pollination in open fields was also investigated, to be compared with the other 2 methods. The experiment consisted of three treatments: Isolation (IS), in which the trees were covered with anti-insect nets; Isolation and Honeybee Pollination (HB), in which in which the trees were caged under anti-insect nets a honeybee hive was placed together with the caged trees; and Open Pollination (OP), in which the trees were completely unprotected. Pollination experiments with honeybees and the use of isolation cages in fruit trees have also been successfully carried out with kiwi [22], plum [6] and sweet cherry [1].

On each experimental tree, ten (10) fruit bearing branches were selected and marked as subjects of each treatment. To carry out the IS treatment, we isolated (Fig. 1A) with the use of insect-proof netting a tree of the 'Regina' and one of the 'Ferrovia' variety together with twenty (20) 0.5 m long blooming branches of the 'Kordia' variety (S_3S_6 group of alleles - pollination group: VI) that were placed in bottles filled with water. With the use of the same net, we also isolated a tree of the 'Lapins' variety, but without any pollinizers (Fig. 1B).

Similarly, for the needs of the HB treatment (Fig. 2A), two trees were jointly isolated by means of insect-proof netting: a tree of the 'Regina' and a tree of the 'Ferrovia' variety together with twenty (20) 0.5 m long blooming 'Kordia' branches. In addition, a tree of the 'Lapins' variety was isolated separately. The difference between treatments (HB) and (IS) is that in the former a honeybee hive was placed inside each cage (Fig. 2B). In the OP treatment, pollination was carried out by both native and honeybee pollinators living in this specific area and, what is more, the transfer of pollen to the flowers of the cultivars under investigation was undertaken by all the pollinizers included in Table 1.

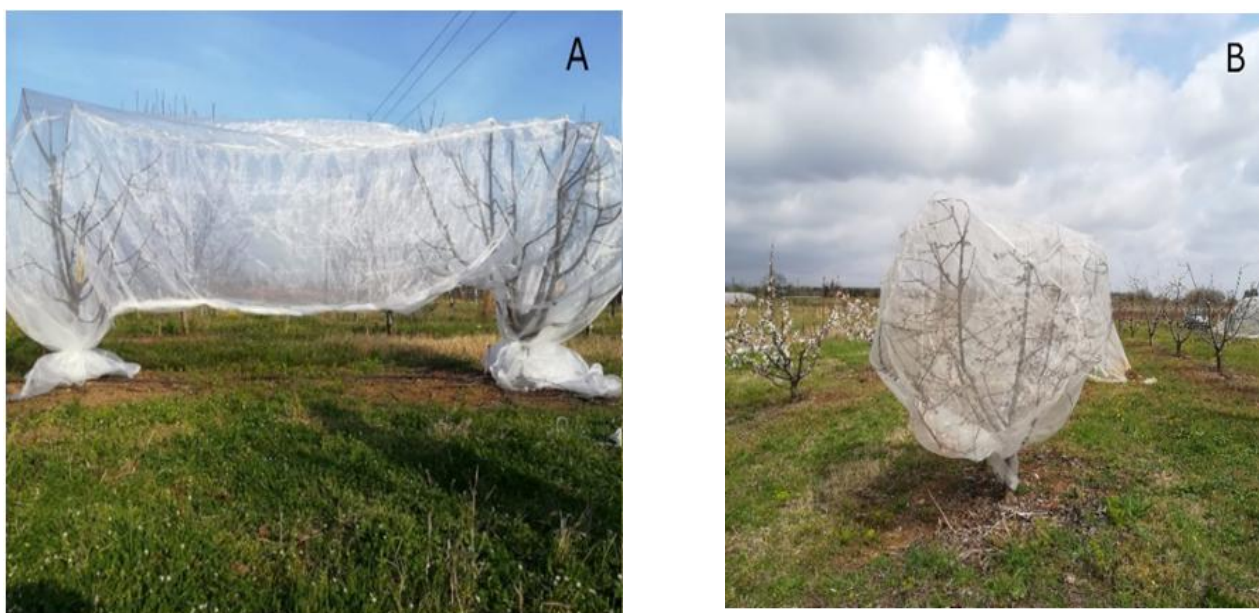


FIGURE 1: Isolation: Ferrovia + Regina (A), Lapins (B)

A great advantage of the method applied is that the measurements conducted concern different branches of the same tree and, consequently, the number of their repetitions can easily be adapted according to the precision that is required each time. In the specific experiment, we assumed that measuring 10 random branches in each treatment, differing in position and orientation was an adequate number for drawing safe conclusions. In addition, the relatively small sample size when combined with homogeneous experimental units, as the ones we selected for our experiment, further ensures valid and robust statistical results. The larger the experiment becomes in terms of the number and/ or size of the individual experimental units deployed, the harder it becomes to either ensure or reasonably assume that these units are all either internally homogeneous or equivalent to each other for the purposes of these comparisons [18].

During the experiment, the following measurements were taken from each selected fruit branch: number of flowers per branch meter, fruit set (%), production (g/m), and fruit diameter (mm).

2.4 Pollinators

For the purposes of the experiment, honeybee hives were used consisting of three bee and brood frames and another two frames of stored pollen and honey (about 6,000 bees). The colonies were introduced under the nets at the onset of bloom. The hives remained inside the HB treatment cages for 8 days, during which they were fed twice with 1:1 sugar syrup to supplement their diet due to lack of nectar.

2.5 Statistical analysis

Statistical analysis was performed with the use of SPSS, Version 21.0 [23] and MS Excel. We used the Kolmogorov-Smirnov test (K-S Test) to examine if our samples are drawn from a population with a normal distribution. For the variables which do not follow a normal distribution, we used nonparametric tests: Kruskal-Wallis median-test, and Mann-Whitney U-test. For normally distributed variables, the differences between two different groups were evaluated with the use of t-test. P-values ≤ 0.05 (5% significance level) were considered significant.



FIGURE 2: Honeybee pollination: Ferrovia + Regina (A), Lapins (B)

III. RESULTS

The 'Ferrovia' fruit set means obtained from treatment IS in 2020 and 2021 (Table 2) were found to be considerably lower than the fruit set means of treatments HB ($P < 0.001$) and OP ($P < 0.001$). The OP treatment conducted in 2020 produced a higher mean fruit set than treatment HB, without this difference being statistically significant ($P = 0.724 > 0.05$); the same occurred in 2021 ($P = 0.153 > 0.05$).

TABLE 2
FERROVIA. RESULTS FOR YEARS 2020 & 2021 (MEAN \pm S.D)

Treatment	Fruit set (%) 2020	Fruit set (%) 2021	Production (g/m) 2020	Production (g/m) 2021	Size (mm) 2020	Size (mm) 2021
IS	4.30 \pm 3.52 ^a	3.60 \pm 2.50 ^a	0.00 ^a	0.00 ^a	-	-
HB	22.9 \pm 7.54 ^b	15.00 \pm 4.26 ^b	229.44 \pm 91.93 ^b	177.33 \pm 80.00 ^b	27.63 \pm 0.51 ^a	28.46 \pm 0.49 ^a
OP	24.10 \pm 7.40 ^b	19.30 \pm 8.05 ^b	263.03 \pm 116.87 ^b	253.71 \pm 92.85 ^b	27.54 \pm 0.83 ^a	26.61 \pm 0.83 ^b

The means of each column followed by a different letter are significantly different at the 0.05 significance level

The average production of 'Ferrovia' in treatment IS in 2020 and 2021 was equal to zero. The few fruits that were formed turned yellow and dropped in the first wave of fruit drop. The 2020 OP treatment had a higher average yield (263.03 \pm

116.87) than treatment HB (229.44 ± 91.93), without the difference being statistically significant ($P = 0.496 > 0.05$); the same also occurred in year 2021 ($P = 0.096 > 0.05$).

In 2020, treatment HB yielded fruit with a nearly equal mean diameter (27.63 ± 0.51) to those resulting from the OP treatment (27.54 ± 0.83). In 2021, mean fruit diameter in treatment HB (28.46 ± 0.49) was significantly greater ($P < 0.001$) than that of the OP treatment (26.61 ± 0.83).

The mean fruit set of the 'Regina' variety obtained from treatment IS in years 2020 and 2021 (Table 3) was significantly lower than the mean fruit set rates of HB ($P < 0.001$) and OP ($P < 0.001$) treatments. In addition, OP treatment had a higher mean fruit set rate than treatment HB in both the years of the experiment, but the difference was not found to be statistically significant (2020: $P = 0.529 > 0.05$; 2021: $P = 0.913 > 0.05$).

The average production obtained from treatment IS in 2020 and 2021 was equal to zero. The few fruits that had been formed turned yellow and dropped in the first wave of fruit drop. The 2020 OP treatment had a higher average production (518.90 ± 111.27) than treatment HB (413.96 ± 139.08), without this difference being statistically significant ($P = 0.105 > 0.05$); the same also happened in 2021 ($P = 0.063 > 0.05$).

TABLE 3
REGINA. RESULTS FOR YEARS 2020 & 2021 (MEAN \pm S.D)

Treatment	Fruit set (%) 2020	Fruit set (%) 2021	Production (g/m) 2020	Production (g/m) 2021	Size (mm) 2020	Size (mm) 2021
IS	6.30 ± 5.03^a	5.10 ± 4.60^a	0.00 ^a	0.00 ^a	-	-
HB	56.90 ± 11.14^b	37.50 ± 9.03^b	413.96 ± 139.08^b	178.66 ± 48.21^b	23.91 ± 0.64^a	24.6 ± 0.50^a
OP	59.00 ± 9.34^b	38.00 ± 11.00^b	518.90 ± 111.27^b	251.57 ± 83.95^b	23.70 ± 0.85^a	23.92 ± 0.78^b

The means of each column followed by a different letter are significantly different at the 0.05 significance level.



FIGURE 3: Regina (2020): Open Pollination (A), HB Pollination (B)

Although the difference in fruit set percentages was rather small, in treatment HB a higher rate of fruit drop was recorded, which resulted in fewer fruits remaining on the trees. In particular, after fruit set, some fruits ceased to grow, turned yellow and dropped in the first abscission wave (obviously a result of unsatisfactory pollination). For instance, we compare two repetitions of treatments OP (2nd-2020) and HB (8th-2020), with an almost equal number of immature fruits (91 and 88, respectively), as regards their final yields (Fig. 3 A and 3 B). The weight of the mature fruits in the 2nd repetition of treatment OP was 526 g, whereas their weight in the 8th repetition of treatment HB was 358 g.

Average production was lower than that in 2020 in both treatments (IS & HB). The OP treatment in 2020 yielded fruit with an almost equal diameter (23.91 ± 0.64) to those obtained from treatment HB (23.70 ± 0.85). In 2021, the average fruit diameter (24.63 ± 0.50) from treatment HB was significantly greater ($P = 0.031 < 0.05$) than the average diameter (23.92 ± 0.78) in the OP treatment.

TABLE 4
LAPINS. RESULTS FOR YEARS 2020 & 2021 (MEAN \pm S.D)

Treatment	Fruit set (%) 2020	Fruit set (%) 2021	Production (g/m) 2020	Production (g/m) 2021	Size (mm) 2020	Size (mm) 2021
IS	3.10 ± 3.41^a	24.80 ± 4.63^a	6.25 ± 14.45^a	192.09 ± 53.61^a	26.00 ± 2.00^{ac}	25.10 ± 0.73^a
HB	26.60 ± 9.95^b	38.90 ± 7.47^b	76.18 ± 43.87^b	323.38 ± 70.42^b	27.53 ± 0.50^{ab}	24.64 ± 0.45^a
OP	33.80 ± 23.07^b	50.10 ± 8.11^b	79.74 ± 51.60^b	456.07 ± 230.02^b	26.25 ± 0.79^c	22.78 ± 1.08^b

The means of each column followed by a different letter are significantly different at the 0.05 significance level

The 2020 mean fruit set rate of 'Lapins' in treatment IS (Table 4) was significantly lower than the mean fruit set rates of treatments HB ($P < 0.001$) and OP ($P < 0.001$). Also, significant differences were recorded in 2021 between the mean fruit set rate of treatment IS and those of HB ($P = 0.001 < 0.05$) and OP ($P = 0.007 < 0.05$). The OP treatment in 2020 yielded a higher mean fruit set than treatment HB, without the difference being statistically significant ($P = 0.383 > 0.05$), and the same occurred in 2021 ($P = 0.059 > 0.05$). In 2021 higher fruit set rates were recorded compared to year 2020.

The average production in treatment IS was significantly lower than the yields of treatments HB ($P < 0.001$) and OP ($P < 0.001$) in 2020 and 2021 (HB: $P = 0.002 < 0.05$; OP: $P = 0.002 < 0.05$). The OP treatment in 2020 and 2021 produced a higher average yield (2020: 79.74 ± 51.60 ; 2021: 456.07 ± 230.02) than that obtained from HB (2020: 76.18 ± 43.87 ; 2021: 323.38 ± 70.42), but the difference was not statistically significant (2020: $P = 0.739 > 0.05$; 2021: $P = 0.089 > 0.05$).

The average fruit diameter in treatment IS did not differ significantly from the fruit sizes yielded in treatments HB ($P = 0.314 > 0.05$) and OP ($P = 0.739 > 0.05$) in 2020. However, significantly greater was the average fruit diameter in treatment IS than that obtained from treatment OP ($P < 0.001$) in year 2021. The average OP treatment diameter in 2020 (26.25 ± 0.79) was significantly smaller than that in treatment HB ($P < 0.001$). The same result was observed in year 2021 ($P < 0.001$). Measurements of the variable "number of flowers per branch meter" were carried out both in 2020 and 2021 (table 5).

TABLE 5
FLOWERING (FLOWERS/M)

Variety	Year 2020 (N = 30)	Year 2021 (N = 30)	Period 2020-2021
<i>Ferrovia</i>	163.25 ± 45.29	170.12 ± 35.03	166.68 ± 40.29
<i>Regina</i>	146.31 ± 34.77	136.99 ± 33.54	141.66 ± 34.20
<i>Lapins</i>	45.07 ± 17.67	150.07 ± 41.32	97.57 ± 61.61

'Ferrovia' had 166.68 ± 40.3 flowers per meter of fruit bearing branch, 'Regina' had 141.66 ± 34.2 flowers and 'Lapins' had 97.57 ± 61.61 . The latter result can be attributed to the very intense pruning done in the winter of 2020, resulting in trees having a small number of flowers.

IV. DISCUSSION

No statistically significant differences in fruit set between HB and OP treatments were found, a fact that reveals the high efficiency of honeybees in the pollination of the 'Ferrovia' cultivar. Differences appear to be due to the following factors: (i) inside the HB treatment cage, there were only two pollinizers, namely a 'Regina' tree and twenty blooming 'Kordia' branches, a fact that restricts pollen supply in terms of time span, diversity and quantity, in comparison to the open pollination; ii) there are marked differences in bee activity between bees isolated in cages (HB) and those that are involved in open pollination [1]; iii) the presence of various kinds of bumble bees living in the natural environment is likely to have improved the performance of honeybees in this cross-pollinated variety [13]. The variation in the fruit set rates of this variety

has also been observed in open pollination by Kazantzis [25], who cites a mean fruit set rate of 14.30%. Generally, in 2021 fruit set rates were lower compared to those in 2020. Treatment HB (15.00 ± 4.26) had the lowest fruit set. The low temperatures recorded in April 2021 (to cite some examples: on 08/04 minimum temperature: 5.5°C, maximum: 12.0°C; on 09/04 minimum: -1.0°C, maximum: 14.0°C; and on 10/04 minimum: 2.5°C, maximum: 17.0°C) appear to have negatively influenced both the flower fertilization process and bee foraging activity. Zhang et al [41] showed that the germination of pollen grains is limited under conditions of low temperatures. What is more, low temperatures slow down the growth of the pollen tube along the style and by the time it reaches the embryo sac, oocytes are highly likely to have lost their fertility [7]. According to Clarke & Robert [8], the most important factors that also affect the observed changeability in bee-pollinator activity include temperature and solar radiation. Still, the OP gave higher fruit set compared to HB, a fact that proves that the insect pollinators of the research area are very effective. However, no record of these pollinators (species diversity or abundance) is known, and it is a subject of further experimentation.

Other factors that have been reported to affect fruit set and should be investigated further are the nutritional state of trees [7], the low and high temperatures during the flowering period [5], [19], [34], [41], as well as the graft-rootstock combination [2]-[4], [11]. As regards the effects of the wind on the pollination of the 'Ferrovia' cultivar, it appears from the results of treatment IS that the wind is not a means of transporting pollen for cherry pollination, and this is also corroborated by James & Measham [24]. The greatest difference in 'Ferrovia' yields was observed in 2021, and this is likely due to the lower rates of fruit set and hence to the lower fruit load born by the HB treatment trees. Einhorn et al. [15] investigated the effects of thinning cherry fruit bearing organs on production, quality, and value of the finished product. The trees that had undergone thinning had a 40% to 54% lower yield compared to the control trees, but produced a higher percentage of large sized fruits.

'Regina' exhibited lower percentages of fruit set (HB: 37.50%; OP: 38.00%) in year 2021, both in comparison with those in 2020 (HB: 56.90%; OP: 59.00%) and with the mean fruit set of earlier observations on the same farm, estimated to be equal to 48% [7]. Experiments by Sagredo et al. [36] have showed that the period of effective pollination of the 'Regina' cultivar lasts approximately 5-6 days, significantly dependent on temperature. In our study, too, it appears that the low temperatures prevailing in April 2021 adversely affected fruit set, exactly as it happened with 'Ferrovia' trees. In treatment HB a higher rate of fruit drop was recorded in the first abscission wave, which resulted in fewer fruits remaining on the trees. The lower average production in 2021 in both treatments (IS & HB) is due to the smaller rate of fruit set taking place in this year. It can also be concluded that the wind does not contribute at all to the pollination of 'Regina' flowers. The lower fruit load born by the HB treatment trees resulted in a significantly greater average diameter in comparison to those in the OP treatment.

Results indicate that the self-fertile 'Lapins' formed fruits without the presence of insect-pollinators in treatment IS, albeit with a significantly lower fruit set compared to HB and OP. According to Klein et al. [26], sweet cherry pollination without insect visitation is only possible with passive self-pollination. Higher mean fruit set rates in open pollination (Open: 54.5% - Caged with bees: 48.6%) were recorded by Andersen & Choi [1]. The differences observed in the fruit set rate were attributed to the shading caused by the nets and the limited bee activity. In the present case, a possible reason that this self-fertile cultivar produced smaller fruit set rates in treatment HB could be that the bees demonstrated very little activity within the cage owing to its exceptionally small size, since the IS treatment concerned solely a single tree. The daily rainfall recorded in the study area between 23/03/2020 and 07/04/2020 (190 mm in total) appears to have negatively affected fruit set in 'Lapins', as compared to year 2021. Somerville [37] claims that during rainfall, bee flights are limited and bees fly only for very short distances. Low temperatures and high atmospheric humidity inhibit bee activity, suppress the dehiscence of anthers and slow down pollen release from open flowers [41]. In addition, Clarke & Robert [8] report that after rainfall, the germination of 'Regina' pollen was reduced from 78% to 28%, and the number of pollen grains transported to the stigma was also smaller.

Significantly reduced was the average production in IS treatment as compared to treatments HB and OP, a fact that is attributed to the lower fruit set percentage. By comparing the results for both years, it can be observed that both fruit set and yield were considerably lower in 2020, as compared to 2021. It became evident from the beginning of the experiment that the intense pruning of 'Lapins' had a negative impact on its flowering in 2020 and this resulted in reduced yields. According to von Bennowitz et al. [39], the highest yields are achieved with no pruning or with soft pruning treatments, while medium- and large-scale treatments cause substantial yield reductions per tree. Fruit diameter differences among treatments are, in all cases, due to the smaller fruit load born by the trees that yielded bigger sized fruit.

V. CONCLUSION

From the results of the present research project it can be concluded that the pollination of cherry flowers with insects is an ecosystem service, essential to both cross- and self-fertile cherry cultivars. Wind is not a means of transporting pollen to sweet cherry trees.

Honeybees are exceptionally effective managed pollinators and thus they constitute a valuable agricultural input that ensures high yields in commercial sweet cherry orchards. At the same time, the presence of other pollinators, mainly bees, is shown to be of great importance. Although in this study no records of other pollinators' diversity have been kept, the maintenance of a landscape suitable for nesting sites for solitary bees and bumble bees seems to support pollination services required for higher yields. Honeybees may be more numerous, but solitary bees may be more efficient. The latter still needs to be investigated, taking into account the particular varieties as well as the particular landscape composition and climatic conditions.

The applied experimental method, was fast, simple, of low labor cost, and yielded robust results that are easily comprehensible by the sweet cherry growers.

One of the disadvantages of the "isolation" method is that for its application the planting schedule of the cultivars under investigation should be carried out in a manner that facilitates their joint isolation in cages, so as to avoid using blooming branches of the pollinating cultivars, but whole trees. Furthermore, big-sized isolation cages must be used so that pollination conditions simulate those prevailing in open fields.

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The Effect of Irrigation Frequency and Amount on the Growth and Yield of Kale (*Brassica Oleracea var. Acephala*)

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Abstract— A study was conducted in which Kale (*Brassica oleracea var. Acephala*) was grown in a Randomized Block Design experiment at the Faculty of Agriculture at Luyengo Campus of the University of Eswatini to compare five different irrigation frequencies (treatments) on the production of kale. The five treatments were: Treatment 0 (T0) the control irrigated daily – applying 5.0 mm, treatment 1 (T1) irrigated after two days, treatment 2 (T2) irrigated after three days, treatment 3 (T3) irrigated after four days and treatment 4 (T4) irrigated after five days. Two litre (2 L) soft drink plastic bottles were used as the irrigation method. The results showed that irrigating kale every two days and applying 14 mm of water was the best option for the Luyengo area compared to applying 5 mm daily. Skipping more than two days between irrigations resulted in significantly lower yields. There were no significant differences in the other measured plant parameters: leaf length, leaf width, plant height and the number of leaves per plant. The results of these parameters for treatment 1 (T1) were higher of that of the other treatments.

Keywords— Kale, yield, Bottle drip, irrigation, frequency, amount.

I. INTRODUCTION

Kale is botanically known as *Brassica oleracea var. Acephala*, belonging to the family Cruciferae / Brassicaceae. It is regarded as a winter vegetable, easy to grow, and the leaves can be continuously harvested throughout its life cycle. It grows well in moist but well-draining soils. Kale tolerates slightly acidic soils with a pH range between 5.5 – 6.5 that has plenty of organic matter such as compost (Hodges, 1991). Cultivars of this vegetable differ primarily in plant size, leaf colour and texture. Some varieties that are sold as "flowering kale" are used as ornamental plants or for decoration.

Eswatini import approximately 37,300 metric tonnes of fruits and vegetable with a value of US\$11,000,000 from South Africa (NAMBOARD, 2018). This is because the annual rainfall distribution in the country is skewed, with the most rainfall 1,500 mm received in the Highveld region and the least 450 mm in the Lowveld region (Dlamini and Khumalo, 2019). The Lowveld is the ideal place for vegetable production, but due to lack of water, rural communities struggle to make ends meet. Vegetable production can only be a success if grown under irrigated conditions. However, the energy requirement associated with irrigation makes its adoption difficult.

Vegetables are a necessity in rural communities as they provide the people with most of the required nutrients. Kale though a highly nutritious vegetable is hardly grown due to lack of water and the technical know-how on its agronomic requirements.

The adoption of low energy agricultural technologies like drip in the country is very slow, as the Eswatini government tends to promote conventional methods of water resource development as opposed to micro irrigation which is ideally suited to small holder farmers (Manyatsi and Magongo, 2008). Drip irrigation can be more efficient than sprinkler and furrow irrigation (Hunsaker et. al., 2019; Bajracharya and Sharma, 2005) since only the root zone of the cropped area is irrigated (Dukes et. al., 2006 and Hartz, 1999). It places water and nutrients where they are needed most with minimal energy requirements.

A majority of the soils where vegetables are grown are sandy with very low water holding capacities. These require frequent irrigation and fertigation to minimize crop stress and to attain maximum production. The main drawback with drip systems is

the frequent emitter blockages (Zhou et al., 2019). Vegetables are easier to grow and many like kale are considered as food components that significantly influence human health and well-being (Dunja et al., 2018). Dunja et al. (2018) also noted that other authors have also recognized that the vegetable kale, among cabbages, was the best source of vitamins (A, B1, B2, B6, C and E), folic acid and niacin, fatty acids, and essentials minerals (especially K, Ca, Mg, Fe and Cu) (Ayaz et al. 2006; Jahangir et al. 2009; Eryilmaz Acikgoz and Devenci 2011; Thavarajah et al. 2016) but their level may depend on the environmental and growing factors (Fadigas et al. 2010; Bjorkman et al. 2011; Westwood et al. 2014).

Since the capital cost of drip irrigation is beyond the reach of many rural farmers (von Westarp et al., 2004; Sandhu et al., 2019; Holmer and Schnitzler, 1997) including Eswatini, the bottle drip system (Dlamini and Khumalo, 2019; Darouich et al., 2014) offers a feasible option for economic production in areas of low rainfall or during periods of water scarcity. A bottle drip system is an easy way of watering plants (Darouich et al., 2014), no costs is involved in purchasing the bottles as old material is useful, no power or piping required to supply the water and it's very easy to make (Isaac, et al., 2013).

The purpose of this study was to evaluate the effectiveness of using spent two (2 L) litre plastic bottles as a medium of irrigation on the growth and yield of kale (*Brassica Oleracea var. Acephala*) grown at Luyengo, Eswatini.

II. MATERIALS AND METHODS

In order to test the response of kale (*Brassica oleracea var. Acephala*) to the method of water delivery by the used two litre (2 L) plastic bottle drip-irrigation systems, a Randomised Design field plot experiment was established in the Agricultural and Biosystems Engineering plot of the University of Eswatini at Luyengo campus. The plot is located in the Middleveld of Eswatini at 21°34' S and 31°12' E at an altitude of about 730 m above sea level. The average seasonal temperature of the area is 18°C. The experiment was conducted during the months of August to September 2021.

The crop was transplanted and allowed to establish for 10 days, in which all the treatments received the same amount of water which was equal to that of the control treatment. There after the treatments were applied and the crop grown for six weeks. The numbers of leaves were counted weekly and simultaneously measurements of leaf height, leaf width and plant height were taken. At the end of the experiment in week six, the wet and dry weight of harvested kale was measured. The data was then analysed to check for differences in the treatments.

2.1 Experiment Design

The experiment was a Randomized Block Design with five treatments that were replicated three times. There were three blocks, each block with three replicates. Kale was planted at a spacing of 45 cm within rows and 90 cm between the rows. There were five plants per treatment, each plant with a two litre (2 L) used plastic bottle as the method of irrigation.

The experiment had five treatments as summarized in Table 1. The daily water use of kale is estimated to range between 3.6 mm to 5.7 mm depending on the location (Chakwizira et al., 2014). A daily water use of 5 mm was selected to be the control.

TABLE 1
THE FIVE TREATMENTS THAT WERE APPLIED IN THE EXPERIMENT

Treatment	Description	Daily Equivalent depth of water applied (mm)
T0	Control – irrigated daily with 350 mL water	5.0
T1	2000 mL water applied every two days	14.1
T2	2000 mL water applied every three days	9.4
T3	2000 mL water applied every four days	7.1
T4	2000 mL water applied every five days	5.7

2.2 Bottle drip equipment

Two litre (2 L) used soft drink plastic bottles with holes 2 mm in diameter drilled on the lids were used to irrigate the kale plants. The aim was to have a uniform discharge from the holes in all the bottles. A hole was dug next to each plant and the bottle buried approximately one-third deep with the bottom facing up (Fig. 1)

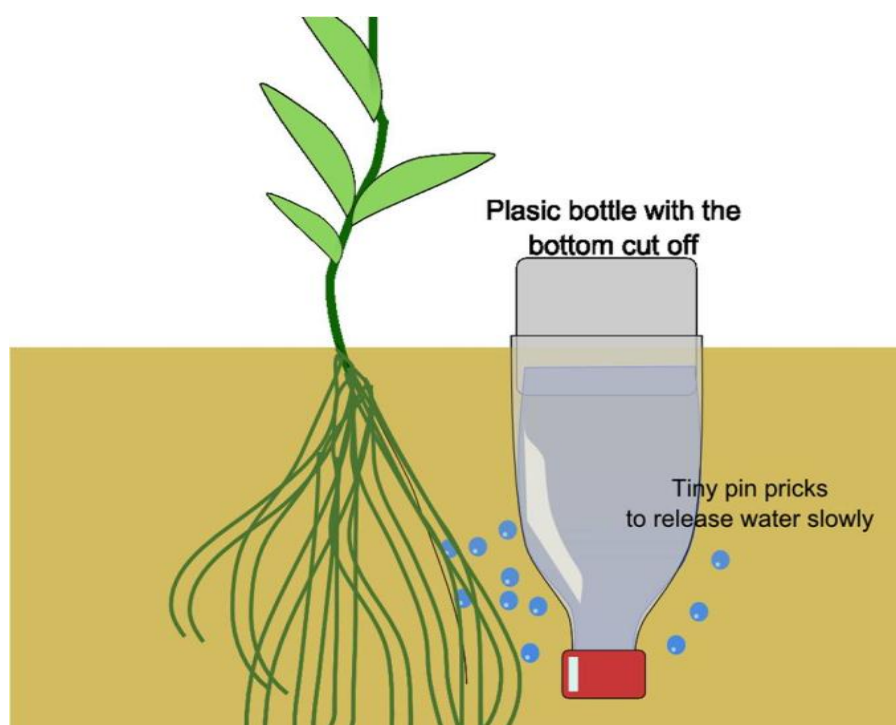


FIGURE 1: An example of a two (2 L) spent plastic cold drink bottle drip irrigation system (Dlamini and Khumalo, 2019)

2.3 Transplanting

Seedlings were obtained from Vickery Seedlings, a local company that supply ready to be planted seedling located at Malkerns. Basal fertilizer dressing was done using N:P:K; 2:3:2 (37) fertilizer at a rate of 100 kg/ha. The seedlings were planted 10 cm away from the bottle drip system. Initially two kale plants were planted and later thinned to one plant a week after transplanting. Top dressing was done when the plants were about 10 cm tall at a rate of 5 grams per plant.

III. RESULTS AND DISCUSSION

3.1 Yield and growth parameters

Results of yield and growth parameters (number of leaves, leaf length (mm), leaf width (mm), plant height (mm), wet and dry weights (g)) are summarised in table 2.

**TABLE 2
MEAN YIELD AND GROWTH PARAMETERS FOR THE KALE EXPERIMENT AT HARVEST.**

	Treatment Means					Level of Significance ($P < 0.05$)	LSD
	T0	T1	T2	T3	T4		
Number of leaves	7.6	8.6	7.8	8.9	7.9	NS	2.8
Leaf Length (mm)	260.1	271.3	266.6	252.7	263.8	NS	79.6
Leaf Width (mm)	169.8	187.0	185.1	176.1	181.0	NS	50.9
Plant Height (mm)	194.2	191.4	231.1	195.6	207.9	NS	88.3
Wet Weight (g)	547.1	653.6	613.0	598.0	532.0	**	2.0
Dry Weight (g)	87.2	105.0	98.5	96.2	85.7	**	1.7

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

The results show that there were no significant differences in all the measured growth parameters except for the wet and dry weights at harvest. There were highly significant differences ($P < 0.01$) in the results for fresh and dry kale mass for treatments T1 and T2 when compared to T0, T3, and T4. The application of 14.1 mm every two days resulted in the best kale yields.

Although all the other parameters were not statistically significant ($P > 0.05$), the treatment that was irrigated every two days showed superior qualities compared to the others. This means that irrigating kale every two days and applying 14 mm was the optimum irrigation schedule for the Luyengo environment. Delaying irrigation until the fifth day was not the best option for the site, as this resulted in the lowest averages for the parameters.

Irrigating kale every day and applying 5 mm resulted in significantly lower yields compared to scheduling irrigation every two days and applying 14 mm. This could mean that for the Luyengo site, the estimated average 5.0 mm of evapotranspiration was not suitable for kale production as this figure included evaporation and kale transpiration.

IV. CONCLUSION

It was concluded that the home-made bottle drip irrigation method could be recommended for rural people in Eswatini who cannot afford to buy commercial drip system for the production of vegetables for household consumption. For the conditions of the experiment, irrigating kale every two days and applying 14 mm was the best option. It was observed that a kale evapotranspiration figure of 5 mm per day was an under-estimate for the Luyengo site. The growth of kale was significantly affected by both the timing and amount of water applied.

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Varietal Evaluation and Preference Analysis of Sixteen Released Rice Varieties in Bhojad, Chitwan, Nepal

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Abstract— A study on varietal selection trials for different varieties of rice (*Oryza sativa* L.) was conducted in the field of Nepal Polytechnic Institute, Bhojad, Chitwan, Nepal with the objective of identifying suitable, high yielding promising rice varieties for the interest of the farming community. The experiment was carried out using 16 different treatments of released rice varieties in Randomized Complete Block Design (RCBD) with the identity on growth parameters like plant height, several tillers per meter square, panicle length, and yield parameters like effective tillers per meter square, filled grains per panicle, sterility percentage thousand-grain in weight, grain yield, straw yield, and Harvest Index were recorded. Data was entered and tabulated using MS-Excel while Analysis of variation and mean separation was done using R-Studio. The variation among varieties was observed for various traits. The variety Bahugunidhan-2 produced the highest grain yield (5.9 t/ha) followed by Ramdhan with the yield of (5.25 t/ha). The statistical analysis showed that the variety Sukha Dhandhan-3 was found earlier in 50% heading (84.67days), and maturity (125days). It was closely followed by Sabitri and Ramdhan which took 87.67 days to 50% heading and 139 and 138 days respectively for days to attain maturity. The information on variation for the traits among studied rice varieties will be helpful to plant breeders in constructing their breeding materials and implementing selection strategies in the future. Inappropriate varietal evaluation and selection in our country have led to the less development of variety with high yield potential. Improvement and participatory selection of promising rice varieties by studying genetic variability, character association between yield traits, and farmer preference analysis help in rice breeding for the selection of best rice varieties in the Chitwan district of Nepal.

Keywords— Harvest Index, Grain yield, Drought, Test weight, Maturity Indices.

I. INTRODUCTION

Rice is the principal staple food for more than 3.5 billion people worldwide (IRRI, 2017). Rice (*Oryza sativa* L.) belonging to Family Poaceae, Sub-family Oryzoideae, tribe Oryzae with chromosome number 24 is a perennial, self-pollinated, and semi-aquatic plant. Rice ranks third most staple crop after Maize & Wheat in the world in terms of production (FAOSTAT, n.d.). Its annual production in the world is 755473800 tons and the yield of 4661kg/ha in the year 2019(FAOSTAT, n.d.). Rice occupies first place in terms of area coverage, production, productivity, and preferences in Nepal and it contributes 15.35% to the AGDP (MOALD, 2020). With the increasing population, the global rice demand will rise to about 765 million tons by 2030 (Akon et al., 2015). Rice contributes nearly 20% to the agricultural gross domestic product (AGDP) and almost 7% to GDP, as well as it supplies 40% of food calorie intake (Neupane & Khadka, 2019). Nepal is one of the important centers of rice genetic resources (MoAD, 2015), having over 1,700 landraces of rice. Rice is cultivated under diversified agro-climatic zones in Nepal from terai (50masl) to mid-hills and high mountains valley (3050masl) in Jumla (the highest altitude of rice growing location in the world) (Kandel, 2018).

The intensity of use of improved seeds and inorganic fertilizers in cereals is very low in Nepal (Report, 2010), resulting in less exploitation of the land production potential. We cannot deny the fact that the use of new, high-yielding varieties, instead

of traditional rice varieties brought huge gains in yield, but the planting of a single variety over large areas year after year may compromise genetic resistance to pests (Khanal, 2017). IDespite65.6% of Nepalese people engaged in agriculture, the major problem here is food insecurity. There is significant role of rice farming in poverty reduction and food security, but ignoring this fact the investment made in the study and research of this crop is very low. As a result, rice yield growth has been negligible in the past two decades. The development of a nation is paced by agricultural development and rice is the most important sub-sector of agriculture in Nepal (Mahato & Adhikari, 2017). The country which used to export rice in the past, now imports about one million tonson of milled rice every year. This causes a serious challenge to rural poverty reduction as well as to food and nutrition security in the country. The problem will become more serious in coming decades.

The attainable yield could not be achieved due to lack of efficient exploitation of use of varieties suitable in accordance to agro ecological niches, in accordance to soil fertility gradient and extent of use efficiency of major fertilizers results in low production of rice (Sarwar et al., 2011). Besides this the, rice cultivating area is decreasing due to human activities like urbanization and industrial expansion. Increasing the productivity of rice is the only option as there is no further chance for bringing more area under cultivation (Lamsal et al., 2018). The production and productivity of rice has not geared up as much as required with traditional system of cultivation despite a lot efforts are being made. By inviting farmers to make decisions in the research process, it is assumed that they will not only adopt but also, more importantly, adapt the available technology to their own needs and environment (Rice & Ashby, 2007). Moreover, varietal assessment leads to the study of yield and yield attributing traits which together with the farmer preference analysis helps in more effective crop breeding program. Also, there is a need to move forward from the Green Revolution to a 'gene revolution,' which is more productive and more 'green' in terms of conserving natural resources and the environment (Atkins & Bowler, 2020).

II. MATERIALS AND METHODS

The research entitled '**Varietal evaluation and performance analysis of promising rice genotypes in Bhojad, Chitwan, Nepal**' was carried out during rainy season of 2020. The trial was carried out at Bharatpur -11, Bhojad, Chitwan, Nepal located between longitudes 83°54' 45'' to 84°48' 15''E and latitudes 27°21'45'' to 27°52' 30''N. Research domain is Rain-fed lowland (Upto 250masl) with rice – mustard – maize / vegetables system. The experiment was laid out in one factorial Randomized Complete Block Design (RCBD) with three replications including 16 treatments with plot size: Gross: 3m × 2m = 6m². Nursery was managed in the field of Nepal Polytechnic College which was raised with the sloppy surface at the middle so as to drain the water after rainfall quickly and the 30cm distance was kept between two consecutive beds. A total of 30 days old seedlings and 2 seedlings per hill were planted in the geometry of 20cmx20cm. The spacing between two consecutive plots was maintained 1m. All the necessary cultivation process and techniques were conducted by taking in consideration to maintain uniform plant population in each plots. Also the recommended application of inputs were done. Data were collected and observed as phenological observation and biometric observations. When most of the treatments reached 80% maturity, all of the varieties were subjected to an evaluation technique called "Preference Analysis" by farmers, extension workers, breeders and other stakeholders, inclusive of both men and women. The preference analysis (PA) of genotypes was done by casting votes for that genotype. The voting box was placed in each plot and sixteen cards i.e. 8 rights and 8 wrong were given in the hands of the farmers (male and female farmers) separately for casting votes. The right vote denotes the superior characters possessed by the genotypes to fit under local conditions while wrong denotes the inferior character. The preference index was calculated for each genotype by using the following formula.

$$\text{Preference index} = \frac{\text{No. of positive votes} - \text{No. of negative votes}}{\text{Total votes cast}}$$

Data entry and tabulation was done by using MS-Excel 2013 and for word processing MS-Word 2013 used. Statistical analysis for the yield and yield attributes were done through F-test and R-test and preference analysis was done on CGIAR model.

III. RESULTS AND DISCUSSION

3.1 Yield and yield attributing traits

The performance of different genotypes for yield and yield attributing traits is presented in table number 1 and 2. Results for effective tiller/m² was highest in Lakla basmati which is followed by the variety Sabitri, Ramdhan, Sukha Dhan-3, Samba

mansulisub-1, Sudodhan kalanamak, Sugandhit Dhan. The tallest plant height was recorded to be variety Sudodhan Kalanamak (165.11cm) followed by lalka basmati, Sugandhit dhan and the shortest height was found in Samba mansulisub-1 (93.22cm). The minimum maturity days was recorded in variety Hardinath-1. It was found that lalka basmati had largest panicle length i.e. 30.11 cm which is statistically at par with variety sabitri, Ram Dhan, Sukha Dhan-3 and Sukha Dhan-4. The variety Chaite-2 had significantly highest 1000 seed weight i.e. 25.6 gram followed by Bahugunidhan-1, Bahugunidhan-2, Sabitri, Ramdhan, Samba mansulisub-1, Sukha Dhan-4, Radha-4 Sukha Dhan-3. Bahugunidhan-1 had highest grain yield i.e. 5.96 ton/hectar followed by Ram Dhan (5.25) and Sudodhan Kalanamak had the lowest grain yield i.e. 2.33 ton/hectar. Bahugunidhan-2 had highest straw yield i.e. 10.4 kg which is statistically at par with Chaite-2 and Sukha Dhan-3 had the lowest straw yield i.e. 6.6 ton/hectare. Likewise, Bahugunidhan-1 had highest biological yield i.e. 16.4 ton/hectar and Sukha Dhan-3 had the lowest biological yield i.e. 10.3 ton/hectar. Moreover, Mithila Dhan has highest harvest index i.e. 40.

3.2 Preference Analysis

The preference score of different rice varieties on participatory varietal selection were presented in table 4.4. The total preference score was found highest in Radha-14 followed by Sugandhit-dhan and Bahuguni-2. Female preference score is highest in all 3 of the variety. According to them, the reason behind this preference is due to early maturity, long panicle, and shorter the plant height. In addition to this the filling nature of grain also ranked it in topmost. The preference score is minimum in Sudo-dhan Kalanamak followed by hardinath-1 and lalka basmati. Preference score is minimum in Sudodhan Kalanamak is due to its late maturity and lodging nature. However, grain yield was maximum in Bahuguni-2, the preference score was maximum in Radha-14 on participatory varietal selection. This indicates Radha-14 have some gene of farmer interest. The preference score and yield of Sudodhan Kalanamak was minimum. This was due to longer growth period of variety. Variation in test weight was proven statistically significant. It may be due to environment factor i.e. temperature effect seed weight and also correlated with moisture percentage (Nirmaladevi et al., 2016).

TABLE 1
YIELD ATTRIBUTING TRAITS OF DIFFERENT RICE GENOTYPES

Treatment	Effective tillers/m ²	Panicle length(cm)	Plant height (cm)
Lalka Basmati	424 ^a	30.11 ^a	154.46 ^a
Sabitri	410 ^{ab}	29.24 ^{ab}	125.47 ^{bc}
Ram Dhan	409 ^{ab}	29.03 ^{ab}	116.2 ^{cd}
Sukha Dhan-3	404 ^{abc}	28.94 ^{abc}	133.59 ^b
Sukha Dhan-4	401 ^{abcd}	28.84 ^{abc}	108.13 ^d
Samba mansulisub-1	399 ^{abcd}	28.77 ^{bc}	93.22 ^e
SudodhanKalanamak	398 ^{abcd}	28.7 ^{bc}	165.11 ^a
SugandhitDhan	397 ^{abcd}	28.57 ^{bc}	155.35 ^a
MithilaDhan	381 ^{bcd}	28.55 ^{bc}	123.41 ^{bc}
Radha-14	367 ^{cde}	27.68 ^{cd}	118.23 ^{cd}
Chaite-2	363 ^{de}	27.02 ^d	127.8 ^{bc}
Bahugunidhan-1	341 ^{ef}	26.92 ^d	126.28 ^{bc}
Radha-4	313 ^{fg}	26.46 ^d	132.8 ^b
Bahugunidhan-2	312 ^{fg}	24.9 ^e	124.3 ^{bc}
Hardinath-1	312 ^{fg}	24.2 ^e	106.99 ^{de}
Swarna-sub1	297 ^g	24.16 ^e	124.01 ^{bc}
F test	***	***	***
CV(%)	6.2	2.87	6.6
LSD (0.05)	38.24	1.32	14.1
Grand Mean	370.4	27.63	127.2

TABLE 2
YIELD AND YIELD ATTRIBUTING TRAITS OF DIFFERENT RICE GENOTYPES

Treatment	Grain yield ton/ha	Straw yield ton/ha	Biological yield ton/ha	HI	Test weight(g)
Lalka Basmati	3.05 ^{efg}	8 ^{cdef}	11.1 ^{fghi}	27.3	18 ^{de}
Sabitri	4.58 ^{abcd}	7.8 ^{cdefg}	12.4 ^{def}	36.1	22.4 ^{abc}
Ram Dhan	5.25 ^{ab}	8.4 ^{bcde}	13.6 ^{bcd}	37.9	22.2 ^{abc}
Sukha Dhan-3	3.76 ^{cdefg}	6.6 ^h	10.3 ⁱ	36.2	21 ^{bcd}
Sukha Dhan-4	2.5 ^{fg}	8.5 ^{bcd}	11 ^{ghi}	22.4	21.4 ^{bcd}
Samba mansulisub-1	4.05 ^{bcde}	7.5 ^{defgh}	11.6 ^{efghi}	34.8	21.6 ^{bcd}
SudodhanKalanamak	2.33 ^g	8.1 ^{cdef}	10.4 ⁱ	22.1	15.3 ^e
SugandhitDhan	3.41 ^{d^{efg}}	7.1 ^{fgh}	10.5 ^{hi}	32.3	20.1 ^{cd}
MithilaDhan	4.55 ^{abcd}	6.7 ^{gh}	11.2 ^{fghi}	40.4	21.4 ^{bcd}
Radha-14	4.53 ^{abcd}	7.3 ^{efgh}	11.8 ^{efgh}	38.2	22.3 ^{abc}
Chaite-2	4.58 ^{abcd}	9.5 ^{ab}	14.1 ^b	32.4	25.6 ^a
Bahugunidhan-1	5.2 ^{abc}	8.5 ^{bcd}	13.7 ^{bcd}	25.4	24.3 ^{ab}
Radha-4	3.36 ^{defg}	8.6 ^{bcd}	11.9 ^{efg}	28.1	21.3 ^{bcd}
Bahugunidhan-2	5.96 ^a	10.4 ^a	16.4 ^a	36.2	23.1 ^{abc}
Hardinath-1	3.91 ^{bcdef}	8.6 ^{bcd}	12.6 ^{cde}	31	20.7 ^{bcd}
Swarna-sub1	5.03 ^{abc}	8.8 ^{bc}	13.9 ^{bc}	36.1	20.8 ^{bcd}
F test	**	***	***	NS	**
CV%	21.5	8.71	6.43	27.04	10.5
LSD (0.05)	1.48	1.18	1.31	14.5	3.74
Grand Mean	4.13	8.1	12.3	32.35	21.3

TABLE 3
PREFERENCE RANKING OF TESTED VARIETIES IN THE EXPERIMENT DURING 2020 (MALE=11, FEMALE= 14)

T.N	Varieties	Good		Total	Bad		Total	PI	Rank
		Male	Female	PS	Male	Female	NS		
T ₁	Lalka Basmati	1	2	3	20	16	36	-0.0325	14
T ₂	Sabitri	2	1	3	7	10	17	-0.0175	14
T ₃	Ram Dhan	6	6	12	0	4	4	0.02	10
T ₄	Sukha Dhan-3	9	6	15	0	0	0	0.0375	7
T ₅	Sukha Dhan-4	5	6	11	5	2	7	0.0225	9
T ₆	SamaMansuli	4	5	9	9	12	21	-0.0075	13
T ₇	SudodhanKalanamak	1	0	1	12	23	35	-0.055	17
T ₈	SugandhitDhan	9	14	23	0	4	4	0.0475	3
T ₉	MithilaDhan	4	8	12	2	0	2	0.03	8
T ₁₀	Radha-14	8	20	28	2	0	2	0.07	2
T ₁₁	Chaite-2	7	4	11	12	9	21	0.005	11
T ₁₂	Bahugunidhan-1	9	10	19	0	1	1	0.045	5
T ₁₃	Radha-4	9	8	17	0	0	0	0.0425	6
T ₁₄	Bahugunidhan-2	9	10	19	2	0	2	0.0475	3
T ₁₅	Hardinath-1	2	5	7	10	22	32	-0.0375	16
T ₁₆	Swarna-sub1	3	7	10	7	9	16	0.0025	12
	Total ballots	88	112	200	88	112	200	0.22	

Note: PS=Preference Score, each farmer provided 8 good & 8 bad ballots, Preference index = (No. of positive votes – No. of negative votes)/ (Total votes cast)

IV. CONCLUSION

From the study, we can mitigate that a proper varietal assessment trial involving participatory preference selection can be proved to be an essential step towards food security and improvement of rice breeding program. As the varieties Bahugunidhan-2 (ranked 3rd in preference ranking) and Ramadhan (ranked 7th in preference ranking) showed higher grain yield per hectare as comparison to others so I, as a researcher suggest for cultivation for high yielding as well as preferred varieties. Likewise, Radha-4 ranked 1st in preference ranking and also had, moderate yield and test weight can also be suggested for cultivation in Chitwan. However, the farmers nowadays took key interest in traits such as plant height, days to maturity, disease pest resistance along with yield components. So, all these factors should be taken into consideration in any kind of research.

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AUTHORS DECLARATION

The authors declare that there is no any conflict of interest. Mr. Bishnu Bilas Adhikari designed the layout and work plan for this research while Babita Dhungana performed cultivation practices, recorded the data, Aakash Adhikari and Babita Dhunagana performed statistical analysis of data, revised the first draft minutely and elaborated the final manuscript for publication. All the authors have read the manuscript and approve the final version.

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Food and Feeding Habit of *Heterotis Niloticus* in Oguta Lake, IMO State, Nigeria

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Abstract— A study on the food and feeding habits of *Heterotis niloticus* (Cuvier, 1829) was carried for a period of six months in Oguta lake of Imo state between January to June 2018. The aim of the study was to investigate the food and feeding habits of *Heterotis niloticus* within the lake. A total of 180 specimens of *Heterotis niloticus* were sampled and examined. The result of the stomach content analysis showed that *Heterotis niloticus* consumed food of animal origin more than those from plant origin with respect to the size of the fish. There is a shift in the food and feeding habit of this fish from carnivorous to omnivorous feeding habit before maturity. The most dominant diet from the juvenile to Adult sizes of the fish were Copepods 61.0%, Cladoceran 58.0%, Ostracoda 48.0%, and Diatoms 46.7%, Frequency of Occurrence (FO) respectively. These finding showed that *Heterotis niloticus* consumed more zooplanktons and other animals based diets than phytoplankton's and other plant based diets.

Keywords— Feeding, Habit lake, Oguta.

I. INTRODUCTION

The importance of fish in the economy and ecology of inland water has generated a lot of interest. Over the years aquaculture has gained a rapid interest due to importance of fish as a cheap source of animal protein, since beef is beyond the reach of an average Nigerian citizen these days (Akegbejo, 1995).

Fish like other animals required adequate nutrition to grow and survive in the wild, nature offers a great diversity of foods; these include: nutrients in solution and a host of different plants and animals. However in ponds, natural food is not sufficient to sustain the fish culture, especially in ponds with high density of fish.

For efficient and effective management in fish farming and in order to avoid high cost of producing fish, there is need for proper and adequate nutrition strategies, which can only be achieved via proper understanding of the food and feeding habit of the fish to be cultured. *Heterotis niloticus* (Cuvier, 1829) of the family Arapaimidae, is widely distributed in Nigeria, most especially in the fresh waters of Nigeria rivers. There is only one species of this genus *Heterotis*, hence species *niloticus* (Akegbejo-Samason, 1995). It constitutes an important food source within the region and comprises a portion of the inland fishes in Nigerian due to its delicacy, as it is widely known, but not popularly used in research and production probably due to its inability to easily adapt to environment changes. *Heterotis niloticus* grow reasonable fast from one to eleven months during culture.

Most Rivers and lakes in Nigeria, contain commercially culturable species of fish but very little information is valuable on food and feeding habits *Heterotis niloticus*, in Oguta lake. It is in this view that a study on the food and feeding habits of *Heterotis niloticus* was carried out in Oguta lake of Imo State.

Heterotis niloticus is a pelagic species, it occurs in shallow waters where it feeds on invertebrates, copepods and chironomids (Hickley and Bailey, 1987). Young ones are found in swampy places among aquatic vegetation (Moreau 1982, Dankwa *et al.*, 1999), adults live in the open water of rivers and lakes, where they can be found in the pelagic zone as well as the littoral zone (Moreau 1982). Its auxiliary branchial air breathing organs enable it to survive in deoxygenated water; the hardness of this fish, together with its great growth rate, make it a candidate for aquaculture in Africa and it has been transported to a

number of countires for this purpose (Bake and Sadiku, 2005). Escapees from ponds into the wilds resulted in established populations which form the basis for Fisheries and Aquaculture. (Akintunde, 1977).

This species is considered as a mud feeder (Hickley and Bailey 1987), but in West Africa it is also seen as a phytoplankton feeder. It feeds mostly on plankton, being the only plankton feeder of the Osteogossidae (Dankwe *et al.*, 1999). It has a superabranarial organ which has a sensory function. During breeding, it creates a circular nest in swamps.

The young's leave the nest after a few days and are guarded by the male (Balon, 1975). *Heterotis niloticus* breeds in the wet season in swamps and flood plains. It builds a circular nest about one millimeter (1mm), in diameter and 20 to 60m deep (Bailey).

The rim of the nest in a high wall is formed out of plank winks, about 15-20cm thick and projecting. Above the water surface, the bottom is a clean platform of clay or mud (Balon, 1975). After the spawning act, the fish leave the way of a hole in wall, through which 5 days later, the young leave the nest and are guarded by the male. The youngs posses external gills for breathing.

Heterotis niloticus is a long-bodied fish with large scales, long dorsal and anal fins set far back on the body and a rounded caudal fin it height is 3.5 to 5 times standard length (SL). It has been reported to reach up to 1m (3.3ft) SL and weight up to 10.2kg (22Ib).

This fish is gray, brown or bronze in colour. Colouration is uniform in adults, but juveniles often have air-breathing organs or less-bronchia, enabling them to survive in oxygen depleted water. A suprachiiae organ allows it to concentrate on small planktonic and food particles and also has a sensory function.

II. MATERIALS AND METHOD

The city of Oguta is divided into two: Oguta 1 and Oguta 2, separated by its popular lake, with the Local Government Headquarters located at Oguta 1. Today, Oguta is better known for its Resort hotel, challenging Scottish-designed golf course. Oguta has attracted lots of visitors because of its popular lake. It is also a home to some fresh water fishes. It has a huge oil and gas exploration going on its cultural diversity. Oguta has history that traces their forefathers to the Oba. Their traditional attires tell a lot about our rich culture. Fish , yam and cassava are some staples food in an average Oguta person's diet.

Neighbouring towns include: Ezi orsu, Izombe (an oil and gas producing community) Akri, Mgbefe, Nkwesi, Nnebukwu, Orsu-Obodo, Egwe and Egbuoma, border town with Anambra State.

2.1 Sampling (Consider Exponge)

Fish samples were obtained between January-June 2018. The samples were collected on weekly bases. A total of 180 fish specimen were sampled during this period. Three categories of fishing gears were used which include: cast nets, surface set gill net and bottom-set gill net.

After capture, the fishes were immediately identified with the aid of (an identification Key) (kef). Thereafter, the specimens were preserved with 10% formalin and it was transported to the Fisheries laboratory of the Imo State Polytechnic Umuagwo in 10% formalin for analyses. Excess water was drained from the fish with filter paper before preservation.

2.2 Preparation of Fish Sample

Dissection of the fish species with the aid of dissection kits.

Weight of stomach content were taken and graded as;

0/4 - empty

¼ - Semi empty

2/4 - half full

¾ - quarter full

Total length, Standard length and Body weight of fish were also measured.

2.3 Examination of Stomach Contents:

Stomach contents of the fish samples were emptied into a Petri dish and diluted with small amount of distilled water, after which a pipette was then used in dropping the mixture to a slide and then the slide is examined with the aid of a microscope.

2.4 Stomach Contents Analyses:

Standard methods described by Sarke (1980), were adopted for stomach contents analyses. Immediately after capture the fishes were kept in ice to prevent regurgitation. They were then measured for total length (T.L), weighed and dissected to extract the stomach which was stored in 4% formalin, or the whole fish was orally administered with 10% formalin to prevent post mortem digestion and then taken to the laboratory for examination. Their foods were evaluated both quantitatively (number of organism) and qualitatively (types of organisms). A combination of frequency of occurrence method and numerical method were adopted in the analysis of the stomach contents. This is to eliminate the inherent bias in the use of one method were separated by taxa and identified to species level and in some cases to higher taxonomic levels. After dissection, the contents of the stomach were placed in separately labeled Petri dishes and the food items examined under a name type of microscope and classified. Counts were made of the number of different food categories was used for the identification of the food items encountered in the stomach was then prepared

2.5 Analysis of Food and Stomach Content:

The stomach contents were analyzed using a binocular microscope to identify the stomach contents. Their foods were evaluated both quantitatively (number of organisms) and qualitatively (types of organisms). The combination of frequency of occurrence method and numerical method (Bake and Sadiku, 2005), were adopted in the analyses of the stomach content. These methods were appropriate because they bring about individual assessment and estimation of majority of food items and feeding intensity were made in line with the size of each specimen and there was no problem of system of standards and adjustment for size of individual. The results were recorded on a raw data sheet. For the fact that very tiny grained particles were found in the gut content, the "occurrence" and point method were used for the estimation of food items.

In frequency of occurrence method, observations were made on each gut and items found were identified and recorded. The total number of gut in which each food item occurred was recorded and taken as a percentage of the result.

TABLE 1
ANALYSIS OF FOOD ITEMS OF *HETEROTIS NILOTICUS* FORM OGUTA LAKE

No. of fish examined	Juvenile 72		Adult 108	
No. of fish empty stomach	21		23	
Food item	% FO	% Nm	% FO	% Nm
Copepods	61.0	33.2	61.3	21.3
Cladeceran	53.0	21.7	58.2	19.3
Ostracode	48.3	18.7	49.4	16.3
Diatoms	46.7	15.8	51.5	15.3
Insect part	30.9	10.8	36.9	3.6
Bivalves	20.3	3.1	18.5	2.3
Plants remains	18.4	10.04	17.9	8.73
Plant detritus	20.7	3.29	21.4	4.83
Sand	25.8	14.1	22.6	9.78
Univellular algae	21.3	6.37	23.4	6.68

The result revealed that the juvenile of *Heterotis niloticus* ingested more of Copepods (61.0%), followed by Cladeceran, (33.0%), Ostracode, (48.3%), with plant remains been the least (15.4%). Adult *Heterotis niloticus* equally ingested more of Copepods just like their juvenile counterparts, followed by Cladeceran (53%) and Ostracode (48.3%)

III. DISCUSSION

The food and feeding habits of *Heterotis niloticus* were examined, 180 stomach contents were then analyzed. Among all the food items, the food items of animal origins were most important in the juveniles diet than those of plant origin.

The most dominant diet of juvenile and adult of *Heterotis niloticus* were:

Crustacean; Cladocerans, (53.0%) for juvenile, 58% for adult, Copepods, 61% for juvenile and 61.3% for adult and Ostracoda, 48.3% for juvenile and 49.4% for adult and diatoms.

The abundance of *Heterotis niloticus* in Oguta lake is overwhelming especially at the period of this, that is (Wet season) due to food availability and environment condition that permits rapid growth.

Fish growth is determined by the combined effect of food quality and quantity. Analysis of composition in stomach of *Heterotis niloticus* from Oguta lake show a predominant microphagous diet plus insect larvae.

The inclusion of sand grains were possibly an incidental ingestion along with insect larvae, annelids, prawn and bivalves, while the high occurrence and preeminence of detritus, suggest frequent bottom feeding on benthic invertebrate, which dominated the diet in the river (Akegbejo-Samsons, 1995).

It was evident that *Heterotis niloticus* was strictly a planktonic microphage in the lentic habitats while it adopted a mud-eating microphagous habit in the river.

Ecologically, these habits seem to be common and characteristic of Osteoglosid fish species (Akintunde, 1977), this adaptiveness to the natural diet is responsible for the success of *Heterotis niloticus* in their feeding; primarily consuming a combination of two or more of crustaceans, insects, plankton and plant detritus, depending on availability and abundance of these foods.

IV. CONCLUSION

From the result of this study *Heterotis niloticus* can be domesticated like other culturable fishes like *Clarias garipinus*, *Hetrobranchus nilotices* as well as *Tilapia do* in the ponds and other artificial settings

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