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Preface

We would like to present, with great pleasure, the inaugural volume-8, Issue-10, October 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, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

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

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

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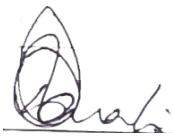

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Animal Science	Agricultural Economics
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Sustainable Natural Resource Utilisation	Management of the Environment
Agricultural Management Practices	Agricultural Technology
Natural Resources	Basic Horticulture
Food System	Irrigation and water management
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Cereals or Basic Grains: Oats, Wheat, Barley, Rye, Triticale, Corn, Sorghum, Millet, Quinoa and Amaranth	Oilseeds: Canola, Rapeseed, Flax, Sunflowers, Corn and Hempseed
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Vegetable crops or Olericulture: Crops utilized fresh or whole (wholefood crop, no or limited processing, i.e., fresh cut salad); (Lettuce, Cabbage, Carrots, Potatoes, Tomatoes, Herbs, etc.)	Tree Fruit crops: apples, oranges, stone fruit (i.e., peaches, plums, cherries)
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Dairy Sheep	Water Buffalo
Moose milk	Dairy product
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Silvopasture	Christmas tree cultivation
Maple syrup	Forestry Growth
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Microbials In Agriculture: A Current Review on the Perspectives and Challenges for Large Scale Implementation

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Abstract**—** *Growing populations, food demand and climate change necessitates the improvement of agriculture in sustainable ways. Microbials provide for an efficient green solution, and a potential replacement for the overuse of chemical pesticides. They have long been researched for their various beneficial effects in crop protection such as improving plant growth, stress tolerance and abetting plant pathogens. Despite their several advantages, the large-scale implementation of microbials is still at its primitive stage. This review attempts to identify the challenges that are barring the improvement of the microbials industry. Both the research and industry sections are explored, to recognize key issues, chokeholds and identify areas of improvement. This review provides a current and updated perspective into the use of microbials in agriculture.*

Keywords— Sustainable Agriculture, Microbials, Biofertilizers, Biopesticides, Market.

I. INTRODUCTION

The Food and Agriculture Organization (FAO) estimates the world's population to reach about 8.5 billion people in 2025 (1). Due to this rapid growth rate in population (approximately 1.05% per year), malnutrition has emerged in roughly 2.4 billion individuals across the world (2). Global food production will need to increase by at least 60% if food security is to be achieved by the year 2050 (3). In addition, an increase in agricultural productivity is also essential in realizing numerous sustainable development goals (SDG) including zero hunger (SDG 2), no poverty (SDG 1), and good health and well-being (SDGs 1 and 2), which can all ultimately benefit life on land (4). It is thus important to upgrade existing cropping systems, despite present limitations of limited resources and reduction in arable land.

Agricultural production, however, faces a number of unforeseen environmental issues such as drought, heat waves and flooding. More significantly, plant pests and pathogens cause a range of plant diseases significantly reducing agricultural output (5). For example, farmers face major food losses annually, ranging from 21.5% in wheat, 30.3% in rice, 22.6% in maize, 17.2% in potato, and 21.4% in soybean, due to pests (5). Downstream effects to human health are inevitable due to decreased yields, loss of species variety and increase in pest mitigation costs (5). Thus, along with an increase in production, a major reduction in food loss due to pests and pathogens is also required.

Currently, agrochemicals are used to increase yields and scale up crop productivity with the goal of intensive farming (4). This is done in attempt to increase agricultural production on current agricultural land, rather than an expansion in arable surface. Farmers rely on traditional agricultural practices which use inorganic fertilizers, pesticides, and other chemical inputs, as they substantially increase yield without the need of more land. Among all, phosphorus and nitrogen fertilizers are commonly used, in combination with herbicides and pesticides to help maintain crop productivity and yields, in addition to managing invasive plants, diseases, and insects (4).

The continuous and excessive use of agrochemicals, however, has led to increased soil salinity and toxicity, hardening of soil, decreased nutrient carrying capacity and water logging (6,7). Additional issues in the form of pesticide resistance prompting the use of higher doses which lead to aggressiveness of disease and pathogen mutations, have pronounced negative environmental impacts and serious implications for food security (7). Not only do they have direct effects on the environment, but they can also be damaging to human health indirectly or directly. Chemical pesticides find their way into drinking water

systems and food products, exposing humans to high levels of toxicity. According to a study published in 2014, many chemical pesticides used throughout the world are far more harmful to human health and the environment than previously assumed (7). A well-known example is DDT which was the first synthetic insecticide to be produced. DDT is highly effective against insects and plant pathogens but was recently suspected to be a probable human carcinogen (7). Numerous other studies have linked chemical pesticides to cancer, Alzheimer's disease, ADHD and birth defects (7). In addition to this, the rising cost of pesticides particularly in less developed countries, and customer demand for pesticide-free food has prompted a search for alternatives. Many fastidious diseases also do not have chemical solutions as they are ineffective or do not exist.

Thus, for all the above reasons and disadvantages of existing farming systems, it is necessary to shift to using of sustainable practices for increased crop output. Sustainable practices have the potential to offer long-term solutions to secure global food security (7). One of the most promising sustainable practices that is being implemented today is the use of microbials for crop growth promotion/ protection. Microbials are a class of biologicals that use living microorganisms for crop protection (6,7). It is a form of biological control that is preventative and/or curative in direct pest control. In addition to crop protection, they work together with the plant microbiome to help improve overall plant growth, nutrient efficiency, and stress tolerance (4,6). Microbial products contain organisms from most microorganism genera (viruses, bacteria, fungal pathogens, yeast and protozoa) (11). Bacteria are the most commonly used microbials due to their lower costs and ease of usage as compared to fungal biological control agents (11).

Microbial products have the potential to boost crop yields and supplement or replace agricultural chemicals and fertilizers (4,6,11). As opposed to chemical pesticides, microbials are made from naturally occurring materials (11). It presents as a very appealing alternative because it would drastically minimize the consumption of agrochemicals. Many companies have begun to use single or multiple microorganisms as biocontrol or biofertilizer products, as well as develop carrier-based inoculants of beneficial strains (12). Large-scale field trials have shown an improvement in crop yield of 10–20% on commercially important crop plants (11). Currently, microbials make up the largest part of the biologicals market and are expected to grow up to 60% of the biological control market by 2025 (12).

Growing consumer interest in organic agriculture, the reduction of synthetic products, and the economic potential of rising countries like India are all major growth factors in the development of the microbials market (14). However, despite the benefits and potential of agricultural microbial products, according to a recent study on microbials, "the scientific literature abounds with numerous potentially highly helpful strains that did not arrive on the commercial market" (15). It was found that, approximately 72% of biocontrol company endeavors failed over a 30-year period ending in 2002 (16).

This can be attributed to many challenges faced in the development of microbial products. Usually, the development of a commercial microbial product is a lengthy process that necessitates a high level of expertise and close collaboration among experts from numerous domains (13). The research domain includes strain isolation, efficiency testing in vitro and in vivo, and trials in natural settings. Following this, the product must be produced on a commercial scale, conserved for storage, and prepared to assure biocompatibility in order to be delivered commercially. Only then, these procedures could be patented for large scale implementation. Even the registration process appears to be an issue as, large number of patents are issued, but only a few products have been registered (6,13). Failures are also caused by underestimating the expenses of creating and marketing microbial goods. The disparity between effective microbial strains and profitable agricultural products shows that unexpected challenges must be overcome.

Microbes will undoubtedly play a role in agricultural revolution in the coming decades, helping to fulfill the needs of a growing population. In order to realize the actual potential of microbes in agriculture, more research is required to improve industry standard of microbials and commercialization. Research and industry implementation always go hand in hand. In this review, we will discuss the major challenges faced by microbial production. To highlight this, a main research question with two sub questions is formulated to delve deeper into how these challenges are currently being overcome, in addition to the current microbials market. What are the challenges faced by translation of microbial products from lab to field? What is the importance of using microbials in enhancing plant growth promotion and crop protection? What are the existing solutions to the current challenges and future perspectives? While some reviews focus either on the research or industry side, this review aims to combine both as they would go hand in hand for ultimate improvement.

II. RESEARCH METHODOLOGY

The review sought to identify, discuss and synthesize recent research into the use of microbials in crop protection. First, literature research in the field using key words: microbials, crop protection, PGPR, challenges, benefits were performed to

identify a problem field to discuss. Since the field of using microbials itself is very recent, for the first section indicating their significance, literature from 1900s to recent is included. For the second section, recent literature from the years 2015-2022 was identified and used.

The inclusion criteria for the articles and research studies were, they have to be (i) full-text paper (either pre-print or published in peer-reviewed journal) (ii) English language (iii) focus on microbials in agriculture (either primary or non-primary research papers).

For the search itself, a simple key-word strategy was used for the particular sections that are needed for the main body. For example, for the section discussing the Global PGPB/PGPR market, the text strings "global microbials market AND challenges OR forecast periods" was entered into the Google search engine to obtain essential results for this review article. The search results were then screened in terms of most relevant to answer the research questions posed in this review article.

III. HISTORY OF MICROBIALS

The idea of utilizing microbes was first proposed almost 150 years ago (17). In a study conducted in 1879, Hagen proposed the dispersal of a disease-causing bacterium on crops to minimize pests (17). In addition to biocontrol, microbials as biofertility inoculants were also used. The commercial application of microbials in biofertilization dates back to Nobbe and Hiltner's (1896) development of a bacterial product named "Nitrogin" to improve agricultural yield of legume crops (18). ''Alnit"" was later discovered by Timonin (1948), which contained *Azotobacter* bacterial compounds to boost agricultural yield of non-legume crops (19).

Hagen as the earliest agromicrobe adopter in 1879 had already stressed particularly on the ease of manufacture, low cost of microbials, and the fact that it is not hazardous to human health (17,20). These factors are still significant today when choosing crop protection products. Following this framework, many other products came to the market. The use of fungal species was first introduced by Metchnikoff in 1880, when he succeeded in the artificial control of *Metharhizium anisopliae*, a pathogenic fungus to reptiles. This was later used in field trials for the control of several insect pathogens (17). Shortly followed the introduction of two bacteria that were used as insect pathogens in the early twentieth century (21). They were later produced and commercialized as the first biopesticide 'Sporein' in 1938, France (20). The first large scale commercially available biopesticides were available in Europe and USA by the 1960s, when agrochemical companies started making significant investments. Early sales in the biocontrol sector were dominated by a single product type containing *Bacillus thuringiensis* (Bt) (6). This product was specifically focused against lepidopterans (e.g., cabbage worms and gypsy moth) (22). Bt product is known as the most used microbial pesticide in world. The estimated total sales for microbial based biocontrol products were close to $400 million USD in 2012, with almost 50% of sales accounted for by Bt-related products (16, 22). Biological control became the fastest growing segment in the plant protection market as companies recognized the advantages of using microbials over chemicals. With a total sales reaching three billion in 2016 and a compound annual growth level of 16%; sales are forecasted to grow to 13.9 billion dollars by 2025, whilst also achieving a pesticide global market share of 29.9% (12).

Over the last two decades, the geographical distribution of biocontrol sales has shifted considerably to cover a larger worldwide market and a higher variety of agricultural crops (16,22). These patterns could be attributed to the expansion of the geographic scope, market sectors, key arable crops, and microbial strain diversity. In addition, growing consumer interest for microbial products in emerging markets such as China and India are major drivers of adoption (6). However, even with continued market growth, the total use remains a fraction of the total worldwide pesticide use. (6,7,13) In the later part of the review, we report on some of the key challenges that are encountered in bringing microbial products to the market.

IV. OVERVIEW AND SIGNIFICANCE OF MICROBIALS IN CURRENT AGRICULTURE

Bacteria, fungi and viruses are the major groups of microorganisms that have been found associated to plants (11). They each benefit the plant in their own way, but also sustain interrelationships between themselves. Traditionally, agricultural application of beneficial microorganisms involved a few types of well characterized microbes such as mycorrhizal fungi or rhizobia bacteria (24). The main group of microorganisms that were found to benefit plants and maintain a long lasting symbiosis were bacteria (11). They are present widely in the plant ecosystem and help the plant in both biocontrol and biofertilization, as opposed to some groups of fungi and virus (that aid mostly in uptake of nutrients). Furthermore, bacteria also participate in complex communities by forming communities through the production of signalling molecules that attract other beneficial microbial communities (4,6,11). They also continuously form biofilms or other networks, that serve as communication or transport networks, benefitting both the plant-host and the microbe (11). The diversity in plant associated microbiomes is touched upon in the next section.

Majority of research studies are focused solely on the ability of the applied microorganisms in the facilitation of specific plant growth promoting traits such as phosphate solubilization, nitrogen fixation and ACC deaminase production. siderophore production, biofilm formation, plant hormone production, biotic, and abiotic stress tolerance or resistance, amongst others (4,6,11). These microorganisms however are usually studied in small, one-on-one investigations in sterile soils and greenhouses (24). The beneficial effects are often not be observed in field situations as they fail to translate in more complex environments (24). This could be attributed to the fact that soil in field plots have more complex microbial communities that are adapted to local eco-environments (24).

V. DIVERSITY OF PLANT MICROBIOME

Diverse microorganisms can colonize different surfaces of the plant; for example- root surfaces (rhizosphere), other aerial parts- phyllosphere microbiome (leaf), anthosphere microbiome (flower), spermosphere microbiome (seed) and carposphere microbiome (fruit) (3). Microbes can even be transferred vertically through seeds (3). These microbes can further be characterized into two categories, epiphytes that stay on the surface of plant organs and endophytes that penetrate plant organs and form beneficial relationships with them (endosphere microbiome) (3). The plant microbiome primarily consists of bacteria that exert highly beneficial effects on plant development by direct or indirect mechanisms (24). They are termed as Plant Growth Promoting Bacteria (PGPB) (24). PGPB that are primarily found in the soil that surrounds the root surfaces are termed as Plant Growth Promoting Rhizobacteria (PGPR) (24). This review would focus on PGPR bacteria as in majority of the cases, beneficial effects are observed by PGPR living on or inside plant roots making them attractive for commercialization. The following section focuses on the specific functions that make PGPR widely popular for commercialization as microbials.

PGPR, that constitute the rhizosphere microbiome are of particular importance in crop protection due to the extensive plant-microbe symbiosis that takes place (3). This is because, the rhizosphere is an integral area of the plant ecosystem, that governs the chemistry of plant nutrient acquisitions (3). The host plant secretes exudates and signaling molecules that can recruit microbial counterparts from surrounding microbial reservoirs (3). In turn, the rhizosphere microorganisms produce vitamins, antibiotics, plant hormones, communication molecules, etc. that encourage plant growth and alleviate abiotic stress (3). The rhizosphere is also one of the major sites that contribute to entry of endophytes into plant roots (3). Though the exact mechanisms and modes of action through which rhizobacteria promote plant growth is not well understood, it is established that PGPR along with integrated nutrient management may be more effective for growth, yield and fertility status under sustainable agriculture (3,4,6). However, the microbiome of the plant is highly subjected to different environmental conditions such as pH, temperature, soil type, moisture and salinity (3). Additionally, microbial community is influenced by plant host factors and microbe-microbe interactions (3). It is due to this reason that different studies report great variation in plant microbiomes between species and within species themselves (3,4,6). This presents as a major challenge to companies working with microbials as they might find it difficult to produce a one-fits-all commercial product that is highly effective to a wide number of species. Companies currently produce a wide range of products with diverse PGPR as detailed in later sections.

VI. FUNCTIONS OF PGPR

Plant growth promoting rhizobacteria can be classified under three broad categories based on their beneficial effects in stimulating plant growth. The categories are biofertilizers, phytostimulators and biopesticides (25), as summarized in the table (table 1) below. It is important to note that many species of PGPR display all three or primarily two categories of beneficial effects (25). This fact makes many species of PGPR more appealing for commercialization.

TABLE 1
THE THREE FORMS OF PGPR CHARACTERIZED BASED ON THEIR BENEFICIAL EFFECTS. ADAPTED FROM P. N. BHATTACHARYYA ET AL., 2012 (25).

PGPR forms	Definition	Mechanism of action
Biofertilizer	Formulations that contain live microorganisms that aid plant growth through increased uptake of nutrients	Biological nitrogen fixation Utilization of insoluble phosphorous
Phytostimulator	Microorganisms that produce phytohormones such as IAA, gibberelins, cytokinins and ethylene	Production of phytohormones
Biopesticide	Microorganisms that promote plant growth through biocontrol of phytpathogens	Production of antibiotics, siderophores, HCN and hydrolytic enzymes Acquired and Induced systemic resistance

PGPR assert their positive effects on plants through two mechanisms- Directly or indirectly (26). Direct mechanisms is the ability of PGPR to provide plants with compounds that are directly produced by them or facilitate nutrient acquisition (26). Direct mechanisms involve the production of phytohormones, nitrogen fixation, increasing iron availability, phosphate solubilization, siderophores and ammonia production, etc (26). These functions are carried out by the production of specific enzymes that induce morphological and physiological changes in the plant host. Indirect mechanisms applies to the ability of PGPR to protect the crop from phytopathogens (26). Indirect mechanisms could involve the production of antibiotics, hydrogen cyanide (HCN), induced systemic resistance (ISR), and production of lytic enzymes such as chitinases, proteases, cellulases and lipases that can lyse the cell walls of many pathogenic fungi (26). Direct mechanisms of PGPR are termed as plant growth promotion effects. They are mainly characterized under biofertilizer and phytostimulator groups. Alternately, indirect mechanisms are termed as plant protection effects and characterized under the biopesticide group. Some of the mechanisms that are important to agriculture and commercialization are touched up on below. The main genera of PGPR that performs each function are highlighted in the next few sections.

6.1 Direct Mechanisms

6.1.1 Biological Nitrogen fixation

Nitrogen serves as the basic building block of plants, animals and microorganisms and is thus the most important nutrient required for plant growth and productivity (26). Nitrogen fixing PGPR fix molecular/atmospheric nitrogen to be further utilized by plants. They can do so in both symbiotic and free living systems. The main genera of symbiotic nitrogen fixers include: *Rhizobium, Achromobacter, Sinorhizobium, Azoarcus, Mesorhizobium, Frankia, Allorhizobium, Bradyrhizobium, Burkholderia, Azorhizobium,* and *Herbaspirillum* (26). Some of the important free living nitrogen fixers include: *Azoarcus sp., Herbaspirillum sp., Gluconacetobacterdiazotrophicus*, and *Azotobacter* (26). Even though they are highly diverse, all rhizobia establish symbiotic interactions with their host plant through highly conserved mechanisms that have been reviewed extensively (26).

Due to their importance, nitrogen fixing PGPR serve as the most important and recognizable examples of biofertility inoculants (6,26). Approximately 90 million metric tons of atmospheric nitrogen is fixed annually by legume crops that are grown globally on an estimated land of 250 million hectares (27). Several marketed products have been shown to affect consistent improvements in yields of legume crops in different studies (6). One study reported yield improvements averaging approximately 120 kg per hectare in *Rhizobia* inoculated soybeans (6, 28). Another study compares the commercial products based on their impact on soybean yields (28). These commercial products are all based on the *Bradyrhizobia* and other formulates or inoculates. Optimize® sold by Monsanto BioAg Alliance had outstanding result among all commercial products. It contains a mixture of *Bradyrhizobium* cells and lipochitooligosaccharide which is a molecule that enhances the soil microbial environment. In their study, they observe that seeds treated with Optimize® consistently show an increase in yield over untreated controls (6). In addition, they find that the use of bioinoculants on soybean crops consistently provides a 4:1 return on investment (6).

Out of the free-living nitrogen fixers, *Azospirillum* MicroAZ-STTM (TerraMax), Mazospirflo-2 (Soilgro) (31) *Azotobacter* Bio-NTM (Agriculture Solutions), and *Gluconacetobacter* have gained interest. They have been shown to increase the yield of various crops such as sunflower, carrot, oak, sugar beet, sugar cane, tomato, eggplant, pepper, cotton, wheat, and rice (6). In a survey of 20 years of global field trials, Okon et al., (1994) found that inoculation with several *Azospirillum* strains boosted crop yields by 5–30% in 60–70% of the trials (32). More recently, Diaz-Zorita et al. (2012) found that on-seed inoculation with *Azospirillum* enhanced wheat and maize yields by 244 kg ha-1 and 514 kg ha-1, respectively, in an expansive multi-year research (33). Except nitrogen fixation, some *Azospirillum* species can also produce plant growth-promoting chemicals in addition to nitrogen fixation, which may play a role in their mode of action (6). Other plant-beneficial features of non-leguminous nitrogen-fixing bacteria include heavy metal clean-up and increased plant tolerance to abiotic conditions like drought (6).

6.1.2 Phosphorous solubilization

Phosphorous is the least mobile nutrient and is the second most important nutrient in crop productivity, after nitrogen (6,26). It is found both in organic and inorganic forms in soil, but are not available to the plant (6). Phosphorus is thus usually substituted in agriculture through chemical fertilizers and manure (6). However, the long term sustainability of current phosphate sources are debated (6,26). Phosphorous solubilizing microorganisms offer a key solution to ensure efficient use of phosphorous in the environment (6). Soil microorganisms that release phosphate from organic and inorganic pools have been commercialized as products that successfully mobilize phosphate from soil's scarce sources, in turn reducing the use of chemical fertilizers (6,26).Some of the key genera that can mobilize phosphate include Pseudomonas spp., Agrobacterium spp., Azotobacter spp., Bacillus spp., Burkholderia spp., Enterobacter spp., Erwinia spp., Kushneria spp., Paenibacillus spp., Ralstonia spp., Rhizobium spp., Rhodococcus spp., Serratia spp., Bradyrhizobium spp., Salmonella spp., Sinomonas spp., and Thiobacillus spp (26).

Bacillus and Pseudomonas account for the most important bacterial genera as they have shown improved agricultural yields (34). They are present in commercial products such as Symbion-P® and JumpStart® respectively. Legget et al., in 2015 summarized maize yield responses to JumpStart® (containing Pseudomonas bilaiae) inoculation (6). Significant yield increases were observed in both small scale and large-scale plots (6). Interestingly however, for phosphorous solubilization, fungal species have been shown to have more solubilizing activity in comparison to PGPR species. Arbuscular mycorrhizal fungi (AMF) are well known fungal species of phosphate-solubilizing microorganisms (6). They may build a network of hyphae that interact with plant roots to increase nutrient delivery and provide plant protection (6). Some of the examples of AMF products include Mycormax® (JH Biotech), BEI (BioOrganicsTM), BioGrow Endo (Mycorrhizal Applications), and VAM (Microbesmart). Although, there seems to be an absence of highly effective commercial phosphate solubilizing inoculants due to lack of research into the mechanisms of phosphorous solubilization (6). In addition, plant and environment compatibility plays a huge role in the commercialization of phosphorous solubilizing products (6). Contrastingly, PGPR products for phosphorous solubilization seems to be more popular than AMF fungal species due to the ease of maintenance of bacteria over fungi in commercialization (6).

6.1.3 Production of phytohormones

Approximately 90% of PGPR are known to produce phytohormones (36). Phytohormones are chemical messengers that influence gene expression and transcription, cellular division, seed germination, flowering emergence, flower sex, leaf senescence, and fruit ripening (36). Plant growth and development are thus greatly regulated by phytohormones and can have an influence in plant phenotype, morphology and metabolism (36). The most well-known phytohormones are auxins, gibberellins, cytokinins, ethylene, and abscisic acid (36). These phytohormones play their own role in plant growth and regulations. For example, auxins and cytokinins are essential regulators of vascular development, root apical dominance, and lateral root initiation, as well as determining root architecture (36).

Bacteria belonging to the genera *Azospirillum, Pseudomonas, Xanthomonas, Rhizobium, Alcaligenes faecalis, Enterobacter cloacae, Serratia marcescens, Mycobacterium sp.,Burkholderia, Azotobacter, Bacillus cereus* and *Bradyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (36). Indole Acetic acid (IAA), which is the most physiologically active auxin in plants is known to be produced by almost 80% of all rhizosphere bacteria (36). In one study, they found that a majority of *Rhizobium* isolates from the field, produce IAA and may serve as PGPR in promoting growth of non-legume plants (36). In another study, IAA-producing strains of *Azospirillum brasilense* and *Bradyrhizobium japonicum* were shown to stimulate early growth promotion of seedlings of corn and soybean (36). In addition, auxins are said to be the most quantitatively abundant hormones produced by *Azospirillum* (37). These studies demonstrate the major influence phytohormone producing PGPR can have in crop improvement. PGPR have also been shown to produce over 30 growth promoting compounds of the cytokinin group, in addition to regulation of ethylene and abscisic acids (36). Most genera not only aid the plant by producing phytohormones, they also perform nitrogen fixation/phosphorous solubilization providing multiple benefits (6). The genera have already been commercialized as microbial inoculants and are detailed in table 2.

6.2 Indirect Mechanisms

6.2.1 Siderophores production

For the majority of microorganisms, iron is an important nutrient, and iron shortage can be fatal (36). Iron is present in the rhizosphere in small levels, and much of it is in a ferric form (Fe+++), which is poorly soluble and not readily accessible to microbes (36). Many PGPR in soils get past this difficulty by the creation of small peptidic molecules called siderophores.

These siderophores contain side chains and functional groups that provide ligands with high-affinity to which ferric ions can bind (36). The siderophores acts as a mechanism through which some PGPR can acquire iron and grow in the rhizosphere. Additionally, through this same mechanism, PGPR can effectively deliver iron to host plants encouraging plant development (26). For example, siderophores produced by Chryseobacterium spp. C138 when delivered to the root were effective in the supply of iron in tomato plant (38). In another study, siderophore producing *Pseudomonas* strain showed significant increase in germination and plant growth (39). In addition, bacterial siderophores can inhibit or reduce pathogen multiplication, by lowering the amount of iron accessible to a pathogen (36). Thus, siderophore producing microorganisms have been shown to have competitive advantage over other microorganisms in the rhizosphere (36). Siderophore production mechanism is an example where PGPR act as both fertilizer and pesticide.

6.2.2 Production of antibiotics and hydrolytic enzymes

The synthesis of one or more antibiotics is the main method used by PGPR to counteract the harmful effects of plant pathogens (26). Antibiotics are low molecular weight chemicals generated by PGPR (26). They effectively inhibit the development of other microbes by interfering with essential enzymes and metabolism, leading to biostatic and biocidal effects (26). Moreover, PGPR's are able to produce one or more antibiotics, which can overcome the ability of the plant to acquire resistance to some specific antibiotics (26). This considerably improves their potential to function as effective antagonistic agents against pathogens as a whole (26). Bacillus spp and Pseudomonas spp. are known to produce many antibiotics including bacillomycin, surfactin, iturin, plipastin, pseudomonic acid, kanosamine, Rhamnolipids, among others (40). However, it was noted that antibiotics produced against one pathogen might not be as effective to control another pathogen on the same plant (26). This factor may limit the use of single spp. PGPR for use as biocides. Additionally, the effects of antibiotic-producing PGPR may be varied in field conditions, and the activity of biocontrol can be altered by methods of cultivation and formulation in the lab (26).

In addition to antibiotics, some PGPRs produce hydrogen cyanide and fungal cell wall degrading enzymes to inhibit fungal pathogens (26). In a study, they found chitinase, β-1,3-glucanase, and chitinolytic enzymes produced by some PGPR appear to inhibit the fungal pathogen *Rhizoctonia solani* (36). Additionally, HCN produced by rhizobacteria is an effective agent of biological weed control, as they have the ability to inhibit the electron transport chain and energy supply to the cell, ultimately leading to apoptosis (41). The production of HCN however is highly regulated, as large amounts could be toxic to the host plant itself (26). This needs to be considered when developing formulations for crop protection.

6.2.3 Induced systemic resistance

PGPR have also been shown to simulate the plants resistance mechanism to pathogens (26). Induced systemic resistance (ISR) allows the plant to have enhanced defensive capacity against infection by one or more pathogens (26). ISR uses plant hormones such as jasmonate and ethylene to stimulate resistance mechanisms in plants (26). Upon contact with pathogens, the plants are primed to defend themselves through effective ISR (26). Some PGPR directly regulate ethylene levels, while others have an effect through simulating ISR (36). Other PGPR can simulate ISR just by the presence of their individual microbial cell components such as lipopolysaccharides (LPS) and flagella, signal molecules, and some antifungal metabolites (36). For example, a study recorded that just the presence of Rhizobium spp. (through elicitation of isoflavonoid phytoalexin) has been associated with disease resistance in alfalfa and common bean. Additionally, ISR is not pathogen specific, thus making PGPR that activate ISR very potent in biocontrol (26).

In addition to the mechanisms mentioned above, PGPR have other effects such as competition in which PGPR compete for the uptake of nutrients with pathogens and predation where the PGPR use the pathogen as a food source. This is particularly observed in *Pseudomonas fluorescens*, when it colonizes of the hyphae of fungal pathogen *Fusarium oxysporum*, also inhibiting their spore germination. All these beneficial effects of PGPR work in tandem with each other and are highly advantageous compared to the few advantageous effects of chemical fertilizers/pesticides, which. Moreover, the use of agrochemicals have major effects on the natural rhizosphere microbiome, in turn harming this harmonious plant-microbe symbiosis.

The functions of PGPR and their direct and indirect effects on the host plant are summarized in the graphic below (figure 1).

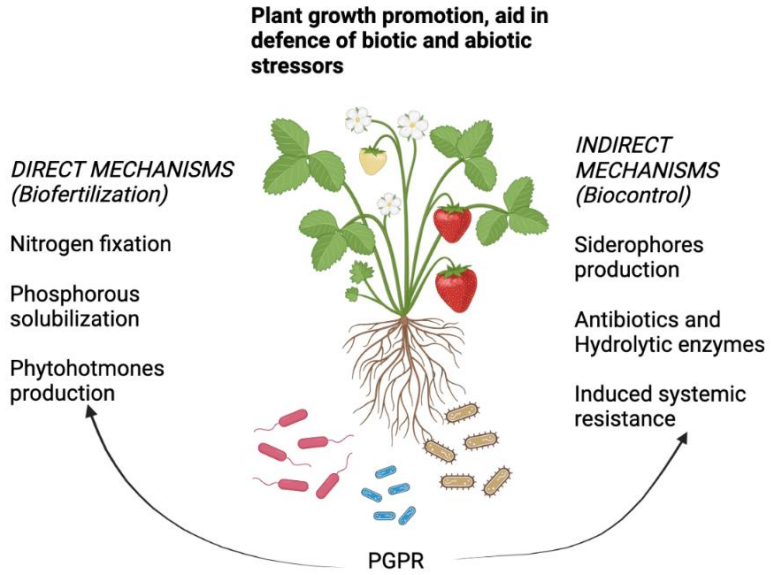

FIGURE 1: A summary of the beneficial effects of PGPR on plant growth.

VII. COMMERCIALIZED PGPR IN AGRICULTURE

According to literature surveyed, *Pseudomonas* and *Bacillus* were the most common bacterial genera identified in plants (26). In this section, we summarize the findings to obtain an overview of the main species of PGPR used in crop protection. The table details the particular crops on which the PGPR is currently being used and their significance. In addition, commercial products with the particular strain of species are highlighted to map which of the species are highly commercialized in comparison to other PGPR which are not yet in the market/new in the market. Ultimately, this section aims to evaluate if there's a correlation between commercialization, crops and function of species. Note: the species that are highlighted in the previous sections to have most effects/ significance are chosen.

TABLE 2

COMMON PGPR STRAINS THAT ARE USED IN AGRICULTURE. THE MAIN CROPS ON WHICH THE PGPR STRAIN IS USED AND THEIR MAIN FUNCTIONS ARE DETAILED. ADDITIONALLY, THE COMMERCIAL PRODUCTS THAT CONTAIN THE PARTICULAR PGPR AS THEIR ACTIVE INGREDIENT ARE MENTIONED

PGPR	Crop	Significance	Commercial Products	Reference
Azoarcus	Rice	Nitrogen fixation leading to increase yield of rice, tolerance to biotic and abiotic stresses		44,45, 46
Azorhizobium	Wheat	Nitrogen fixation		44,45, 46
Azospirillum	Wheat, Maize,Rice, Soybean Sugarcane	Nitrogen fixation	TwinN,(Australia) SymbionN, CALSPIRAL, Sadar Biofertilizers (India) Azo-green, Gmax,Nitromax	44,45
Azotobacter	Wheat, barley, oats, rice, sunflowers, maize, line, beetroot, tobacco, tea, coffee and coconuts, soybean, sugarcane	Nitrogen fixation	Azotobacterin, Ekophit (Southern and Eastern Russia), TwinN (Australia) Phylazonit-M (Hungary), SymbionN, CALZOTO, Sardar Biofertilizers (India); Dimargon1 (Colombia) Biogold, GmaxNitromax, Kisan Azotobacter, Astha azo, Sanjivini- N2, Nitrofix, BIO N MORE	42, 44, 45, 46

Bacillus	Potato, cucumber, pepper, wheat, eggplants, maize, peanuts, cauliflower, sugarcane, chickpea, soybean, tomato, cotton, barley, mungbean, grapes, apple	Auxin,cytokinin and gibberelin synthesis, potassium solubilization, induction of plant stress resistance, antibiosis effects	Bamil and Omug (Russia), Ekud (Russia), Pudret (Russia), Bactophosphin (Russia), Xin Sheng Li (Japan), Serenade (USA), SymbionN (India), Phylazonit-(M) (Hungary), UPMB (Malaysia), Probio96 (Iran) PIxPlus, Sonata ASO, Ballard, Epic, HiStick NT, Kodiak, Rhizoplus, Subtiex, Quantum 4000, Rhapsody, System 3, Companion, Bioyield, Rhizovital, Biotilis Gmax Phosphomax, KisanPSB, Astha PSB, UPAJ- K, eco-potash	42, 43, 44, 45, 46
Beijerinckia	Sugarcane	Nitrogen fixation		44,45
Burkholderia	Rice, Mint, Sugarcane, Chickpea, Apple		Blue Circle, Deny, Intercept	44,45
Chryseobacterium	Tomato	Siderophore production		44,45
Frankia	Alnus, tomato	Nitrogen fixation		44,45
Glomus *(Mycchoriza)*	Rice, cotton, soybean, corn, coffee, sorghum, sugarcane, tomato, banana	Nitrogen fixation	EcoMic (Cuba), Microfert (Cuba, Mexico), MYCOGOLD (Malaysia), Agri VAM, bio e rich	44,45, 46
Herbaspirillum	Sugar cane, bean, rice, sorghum	Nitrogen fixation		44,45
Mycobacterium	Maize	Induction of plant stress resistance		44,45
Paenibacillus	Lodgepole pine, black pepper	IAA synthesis, Potassium solubilization		44, 45
Phyllobacterium	Strawberries	Phosphate solubilization, siderophore production		44,45
Pseudomonas	Mung beans, wheat, cotton, maize, potato, tomato,	Chitinase and β-glucanases production, Induction of plant stress resistance, Antibiotic production, Siderophore production	CALMONAS (India), FOSFORINA (Cuba) BioJect, Spot-less BioJect, AtEze, Cedomon, Blight Ban A506, Conquer, Victus, Biosave (10,11, 100, 110, 1000), Proradix, BioPower Lanka, Gmax FYTON, Astha PF, SKS PF	42, 43, 44, 45, 46
Rhizobia	Legumes, peanuts	Nitrogen fixation, Induction of plant stress resistance, Hydrogen Cyanide production	R-Processing Seeds (Japan), Hyper-coating seeds (Japan)	44, 45
Rhizobium	Rice, pepper, tomato, lettuce, carrot, mung beans	Nitrogen fixation, IAA synthesis, ACC deaminase synthesis, phosphate solubilization, siderophore production	Mamezo (Japan), Nitrogin Gold (USA), SymbionN (India), CALOBIUM (India), Rizotorphin (Russia), Rhizobia, Sanjivini N1, Astharhizo	44, 45, 46
Bradyrhizobium	Pigeon pea Soybean,	Chitinase and B-glucanase production	Optimize® (USA), Vault® (USA), ExcalibreTM (USA), MycoGoldTM (USA)	44, 45
Sphingomonas	Tomato	Gibberelin synthesis		44, 45

Streptomyces	Indian lilac, soybeans, Cotton	Siderophore production	EM1R (Japan), EM Bokashi (Japan), Pixplus	44, 45

From table 2, it is apparent that *Bacillus* species is the most prevalent in this market. Many of the *Bacillus* strains (such as *B. cereus, B. pumilus, B. subtilis, B. amyloliquifaciens*) have desirable characteristics that make them particularly suitable as a product for commercialization (48). They are ubiquitous in soils, exhibit high thermal tolerance, and their rapid growth in liquid culture combined with ease of maintenance are advantageous (48). Additionally, they are considered to be a safe biological agent, having high potential in both the biocontrol and plant growth promotion sector (48). Nitrogen-fixing PGPR turn to be the second most highly commercialized strains. However, compared to Bacillus spp., nitrogen fixing PGPR are accounted for mostly in the plant growth promotion sector (6,26,43). Commercialization of Pseudomonas spp. appears to be not as expansive when compared to Bacillus spp. Although, they have similar desirable characteristics as Bacillus spp,, their low implementation rates could be because application of *Pseudomonas* is at infancy stage despite being extensively researched (6,43).

Overall, research and commercialization in general seems to be focussed on free-living rhizobacterial strains, especially to *Pseudomonas* and *Bacillus* (49). Unique associations are present between nonsymbiotic endophytic bacteria (such as *Frankia, Allorhizobium* and endophytic rhizobium) and host plants that leads to a more pronounced growth-enhancing effect on host plants (49), but not much information about them is found in literature.

VIII. CHALLENGES FACED BY MICROBIALS

The previous sections imply that there is significant evidence supporting the use of microbials in agriculture, with microbials outperforming chemicals. However, some concerns persist. These implementation barriers for microbials have been addressed by many authors, which are outlined by Gelernter et al., in 2005 and (23) and Ravensberg et al., in 2011 (20). The main reasons that were reported were: variable efficacy and quality of the products, their cost-performance level, cumbersome registration process, competition with agrochemicals, underestimation of the required investment and implementation time, overestimation of market size and market adoption rate, and lax collaborations between product developers and academic researchers in the industry (10, 20). Some of these challenges persist today. Thus, in this section, we would delve deeper into these challenges. The challenges identified can be broadly classified under three categories: Research and development, the global market and product registration. In this section of the review, we delve deeper into these categories to identify the bottlenecks that are preventing the widespread use of microbials.

8.1 Research and development

Much research has been dedicated to exploring the benefits of PGPR and the soil microbiome on host plants, but there is little evidence of translation of these effects in large scale applications (on-field) (6). Research by itself cannot stand alone and must go in hand with development and commercialization for effective use of the research. Several groups have criticized this issue in the applications of microbials in the agricultural market (20,49). Early on in 1997, Dent stated that the known information on microbial biological control was built up in a haphazard way (49). Individual scientists concentrated on their own studies and interests in comparison to the chemical pesticide business which collaborated with large R&D departments to produce crop protection products (49). More recently, Ravensberg noted that little had changed since then, and proposed that academic scientists and biopesticide developers must begin collaborating early in the product's research and commercialization phase (20).

Though research is more intensive since the first introduction of microbials in the market, important challenges remain. One of the main limiting factors preventing more widespread use of PGPB/PGPRs is their selectivity (13). Conventional agrochemicals often have a broad spectrum of effects on a variety of species (13). On the other hand, PGPB/PGPR tend to be quite focused (13). Previous sections (and table 2) have pointed out the plant host specificity of some PGPR. The PGPR *Azoarcus* for example is highly beneficial for rice crop, but is not found in the microbiome of other crops. For this reason, there are no commercial products existing with *Azoarcus*, albeit rice being a major commercial crop. Additionally, under field circumstances/ in complicated field environments where several organisms act concurrently, the use of PGPR might lead to varied quality and efficacy (13).

Second, the screening strategies of the microbial inoculants must be improvised. This is because the rhizosphere accounts for a diverse habitat for growth of microorganisms and community structure of plant roots (6,13). With this environment changing constantly due to stressors or other factors, it is important to involve differential quantitative and qualitative techniques to identify microbes (49, 50). Additionally, some PGPR such as Pseudomonas spp and *Bacillus subtilis* form biofilms and others are endophytic, which could make them tough to cultivate (50). Screening would lead to better characterization of species that have advantageous effects and overall plant-microbe symbiosis, in addition to discovery of novel species and better understanding of inter-species interactions. With recent developments in multi-omics technologies, other improved computational technologies that have been accordingly reviewed by JM barea, 2015 (51) and Godinez et al., 2021 (52), screening can be performed on a large scale and optimized.

The third issue that arises is the efficacy of the current microbial products on the market (4,6,7). Products have a maximum shelf-life of two-years, and this can be attributed to the active ingredient being live microorganisms (4,6,7). Moreover, in the case of biocontrol products, resistance of pathogens presents as a problem (26). Pathogens develop defense mechanisms that battle host defenses, sometimes with disease turning more aggressive compared to before (26). This is usually observed in microbial products with single species strains, and microbial products are rendered ineffective in such cases (4,6,7,26). Thus, the durability of the biocontrol products must be improved to contain either polymicrobial formulations and/or effective delivery systems (26, 36). The use of polymicrobial formulations will benefit the rhizosphere ecosystem and overall plant health, by combining different effects of the individual species (36). Commercial products containing multiple species of nitrogen-fixing microorganisms currently exist, however they are not commonly combined with other species such as *Bacillus* and *Pseudomonas* for biocontrol and biofertilization (6). Using such combinations would prove to be extremely beneficially for both the crops and the market, but this is an area of nascent understanding (36).

In addition to the issues mentioned above, efficacy of the microbial inoculants needs to be improved for overall research and development (4,6,7). The effectiveness of microbial plant protection solutions can be influenced by a variety of circumstances. Temperature, humidity, wetness (for example, in the soil or on leaf surfaces), plant growth stage, and other factors can influence microorganism behavior in a variety of ways (6). To integrate these, lab trials should be followed by wide-scale field trials to establish the complete effectiveness of the product (6,7).

Laboratory studies may offer information on the mode of action, susceptibility of target pests or hosts, including multiple life stages when applicable, dosage response behavior, and the impact of environmental, agronomic, and other conditions on the product (6,13). For proper dissemination of information to farmers and consumers, the overall conditions required for the microorganism(s) that make up a product's active ingredient to live, proliferate, colonize, or infect target species should be identified when possible, and advise given on the proposed product label if possible (6,13). Farmers could then make informed decisions in the use of microbials over agrochemicals (6,13). Moreover, polymicrobial formulations can be combined with effective delivery systems cold improve vastly the efficacy of microbials. The different delivery systems and their advantages/disadvantages are detailed in Bashan et al., 2015 (15).

8.2 Global PGPB/PGPR market

PGPB/PGPRs are commercialized as biofertilizers and biopesticide products (13). Biofertilizers and biopesticides are generally characterized by the product type, active ingredients, crop type, application and geography (13). In a market research report published by Transparency Market research, it was estimated that the Global Biofertilizers Market size was at USD 3,491.19 million in 2021 and expected to reach USD 3,842.76 million in 2022 (53). The market is projected to grow at a CAGR (compound annual growth rate) of 10.32% to reach USD 6,295.31 million by 2027 (53). For biopesticides, the global market in terms of revenues was estimated to be worth about 5.5 billion USD in 2022 (54). The market is expected to reach $9.6 billion by 2028, at a CAGR of 11.7% during the forecast period of 2021 to 2028 (54).

Over the projected period, North America is expected to lead the worldwide biopesticide/biofertilizer market in terms of demand (53,54). This demand can be owed to the growth of organic products, adoption of advanced irrigation systems and severe concerns towards the excessive use of chemical fertilizers (6,13). For the same forecast period, it is projected that the region of Latin America and Asia Pacific will be the most upward biofertilizer/biopesticide growth market (53,54). This trend is not predicted in Europe due to the long and cumbersome registration process and regulatory issues, even though the adoption of organic markets is high (6,13). Comparatively, in under-developed and developing countries such as India and Africa, slower growth could be attributed to high costs and general adoption of organic products in the market (14,26).

The current biofertilizer and biopesticide market however, represents just about 2.5-5% of the total agrochemicals market (13). Biofertilizers and biopesticides are mainly promoted as supplementary and complementary inputs and not as a replacement to chemicals (14,26). Farmers alternately choose chemical fertilizers as they remain cheap and easily accessible (6,13) As a result, agriculture still remains chemically intensive, and majority of farmers choose not to spend on additional inputs to reduce costs (6,13). Cumulatively, two key issues for the market can be distinguished: first, the biologicals programs have not yet succeeded in demonstrating the cost-effectiveness of the product to encourage governments to invest more and academics to carry out more research, which would accelerate the market development (7, 53). Second, land managers and farmers only see slow progress or initially no impact on yields and do not immediately see the financial benefits of microbials, compared to their usual pesticides that they see as reliable and predictable (53). To overcome this, programs and governmental initiative should be taken for proper dissemination of information to farmers and consumers (14,26). Furthermore, collaborations between private companies and small-scale industries, aimed at reducing costs can provide useful for these markets (6,7,13).

8.3 Challenges with product registration

Microbial product development is guided by regulatory frameworks and product registrations all around the world (55). The legislative framework for producing novel microbial products differs depending on the nation, the product's features, and its intended use (55). These regulations are strict as some PGPR have posed a risk to human health (7,55). This is because some microbial biocontrol agents have been reported toxic and pathogenic to non-target organisms (7,55). These national and international restrictions must be considered at every stage of the product development cycle, even the earliest stages, because regulations also specify the environments from where natural microorganisms can be harvested (55).

The registration process has long remained a hurdle in the microbials development process, and still appears to be so (20,55,56). The registration process in the European Union is complex for new active substances as they are categorized the same as chemical pesticides (55,56). This means that biopesticides would undergo the same regulatory measures as the registration of chemical pesticides (55,56). The guidelines currently used to evaluate biopesticides were originally developed for chemical pesticides and are mostly not appropriate for microorganisms (55,56). Furthermore, biofertilizers and biopesticides are grouped together as plant-protection products (PPP), which means that both go through the same process as for agrochemicals (55,56). This makes the process long and cumbersome, taking an additional 1.62 years (43%) on average, compared to procedures in USA (55,56). Furthermore, in the EU, the active substance is evaluated first, and only then begins the process for registration as a plant protection product (55,56). In contrast, the USA framework has a separate registration for biopesticides (56). The active substance and plant protection product are simultaneously evaluated, making the process less heterogenous, more flexible and less time (56). Also, the USA system uses ‘data waivers’, financial exemptions and conditional registrations to promote the registration process (55,56). This can be reflected in commercialization and in the market, giving a plausible reason as to why North America leads the biopesticide/biofertilizer market (53,54). In other developing countries such as India and Africa however, the registration process is more streamlined, but what remans challenging is the market (as discussed previously) (14,26).

This registration process itself might prove challenging for many companies and start-ups that want to bring a product into the market (6,26). With the recent implementation of Integrated Pest Management strategies in the EU, microbials are characterized as low risk substances (56). However, one requirement for low-risk substances, that is still to be elaborated, is that their half-life in the soil should be less than 60 days (56). This may be disadvantageous to researchers as the development of such a product will have to exclude some microbial pesticides such as rhizosphere competent antagonists of soil borne pathogens (56). Additionally, the long wait until the company can make profits could prove to be discouraging for the microbial market as a whole (6,14,26). Nevertheless, this year marks a huge change in the process for EU as they implement new regulations regarding biofertilizer products (55,56). These new harmonized regulations will come into force on 16 July 2022, and will differentiate biofertilizer products from biopesticides, allowing for a differentiated regulation process (55,56). The length of the registration period is also reportedly decreasing faster in the EU compared to the US (55,56).

IX. CONCLUSION AND FUTURE PERSPECTIVES

Microbials have long been used in agriculture, for their beneficial effects (6). They also present as an appealing, cost-effective alternative to agrochemicals (6,7). Microbes are simple target for companies working in organic ‘green’ agriculture and looking for a replacement for agrochemicals. Alternatively, developing interest in organic and sustainable farming in the past few years, has led to the characterization of more strains and species of bacteria that are beneficial in plant growth (24).

However, their potential applications in sustainable agriculture are still at the infancy stage. Fundamental challenges are present that bars the development of the whole microbials industry. In this review, we identify the challenges in each section of the industry to find areas of improvement. In the first section, we discuss the importance of microbials used in agriculture. We map out the important species of PGPR identified by research and compare to the active strains that are present in the current market. An interesting finding is that, although research has developed considerably, the market is still focused on *Bacillus*, *Pseudomonas* and nitrogen-fixing species. In the second section, the review focuses on detailing the key issues that are present for microbials in the sectors of research and development, market and registration. In this section, it becomes apparent that these three sectors are currently divided with various barriers within them.

For the future of microbials, it is logical that the sectors should not act independently to overcome these barriers. With more collaboration and dissemination of information amongst all participants (researchers, consumers, farmers), the potential of microbials would significantly improve. Recently, programs in the EU specify that member states have been encouraged to use rural development programs (funded under the Common Agricultural Policy) to provide financial incentives to farmers to start implementing microbials (56). With these improving regulations, discovery of new technologies and increasing adoption of sustainability worldwide, microbials can effectively replace agrochemicals in the near future. Overall, microbials now represent a significant division in the agriculture sector, and the future of microbials in sustainable agriculture remains attractive.

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Studies on Seasonal Incidence and Management of Early Shoot Borer and Top Shoot Borers using New Insecticides in Sugarcane (*Saccharum officinarum L.*)

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Abstract— *The present investigation was carried out to Studies on seasonal incidence and management of early shoot borer and top shoot borers using new insecticides in sugarcane (Saccharum officinarum L.), at Institute of Agricultural Sciences, BHU, Varanasi, UP, India to assess the chemical control of sugarcane pest Chilo infuscatellus (Snellen) and Scirpophaga excerptalis (walker) with seven insecticide viz. Novaluron 10 EC @ 100 g a.i. ha-1, Fipronil 5 SC @ 150 g a.i. ha-1, Lambda-cyhalothrin 5 EC @ 25 g a.i. ha-1, Rynaxypyr 20 SC @ 40 g a.i. ha-1, Acetamiprid 20 SC@ 10 g a.i. ha-1, Spinosad 45 SC @ 100 g a.i. ha-1 and Emamactin benzoate 5 SG @ 10 g a.i. ha-1 and compared with untreated control using randomized block design with three replications and observations of dead heart per 10 hills recorded 1 day before spray and 7th, 15th, 30th days after spray. It was observed that infestation both early shoot borer and top shoot borer started from 23rd standard week with 2.45 and 1.23% dead heart per 10 hills respectively with the corresponding maximum, minimum and rainfall was 36.120C, 27.520C and 0.00 mm while the morning and evening RH 71% - 52%. The studies on efficacy of newer insecticide molecules on early shoot borer revealed that the Rynaxypyr 20 SC recorded lowest post treatment mean of (13.85% - 7.13%) over the other insecticidal treatments. On top shoot borer Rynaxypyr 20 SC showed lowest damage of (5.77% - 5.50%) over the other insecticidal treatments. yield of plot treated with Rynaxypyr 20 SC was higher (61.68 t/ha) than that of any other treatments.*

Keywords— Sugarcane, Seasonal incidence, Early shoot borer and Top shoot borer, Efficacy of newer insecticides.

I. INTRODUCTION

Sugarcane (Saccharum officinarum L.) is a tropical plant belonging to the family gramineae. Sugarcane originated in New Guinea, where the cultivated canes were of two main groups: (a) thin, hardy north Indian types Saccharum barberi and the Chinese Saccharum sinenses and (b) thick, juicy noble canes S. officinarum. S. officinarum is highly prized cane. The origin of S. officinarum is Indo-Myanmar China border with New Guinea as the main centre of diversity. The S.officinarum are called the "noble canes' due to thick, juicy, low-fibred canes of high sucrose content. The origin of Saccharum robustum is New Guinea. The origin of Saccharum spontaneum is subtropical India. For cultivation of sugarcane, loam soil of 10 - 15 percent moisture content is suitable. If the moisture content in soil is low, proper moisture should be maintained. Till deep by disc harrow followed by 2 - 3 light plough and levelling. Generally the distance between row to row is kept at 90 cm and setts of three buds are used, 37.5 thousand setts or according to thickness 60 - 65 quintal setts per hectare is used. Before sowing, soak

the setts of 2 or 3 buds in water and then treat with mercury chemical (Ariton 6 percent or Anglol 3 percent) of 0.25 percent solution. For seed processing, Bavistin 0.1 percent solution can be used.

Byproducts like molasses is the main raw material for alcohol and alcohol based industries, paper industry, fuel purposes and green tops are used as cattle feed. About two percent of the sugarcane is used as raw and juice (beverage) purpose. Sugarcane contains about 65 percent juice and juice contains 77.88 percent water, 8 - 12 percent sucrose, 0.3 to 3.0 percent reducing sugar, 0.5 - 1.0 percent organic substance and 0.2 - 1.0 percent ash (Sundra, 2001). Theoretically sugarcane gives a yield of 450 t ha-1 per year (Moore 1998) but the average yield of the country is only around 70 t ha-1. The average yield is higher (80 t ha-1) in tropical region than the sub-tropical 55 t ha-1. The low production and the productivity are the end results of various factors. Among the factors responsible, the insect pests problems are prominent (Purbeyet al, 2000). The production and productivity of the sugarcane is affected by many factors viz, soil type, selections of variety, fertilizer management, irrigation management and damaged caused by pests. Sugarcane is attacked by insects however 15 pests are reported to cause considerable loss in yield. The early shoot borer, top shoot borer, Internode borer, white grub, sugarcane pyrilla, white wooly aphid, scale insect and termites these are major pest of sugarcane but the early shoot borer are worst pest responsible for severe damage in early growth stage and yield loss.

Sugarcane by virtue of its long duration is infested by a large number of pests. Many pests start infesting the cane right from the very first day when setts are planted in the soil, and till the crop is harvested. The crop is attacked by a large number of insect pests (David 1990) and among them Lepidopteron tissue borers are considered to be the most destructive. Out of about a dozen tissue borers, damaging sugarcane crop in India, top borer Scirpophaga excerptalis (Walker & Plassey), Chilotumidiscostalis are the most injurious particularly in Bihar (Purbeyet al., 2000) and shoot borer, Chiloinfuscatelhis in Uttar Pradesh and Assam. Among different borers, shoot borer causes 22 - 33 percent in yield loss (Patil & Hapse, 1981), top borer causes 86 percent in yield loss, stalk borer causes 6.11 - 10.61 percent in yield loss (Jena &Patnaik, 1996), and root borer causes upto 34.20 percent in yield loss (Pandey et al., 1996). In sucking insects pests Pyrillacauses 28.10 percent in yield loss and 1.60 units of sucrose in juice (Agarwal, 1969), white fly causes a loss of 23.40 percent in yield & 2.90 units of sucrose in juice (Khanna, 1948). Mealybug causes a decrease in sucrose content by 24.10 while the reduction in brix is 16.20 percent (Kalra& Sidhu 1964) and the thrips cause a loss of 30.77 percent on central leaves of 90 days old crop (Gupta 1996). The damage and loss caused by top borer, (Scirpophaga excerptalis walker) are due to the mortality of shoots and canes and also due to the arrest in growth of the later. The mortality of young shoots may go even upto 100 percent as observed in Punjab and Bihar (Anon., 1939; Agarwala and Prasad, 1956). In Tamilnadu, due to low incidence of the pest, 10 percent of the shoots die, while in 3-4 percent further growth is suppressed (Doss, 1954). As the crop grows, the percent mortality of shoots/canes due to borer infestation decreases (Anon., 1939; Agarwala and Prasad, 1956; Agarwal and Siddiqui, 1964). Most of the insecticides used on agricultural crop belong to any one of the following chemical group viz. organophosphate, carbamates and pyrethroids. The wide spread use of structurally similar preparation which have same mode of action carries the risk resistance development. To overcome this, discovery of newer classes of insecticide molecules which belongs to formulation technology, active at low doses and least exposure to an environment and their incorporation in integrated pest management system is gaining importance. Increasing the area under sugarcane crop at one hand and relative paucity of the information regarding new molecules on other hand, the present investigation were therefore undertaken to evaluate the new generation, low dose ecofriendly pesticides viz., Novaluron, Lambda-cyhalothrin, Rynaxypyr, Fipronil, Spinosad, Emamectin benzoate and Acetamiprid with following objectives.

Objective of research:

1) To study these Seasonal incidence of early shoot borer and top shoot borer.
2) To study the efficacy of new insecticide against Early shoot borer, Chilloinfuscatellus (Snellen) and Top shoot borer, Scirpophaga excerptalis (walker).

3) To study the impact of insecticidal treatments on sugarcane yield.

II. MATERIALS AND METHODS

The field experiment on the topic entitled "Studies on seasonal incidence and management of early shoot borer and top shoot borers using new insecticides in sugarcane (Saccharum officinarum L.)"were conducted at the Agricultural Research Farm of Institute of Agricultural Sciences, B.H.U, Varanasi during Kharif 2018 - 2019.

2.1 Experimental site

Varanasi lies between 240 56' N to 250 35' N Latitude and 820 14' E to 830 24' E Longitude and the elevation is 141.3 m above the mean sea level, almost in the center of Indo-gangeticbelt. It possesses sub-tropical climate and experiences annual mean precipitation ranging 75 to 100 cm (approx.), most of which is received during kharif season. Mean maximum temperature experienced during the experiment was 41.00C during 2nd week of June and the mean minimum temperature was 4.70C during 3rd week of January. The maximum rainfall experienced was 154.8 mm during 3rd week of August.

TABLE 1
DETAILS OF THE FIELD EXPERIMENT

Crop	Sugarcane
Variety	Co 0239
Design	Randomized Block Design (RBD)
Treatments	8
Replication	3
Plot size	6x3 m^2
Total No. of Plots	24
Number of rows per plot	4
Row to row distance	90cm
Planting material	3 budded setts, 4 setts/meter
Date of sowing	10 April 2018
Date of Insecticides spray	First spray 25 June 2019
	Second spray 22 August 2019
Date of harvesting	12 April 2019

Fertilizer application: The fertilizers were applied at the rate of 120:80:60 kg ha-1 N, P2O5 and K2O in the form of urea, single super phosphate and murate of potash. Full dose of phosphorous and potash and half dose of nitrogen should be applied at the time of planting and rest of the nitrogen after 80- 90 days of planting.

Field trial was conducted to determine the appropriate phenological stage of sugarcane for applying insecticides in order to achieve most effective chemical to control sugarcane early shoot borer and top shoot borer during crops seasons of 2018 - 2019 at experimental site of Agricultural Research Farm, Institute of Agricultural Science, Banaras Hindu University. The experiment consists of eight treatments including control and was laid out in randomized block design (RBD) with 3 replications for evaluating their comparative efficacy against sugarcane early shoot borer and top borer. The sugarcane variety Co 0239, a recommended early was planted on 9th April during 2018. The plot size was kept 6 x 3 m2 with row to row spacing at 0.9 m. The path between replication and subplots were maintained 1.5 m and 1m, respectively. Three budded cane setts were planted eye to eye keeping 4 sets in each row. The uniform agronomical practices were followed as per the recommendation for the crop in this area. The treatment details are as follows:

TABLE 2
DETAILS OF VARIOUS INSECTICIDAL TREATMENTS FOR FIELD EXPERIMENT ON SUGARCANE.

Treatments	Technical name	Formulation (percent)	Dose (g a. i./ha)
1	Novaluron	10 EC	100
2	Fipronil	5 SC	150
3	Lambda-cyhalothrin	5 EC	25
4	Rynaxypyr	20 SC	40
5	Acetamiprid	20 SP	10
6	Spinosad	45 SC	100
7	Emamectin benzoate	5 SG	10
8	Untreated Control	-	-

2.2 Method of application of treatments

The required quantity of spray solution was calibrated by spraying the control plot with water alone. Spraying solution of insecticides per plot of different concentrations were worked out at the time of spraying and mixed in clean water. The spraying of insecticides was carried out during morning hours by electric operated knapsack sprayer. All the three plots of treatment in three replications were treated at a time. The care was taken to cover all the plant parts thoroughly. The spray pump was thoroughly washed with water while switching on one insecticide to another.

2.3 Methods of recording observations

2.3.1 Observations of Meteorological Data

Daily meteorological observation with regards to the temperature (°C) i.e., maximum and minimum, relative humidity (percent) at 07:00 am and 14:00 pm and precipitation (mm) prevailing at Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi were recorded during the course of investigation i.e., 2018 - 2019. Data so obtained were finally merged together to obtain the average of weather parameters viz.; temperature (°C) and relative humidity (percent) and rainfall (mm) on monthly basis from January to December. Correlated with the occurrence of the pest population. A correlation coefficient method was adopted to work out the relationship between the occurrence of the pest and the weather parameters

2.3.2 Bioefficacy of new insecticide molecules against early shoot borer, C. infuscatellus.

The efficacy of various insecticides against early shoot borer was judged on the basis of the percent dead heart at vegetative stage. The spraying was done on ETL basis. The granular application was done in endemic area on ETL basis. The percent incidence (dead heart) was calculated as follows:

$$\text{Incidence (percent)} = \frac{\text{No. of infested plants (dead hearts)}}{\text{Total number of cane observed}} \times 100$$

Simultaneously, the meteorological parameters viz; ambient temperature in °C (maximum and minimum), relative humidity percent, at 07:00 a.m. and 14:00 p.m. and rainfall (mm) were also recorded during the investigation from the observatory of Agricultural experimental farm of Banaras Hindu University. Finally correlation between pest incidence and weather parameters were worked out.

2.3.3 Record of yield

Harvesting was done on 12th April 2019 plot wise. The yield per plot subjected to respective treatments was extrapolated to tones per hectare. The yield data in each treatment was recorded separately and subjected to statistical analysis to test the significance of mean yield variation in different treatments. The percent increase in yield over control in various treatments was calculated by using the following formula.

2.3.4 Statistical analysis

The ANOVA of data recorded during the experiment was made for the insect pests under study and the calculated ‘F’ was compared with tabulated 'F' at 5 percent level of significance. The significance of difference between treatments was judged by CD at 5 percent level of significance.

TABLE 3
EFFECT OF INSECTICIDAL TREATMENTS ON CHILLO INFUSCATELLUS (SNELLEN) AFTER FIRST INSECTICIDAL SPRAY.

S.N.	Treatments	Dosage (g a.i./ha)	Average per cent dead hearts by early shoot borer				Post treatment mean
			1 DBS	7 DAS	15 DAS	30 DAS	
1	Novaluron 10 EC	100	23.10* (4.91)**	20.20 (4.60)	16.20 (4.15)	13.50 (3.81)	16.69
2	Fipronil 5 SC	150	22.10 (4.80)	17.40 (4.29)	14.20 (3.90)	10.90 (3.45)	14.22
3	Lambda-cyhalothrin 5 EC	25	23.20 (4.91)	18.10 (4.37)	15.40 (4.05)	11.90 (3.60)	15.20
4	Rynaxypyr 20 SC	40	23.30 (4.93)	17.10 (4.26)	13.30 (3.79)	11.00 (3.46)	13.85
5	Acetamiprid 20 SP	10	23.70 (4.96)	21.50 (4.75)	20.20 (4.60)	15.60 (4.07)	19.13
6	Spinosad 45 SC	100	24.10 (5.01)	21.10 (4.70)	16.30 (4.16)	13.60 (3.82)	17.06
7	Emamectin benzoate 5 SG	10	23.60 (4.95)	19.80 (4.56)	20.60 (4.64)	15.30 (4.04)	18.60
8	Untreated Control		25.10 (5.10)	23.40 (4.94)	23.30 (4.93)	22.10 (4.81)	23.01
C.D. at 5%			N/A	0.148	0.210	0.193	
S.Em. ±			0.06	0.049	0.069	0.064	

Mean of three replication, ** Figures in parentheses are square root transformed values, DBS- Days before spray, DAS- Days after spray.

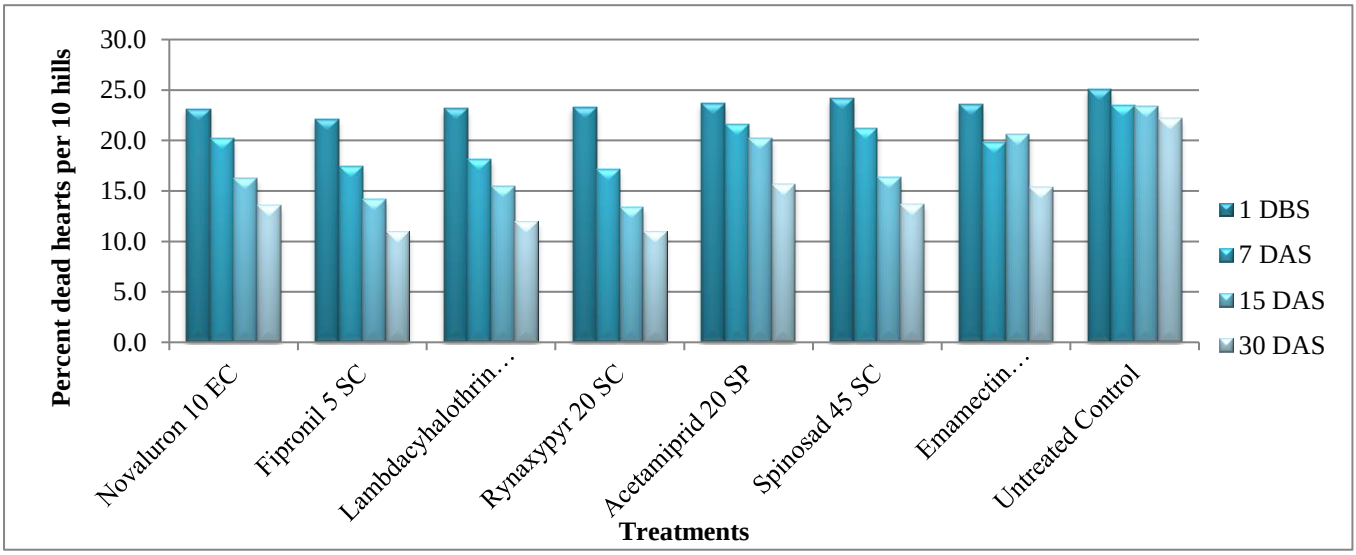

FIGURE 1: Response of insecticidal treatments against *Chilo infuscatellus* snellen after first spray
DBS - Days before spray, DAS - Days after spray

TABLE 4

EFFECT OF INSECTICIDAL TREATMENTS ON *CHILLO INFUSCATELLUS* (SNELLEN) AFTER SECOND INSECTICIDAL SPRAY

Sl.No.	Treatments	Dosage (g a.i./ha)	Average per cent dead hearts by early shoot borer				Post treatment mean
			1 DBS	7 DAS	15 DAS	30 DAS	
1	Novaluron 10 EC	100	24.40* (5.03)**	14.60 (3.94)	10.10 (3.33)	8.30 (3.05)	11.03
2	Fipronil 5 SC	150	23.20 (4.92)	8.80 (3.13)	8.00 (3.01)	7.50 (2.91)	8.14
3	Lambda-cyhalothrin 5 EC	25	23.40 (4.93)	7.40 (2.90)	7.70 (2.95)	6.40 (2.72)	7.22
4	Rynaxypyr 20 SC	40	24.50 (5.04)	7.50 (2.91)	7.50 (2.91)	6.30 (2.71)	7.13
5	Acetamiprid 20 SP	10	24.70 (5.07)	13.50 (3.81)	12.70 (3.72)	13.10 (3.76)	13.14
6	Spinosad 45 SC	100	25.70 (5.16)	15.40 (4.06)	9.90 (3.30)	8.90 (3.15)	11.46
7	Emamectin benzoate 5 SG	10	23.60 (4.95)	15.20 (4.02)	11.40 (3.52)	10.90 (3.45)	12.54
8	Untreated Control		27.20 (5.31)	23.18 (4.91)	19.90 (4.58)	16.30 (4.16)	19.85
C.D. at 5%			0.134	0.156	0.156	0.156	
S.Em. ±			0.044	0.052	0.052	0.052	

Mean of three replication, ** Figures in parentheses are square root transformed values, DBS- Days before spray, DAS- Days after spray.

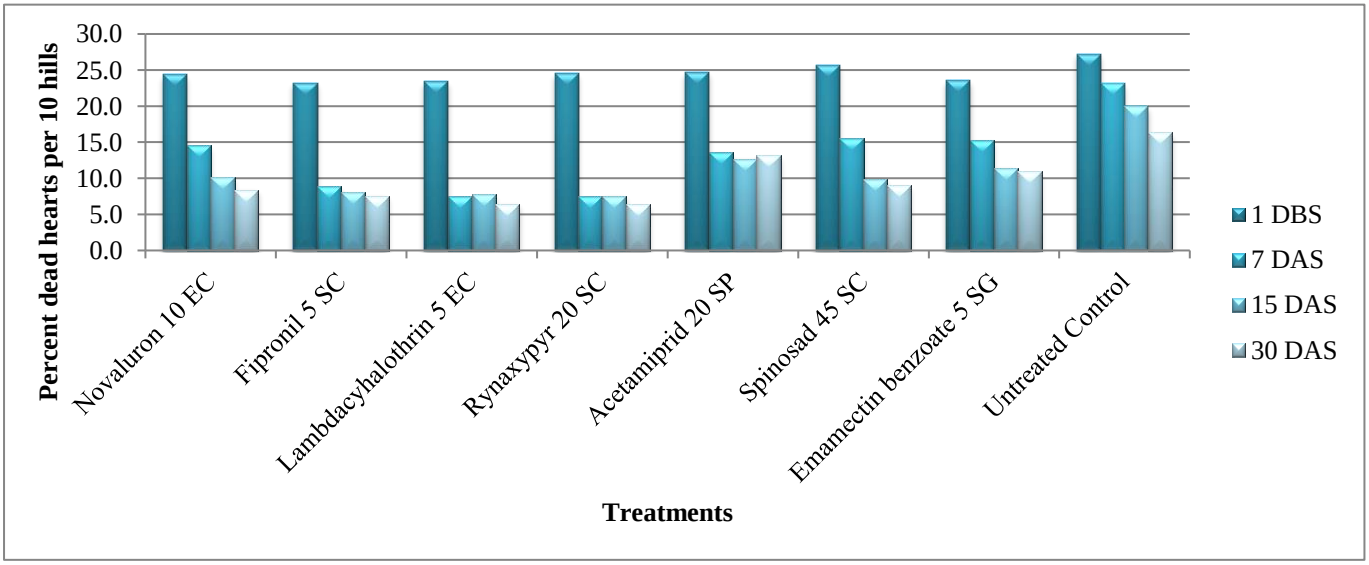

FIGURE 2: Response of insecticidal treatments against *Chilo infuscatellus* snellen after second spray

TABLE 5
INFLUENCE OF ABIOTIC FACTORS ON SEASONAL INCIDENCE OF EARLY SHOOT BORER (*CHILLO INFUSCATELLUS SNELLEN*) OF SUGARCANE

Standared week no.	Rainfall (mm)	Temperature (^{0}C)		Relative humidity (%)		Early shoot borer infestation (%)
		Max.	Min.	Morn.	Even.	
22	0.00	36.15	26.35	69	50	0.00
23	0.00	36.12	27.52	71	52	2.45
24	2.42	41.24	27.33	60	36	5.82
25	0.00	40.42	28.36	63	36	9.62
26	33.30	33.95	26.75	80	61	14.52
27	8.00	35.54	27.94	77	57	10.21
28	11.65	35.59	26.23	83	58	13.54
29	78.43	33.38	25.45	86	66	12.34
30	91.45	28.46	23.62	88	87	10.24
31	86.84	28.17	22.86	93	88	8.37
32	26.64	31.85	24.73	92	77	5.38
33	20.44	33.36	25.34	88	70	3.24
34	154.87	31.11	24.31	91	81	1.42
35	118.48	32.21	24.32	93	77	0.00

TABLE 6
CORRELATION BETWEEN WEATHER PARAMETERS AND EARLY SHOOT BORER (*CHILO INFUSCATELLUS SNELLEN*) INFESTATION

Insect pest	Weather parameters				
	Rain fall (mm)	Relative humidity		Temperature	
		Morning	Evening	Maximum	Minimum
Early shoot borer (%DH)	-0.17666	-0.02310*	-0.07366	0.01698**	0.17091

DH - Dead hearts
***** correlation is significant at the 0.01 level.***
**** correlation is significant at the 0.05 level.***

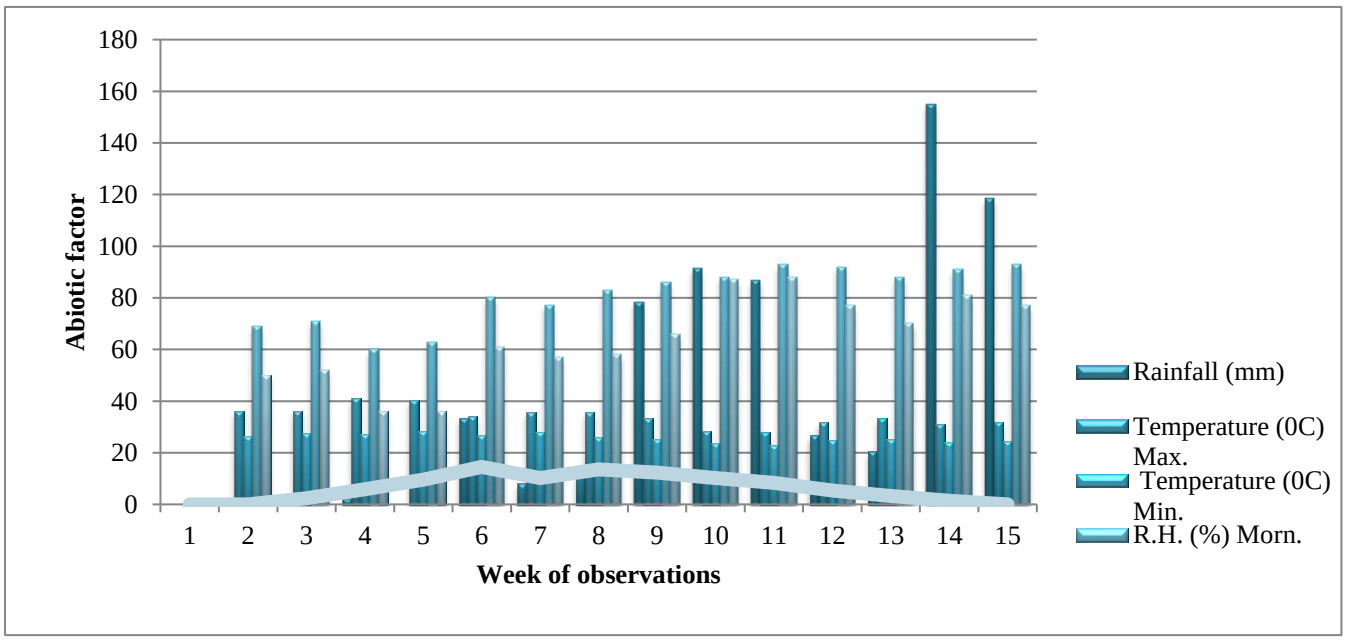

FIGURE 3: Influence of abiotic factor on the infestation of sugarcane *Chilo infuscatellus* snellen

TABLE 7
EFFECT OF INSECTICIDAL TREATMENTS ON *SCIRPOPHAGA EXCERPTALIS* AFTER FIRST INSECTICIDAL SPRAY

S.N.	Treatments	Dosage (g a.i. / ha)	Pre count (%)	Average per cent dead hearts by top shoot borer			Post treatment mean
			1 DBS	7 DAS	15 DAS	30 DAS	
1	Novaluron 10 EC	100	9.56* (3.24)**	8.42 (3.06)	8.66 (3.06)	7.42 (3.10)	8.17
2	Fipronil 5 SC	150	8.58 (3.09)	7.49 (3.08)	6.18 (2.90)	6.36 (2.67)	6.68
3	Lambda-cyhalothrin 5 EC	25	8.44 (3.07)	7.64 (3.05)	7.52 (2.93)	7.45 (2.91)	7.54
4	Rynaxypyr 20 SC	40	8.33 (3.05)	6.42 (3.05)	5.47 (2.71)	5.43 (2.53)	5.77
5	Acetamiprid 20 SP	10	8.85 (3.13)	8.49 (2.89)	7.95 (3.07)	6.60 (2.99)	7.68
6	Spinosad 45 SC	100	9.37 (3.21)	8.52 (3.23)	8.41 (3.08)	8.45 (3.06)	8.46
7	Emamectin benzoate 5 SG	10	9.53 (3.24)	8.42 (3.26)	8.72 (3.06)	8.78 (3.11)	8.64
8	Untreated Control		10.02 (3.31)	11.32 (3.39)	11.47 (3.50)	12.50 (3.53)	11.76
C.D. at 5%			N/A	0.261	0.307	0.212	
S.Em. ±			0.07	0.085	0.1	0.069	

Mean of three replication, ** Figures in parentheses are square root transformed values, DBS- Days before spray, DAS- Days after spray.

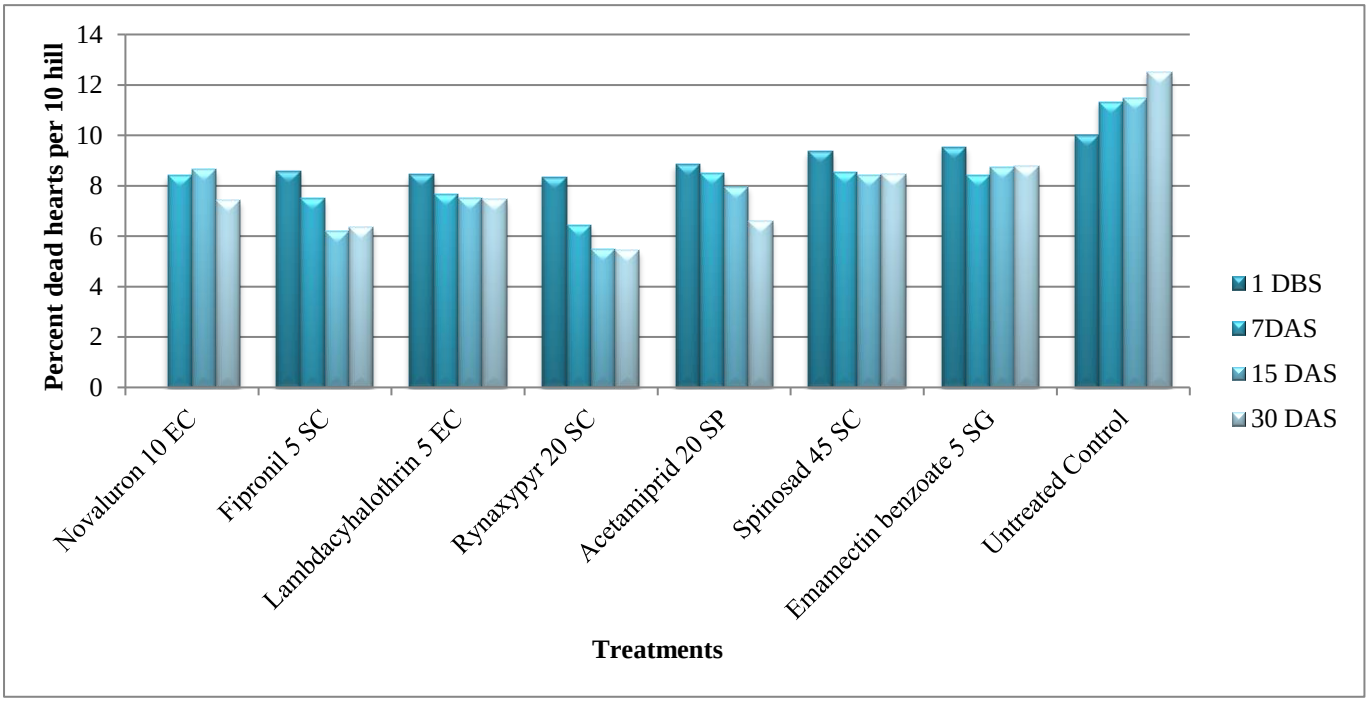

FIGURE 4: Response of insecticidal treatments against *Scirpophaga excerptalis* (Walker) first spray
DBS - Days before spray, DAS - Days after spray

TABLE 8

EFFECT OF INSECTICIDAL TREATMENTS ON *SCIRPOPHAGA EXCERPTALIS* AFTER SECOND INSECTICIDAL SPRAY

S.N.	Treatments	Dosage (g a.i./ha.)	Pre count (%)	Average per cent dead hearts by top shoot borer			Post treatment mean
			1 DBS	7 DAS	15 DAS	30 DAS	
1	Novaluron 10 EC	100	8.65* (2.76)**	6.46 (2.95)	5.34 (2.72)	4.31 (3.05)	6.25
2	Fipronil 5 SC	150	6.89 (2.80)	5.45 (2.97)	4.56 (3.07)	6.44 (2.92)	5.83
3	Lambda-cyhalothrin 5 EC	25	7.62 (2.93)	6.42 (2.90)	5.65 (2.71)	4.66 (3.17)	6.15
4	Rynaxypyr 20 SC	40	6.78 (2.78)	5.33 (2.75)	5.63 (2.51)	4.14 (2.56)	5.50
5	Acetamiprid 20 SP	10	8.07 (3.01)	7.80 (2.90)	7.44 (2.96)	7.01 (2.90)	7.67
6	Spinosad 45 SC	100	7.70 (2.94)	6.10 (2.89)	6.01 (2.66)	5.43 (2.64)	6.33
7	Emamectin benzoate 5 SG	10	8.52 (3.08)	7.54 (2.89)	6.69 (2.91)	6.49 (2.77)	7.38
8	Untreated Control		9.24 (3.28)	9.54 (3.19)	9.66 (3.23)	9.80 (3.26)	9.66
C.D. at 5%			0.22	0.202	0.297	0.255	
S.Em. ±			0.072	0.066	0.097	0.083	

**** Mean of three replication,** Figures in parentheses are square root transformed values, DBS- Days before spray, DAS- Days after spray.***

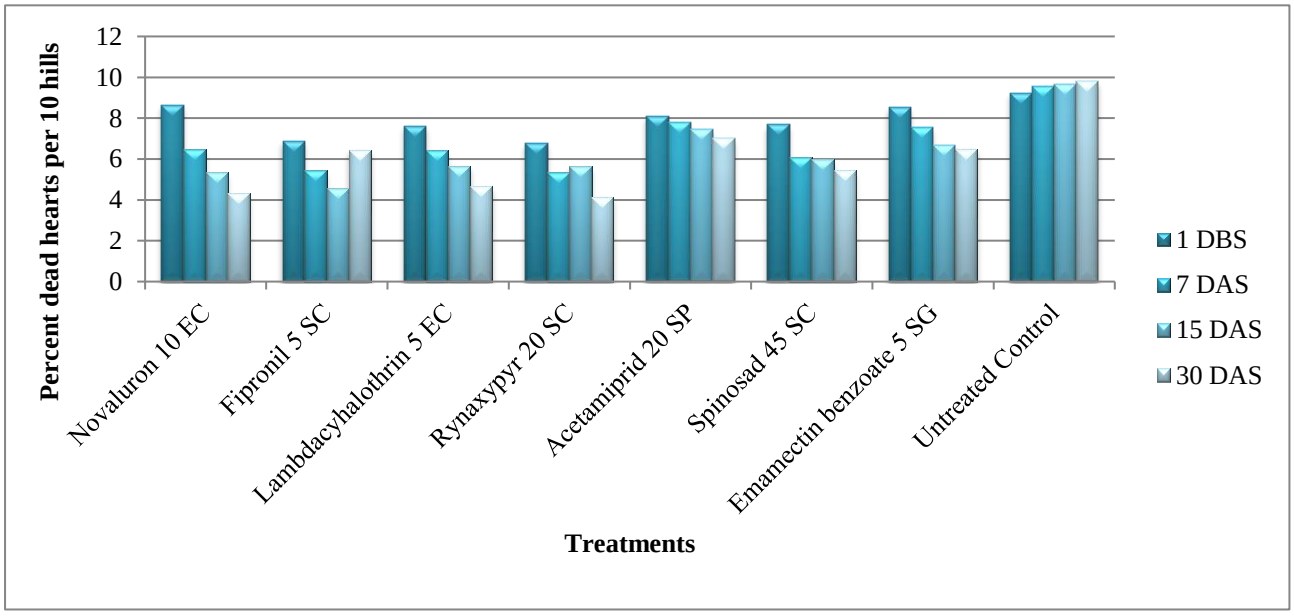

FIGURE 5: Response of insecticidal treatments against *Scirpophaga excerptalis* (Walker) second spray

DBS - Days before spray, DAS - Days after spray.

TABLE 9

INFLUENCE OF ABIOTIC FACTORS ON SEASONAL INCIDENCE OF TOP BORER (SCIRPOPHAGA EXCERPTALIS) OF SUGARCANE

Standard week No.	Rainfall (mm)	Temperature (^{0}C)		R.H. (%)		Top borer infestation (%)
		Max.	Min.	Morn.	Even.	
22	0.00	36.15	26.35	69	50	0.00
23	0.00	36.12	27.52	71	52	1.23
24	2.42	41.24	27.33	60	36	3.56
25	0.00	40.42	28.36	63	36	4.34
26	33.30	33.95	26.75	80	61	3.69
27	8.00	35.54	27.94	77	57	5.85
28	11.65	35.59	26.23	83	58	6.02
29	78.43	33.38	25.45	86	66	12.34
30	91.45	28.46	23.62	88	87	9.24
31	86.84	28.17	22.86	93	88	8.96
32	26.64	31.85	24.73	92	77	10.24
33	20.44	33.36	25.34	88	70	9.23
34	154.87	31.11	24.31	91	81	6.56
35	118.48	32.21	24.32	93	77	5.62
36	94.67	30.65	23.63	91	79	4.37
37	0.00	32.42	23.68	88	68	2.84
38	53.42	32.53	22.86	88	65	1.66
39	0.00	33.44	25.95	88	63	1.13
40	0.00	34.25	20.87	83	51	1.09
41	0.00	31.64	20.34	89	61	1.20
42	0.00	33.43	16.56	84	40	0.00

TABLE 10

CORRELATION BETWEEN WEATHER PARAMETERS AND TOP BORER (*SCIRPOPHAGA EXCERPTALIS*) INFESTATION

Insect pest	Weather parameters				
	Rain fall (mm)	Relative humidity		Temperature	
		Morning	Evening	Maximum	Minimum
Top borer (%) DH	-0.51872	-0.35426*	-0.58808	0.33343**	0.23221

DH - Dead hearts

***** correlation is significant at the 0.01 level.***

**** correlation is significant at the 0.05 level.***

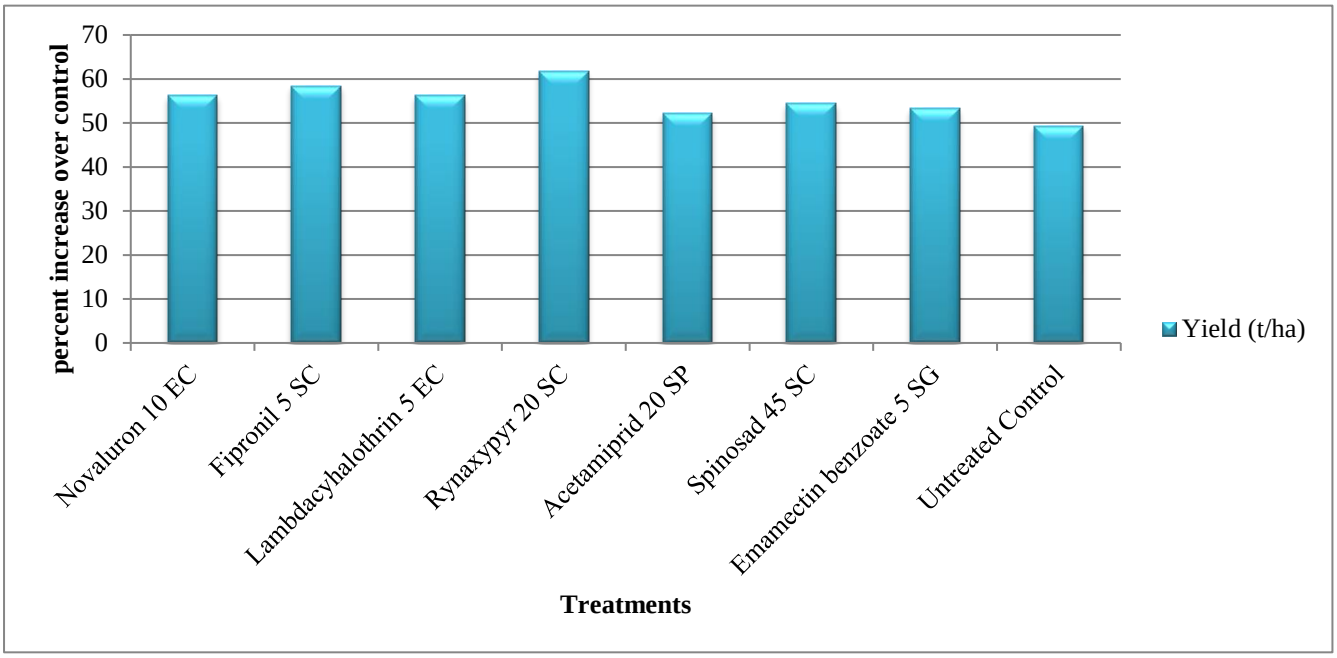

FIGURE 7: Impact of insecticidal treatments on Sugarcane yield

III. SUMMARY AND CONCLUSION

Seasonal incidence of insect pests on sugarcane was studied on a separate bulk plot in the same field having isolation distance. Weather data was also recorded simultaneously from the meteorological observatory available at the Agricultural Research farm work out the relationship between the occurrence of insect pests and weather parameters. To study the bio efficacy, two sprays of test insecticides viz., Rynaxypyr 20 SC, Fipronil 5 SC, Lambda-cyhalothrin 5 EC, Novaluron 10 EC, Spinosad 45 SC, Emamectin benzoate 5 SG and Acetamiprid 20 SP. were assessed against the major insect pests and the data thus obtained have been subjected to suitable transformation before being statistically analyzed. Studies on the incidence of C. infuscatellus revealed that the percent dead hearts were observed to be highest 26 th standard week (14.52 percent infestation) and S. excerptalis its peak population during 29 th standard week (12.43 percent infestation).

The correlation studies between the incidence of C. infuscatellus and weather parameter revealed that percent dead hearts had showed a significant positive correlation with maximum temperature (r=0.01698) and minimum temperature (r=0.17091). A non- significant negative correlation with morning RH (r=-0.02310), evening RH (r=-0.07366) and rainfall (r=-0.17666). The correlation studies between the population's buildup of S. excerptalis and weather parameter revealed a significant positive correlation with maximum temperature (r=0.33343) and minimum temperature (r=0.23221). A non-significant negative correlation with rainfall (r=-0.51872) and evening RH (r=-0.58808). A significant negative correlation with morning RH (r=-0.35426). Regarding the efficacy of insecticides it was observed the Rynaxypyr 20 SC was found to be effective against the insect pests under study viz., C. infuscatellus (13.85 percent and 7.13 percent post treatment mean) and S. excerptalis (5.77 percent and 5.77 percent post treatment mean). It is the only insecticidal treatment among all the treatments assessed which proved to be most effective against both pests in sugarcane. The insecticides Fipronil 5 SC were found to be effective next to Lambda-cyhalothrin 5 EC. Among all insecticidal treatments, application of Acetamiprid 20 SP recorded a low efficacy compared with other insecticides but significantly superior over control.

The yield was found to be highest in Rynaxypyr 20 SC treated plot (61.68 t ha -1) and was followed by Fipronil (58.36 t ha -1) and Lambda-cyhalothrin 5 EC (56.25 t ha -1) treated plot. Among all the insecticides a low yield was recorded in plots treated with Acetamiprid 20 SP (52.14 t ha -1) but the yield was significantly higher than the mean yield recorded in untreated control plots. From the above observations, it could be concluded that; High incidence of C. infuscatellus were observed (14.52 percent infestation) during fourth week of June (standard week no. 26) whereas, in case S. excerptalis peak population was observed (12.34 percent infestation) during third week of July (standard week no. 29) and the population dynamic were high during the vegetative phase of crop growth. Bio-efficacy of insecticidal treatment against major insect pest of sugarcane showed that Rynaxypyr 20 SC was first best insecticidal treatment against the pest's viz., early shoot borer (13.85 percent and 7.13 percent post treatment mean) and top shoot borer (5.77 percent and 5.5 percent post treatment mean). Besides Fipronil 5 SC, the next best insecticidal treatments were Lambda-cyhalothrin 5 EC, Novaluron 10 EC and Spinosad 45 SC.

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Effect of Plant Growth Regulators on Chlorophyll Content and Relative Water Content of Green Gram under Salinity

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Abstract— *An investigation was carried out in green gram CO8. The objective of the experiment was standardization of NaCl to study the effects of salinity during seed germination of green gram and to study the response of green gram treated with plant growth regulators to salinity. In laboratory study, the standardization of NaCl was done first by using 75mM, 100mM, 150mM and 200mM NaCl. Among the four concentrations, 50% germination was observed in 150mM NaCl and it was standardized for further experiment. The plant growth regulators used were T3: NAA 100ppm, T4: NAA 200ppm, T5: kinetin 50ppm, T6: kinetin 100ppm, T7: GA₃ 100ppm, T8: GA₃ 200ppm along with T1: Absolute control and T2: Control (150mM NaCl). The experimental results showed a decrease in seedling growth due to salinity but with the seed treatment with the above mentioned PGRs showed an increased stress tolerance index, chlorophyll content and relative water content. The seeds treated with GA₃ 200ppm recorded the maximum stress tolerance index (STI) (83.09) as well as higher relative water content (88.44%). While the seeds treated with kinetin 100ppm recorded the maximum chlorophyll content (1.22 gm/g). The whole study revealed that, in laboratory condition, with the imposition of salinity stress by 150mM NaCl, the seed treatment with GA₃ 200ppm responded better compared to other treatments.*

Keywords— Greengram, PGR, NaCl, Stress tolerance index, Chlorophyll content, Relative water content.

I. INTRODUCTION

Green gram (*Vigna radiata*), also called Mungbean is a pulse crop from botanical family of Fabaceae. It is a warm season, frost-intolerant plant and suitable for being planted in temperate, sub-tropical and tropical regions. The most suitable temperature for mung bean's germination and growth is 15-18 °C. It has high adaptability to various soil types, while the best pH of the soil is between 6.2 and 7.2. Since, it is a short-day plant, long day condition will delay its flowering and podding.

Salinity is one of the most important abiotic stress factors limiting plant growth and productivity (Flowers, 2004). Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations affect seed germination, induce water deficit, cause ionic imbalance of the cellular ions resulting in ion toxicity and osmotic stress (Khan and Panda, 2009). Specific effects of salt stress on plant metabolism have been related to the accumulation of toxic Na+ and Cl- ions or to K+ and Ca2+ ions depletion (Sreenivasulu *et al.*, 2000). As a consequence of ion imbalance and hyperosmotic stress, which are primary effects of salt stress, secondary stress such as oxidative damage may occur (Rahman *et al.*, 2018). Plant hormones, also known as phytohormones, are small chemical messenger that are produced within the plant at extremely low concentrations and plays a crucial role in plant growth and development by co-ordinating their cellular activities. It control all aspects of plant growth and development, from embryogenesis, regulation of organ size, pathogen defence, stress tolerance and up-to reproductive development. Aziz Khan *et al*., (2009) concluded that phytohormones are known to play vital roles in the ability of plants to acclimatize to varying environments, by mediating growth, development, source/sink transitions and nutrient allocation.

Auxin plays an important role in cell elongation in the shoot, apical dominance, root initiation, prevention of abscission, induction of parthenocarpy, stimulation of respiration, activate cell division and induce callus formation, induce vascular differentiation in plants. NAA is a synthetic plant hormone in the auxin family and is an integral component in many commercial plant rooting horticultural products. It is a rooting agent and used for the vegetative propagation of plants from

stem and leaf cuttings. It is also used for plant tissue culture. Kinetin is a cytokinin derivative which promotes cell division and plant growth. It has been shown to naturally exist in DNA of organisms including humans and various plants. While kinetin is used in tissue cultures to produce new plants, it is also found in cosmetic products as an anti-aging agent. Gibberllic acid, a plant hormone stimulating plant growth and development is a tetracyclic di-terpenoid compound. GAs stimulate seed germination, trigger transitions from meristem to shoot growth, juvenile to adult leaf stage, vegetative to flowering, determines sex expression and grain development along with an interaction of different environmental factors viz., light, temperature and water (Sivakumar R *et al.*, 2018).

II. MATERIALS AND METHODS:

The present investigation was carried out to evaluate the effect of plant growth regulating chemicals in alleviating the effect of salinity in Green gram CO8. The effect of salinity was evaluated by seed soaking method of plant growth regulators. The research trail was carried out as a laboratory study at Crop Physiology Lab, Department of Crop Management, Thanthai Roever Institute of Agriculture and Rural Development, Perambalur. The experiment was laid out under completely randomized block design with eight treatments and three replications. The seeds of Greengram CO8 were placed in petriplates. The petridishes for the experiment were sterilized using 0.01 per cent HgCl2 and 70 per cent ethanol and finally repeated washing with distilled water. The salinity was imposed through using NaCl at different concentrations viz., 75mM, 100mM, 150mM and 200mM. Among these concentrations of NaCl used, there was no seed germination in 200mM. In 75mM and 100mM, almost all the seeds were germinated along with control. However, only 50 per cent of the seeds were germinated in 150mM concentration of NaCl compared to control. Hence, NaCl 150mM was standardized to carry out the experiment to evaluate the effect of plant growth regulators in mitigation of salinity stress effect in greengram. Seeds were soaked in T3: NAA 100ppm, T4: NAA 200ppm, T5: kinetin 50ppm, T6: kinetin 100ppm, T7: GA3 100ppm and T8: GA3 200ppm. After that, seeds were dried up under shade for four hours. These treated seeds were later placed on germination sheet in each petriplates separately, untreated seeds in control and absolute control. The germination paper was moistened at regular intervals with NaCl 150mM solution for salinity and water for absolute control. The petriplates were kept in laboratory under room temperature. The seeds were allowed to germinate by pouring the NaCl 150mM solution of approximately ten ml each once in three days. Distilled water was used for maintaining the absolute control. Stress tolerance index of the seeds was calculated using the following formula proposed by Dhopte and Livera-Muñoz (1989). The contents of chlorophyll 'a', 'b' and total chlorophyll were estimated by adopting the procedure of Arnon (1949) and the content was expressed as mgg-1 of fresh weight. The relative water content (RWC) was estimated according to Barrs and Weatherley (1962) and calculated by using following formula and expressed as per cent.

III. RESULT AND DISCUSSION

The results showed the significant differences among the treatments. The highest stress tolerance index was noticed in GA3 200 ppm (T8) treatment (83.09 %) followed by GA3 100 ppm (T7) (79.54, the lowest stress tolerance index was recorded by NAA 200 ppm (T4) (24.31%). Stress tolerance index (STI) indicates the tolerant potential of the plants during stress. In this present investigation GA3 200ppm (T8) noticed up to (70%). High stress tolerance index compared to control followed by GA3 100ppm (T7) (6.8%) **(Table 1)**. This increment may be due to GA3 200ppm (T8) induced germination, vigour index, shoot and root length under saline environment.

TABLE 1
EFFECT OF PGRS ON STRESS TOLERANCE INDEX (%) OF GREEN GRAM UNDER SALINITY

S.No	Treatments	Stress tolerance index (%)
1	T2: Control (Salinity)	15.22
2	T3:NAA 100ppm	31.31
3	T4:NAA 200ppm	24.31
4	T5:Kinetin 50 ppm	28.95
5	T6:Kinetin 100ppm	38.54
6	T7:GA3 100 ppm	79.54
7	T8:GA3 200ppm	83.09
Mean		42.99
SEd		2.70
CD (P=0.05)		5.78

The chlorophyll content was reduced by the salinity compared to control. Significant difference was noticed in all the treatments with respect with respect to chlorophyll extent. GAʒ 200ppm (T8) was obtained the highest chlorophyll content (1.090 mg\g). Seed treatment with PGRs showed a significant increment in chlorophyll content. Among the PGRs, highest chlorophyll content was obtained in seed treatments with GAʒ 200ppm (T8) (1.09 mg\g) followed by kinetin 100ppm (T6) (1.22mg\g) and GAʒ 100ppm (T7) (0.76 mg\g). However the lowest chlorophyll content was observed in the treatment NAA 100 ppm (T3) (0.43mg\g) followed by NAA 200 ppm (T4) (0.69 mg\g) **(Figure 1)**. Chlorophyll content of the absolute control (T1) is (0.90mg\g). A Decrease in photosynthesis pigment content of green gram plants under salt stress was observed. There was a decrease of 90% of Chlorophyll a in response to the 150 mM NaCl treatments. When respectively compared to the control. In the case Chlorophyll b decrease was 93% in response to 150mM NaCl treatments respectivety, compared to control total Chlorophyll was reduces by 92% under high salinity. The reduction in leaf Chlorophyll content under Nacl stress has been attributed to the destruction of Chlorophyll pigments. In the present investigation kinetin 100ppm (T6) is high Chlorophyll content 92% compared to control. Kinetin improves the Chlorophyll content similar response Cengiz Kaya et al. (2010) start that foliar application kinetin improved the Chlorophyll level in salinity stressed.

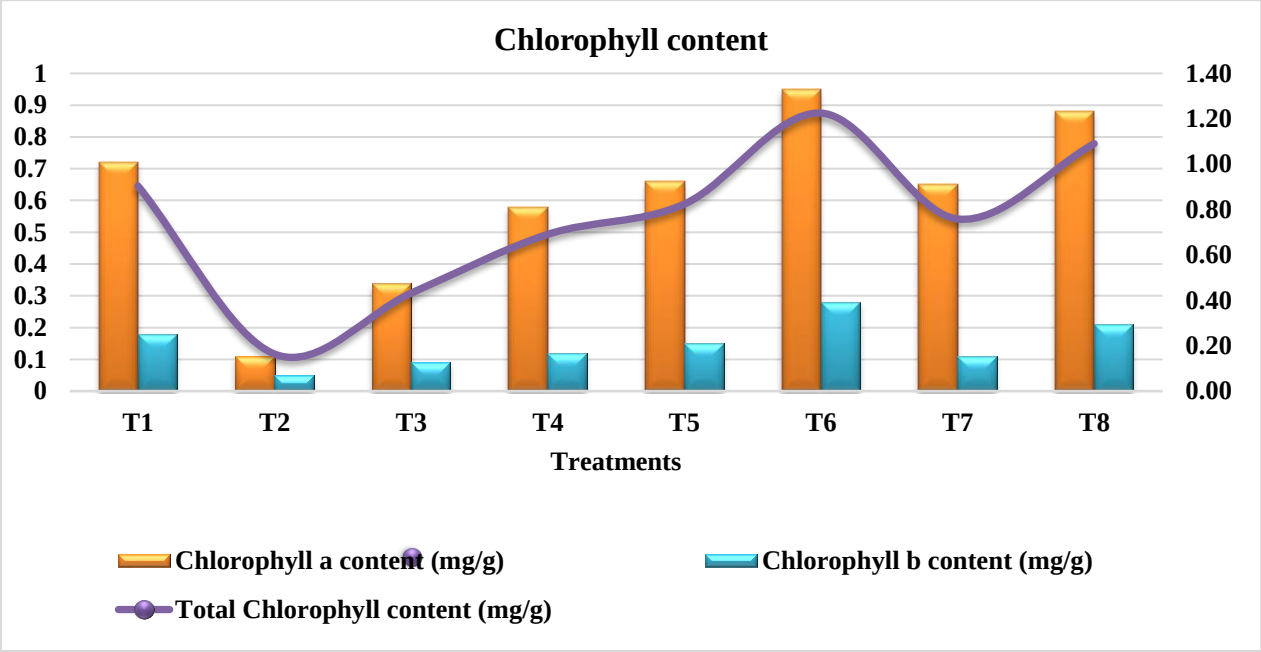


FIGURE 1: Effect of PGRs on chlorophyll a and chlorophyll b of green gram under salinity

Significant differences were noticed in all the treatments with respect to relative water content. The GAʒ 200ppm (T8) treatment recorded the higher RWC (88.44%). The control (T2) showed the lowest RWC (61.85%). Among the PGRs, there was a significant increment in RWC. Highest RWC was observed in seed treatment with GAʒ 200ppm (T8) followed by kinetin 100ppm (T6) (82.33%) and GAʒ 100ppm (T7) (80.70%). The lowest RWC in treatments is NAA 100ppm (T3) (68.43%) followed by NAA 200ppm (T4) (72.12%) **(Figure 2)**. The absolute control (T2) RWC is (82.10%). Relative water content was decreased up to 26.59% by salinity stress. This might be due to high level of sodium chloride changed the water potential and decrease the water absorption of the plant, hence Relative water content is decreased. Reduction in Relative water content is a common effect of salinity stress. Mohammed arifsadik polash et al. (2018) stated, When the plants are subjected to salinity they faced an osmatic challenge that reduces water uptake by roots. In the present investigation GAʒ 200ppm (T8) increase the Relative water content up to 26.59% compared to control. Relative water content declined with water deficit similar reports have been made for many species during water stress condition. Such a decrease in Relative water content is due to unavailability of water in the soil (Shalhevet 1993), both 25& 50 Mg\L. GAʒ treatments elevated water status in water stressed plant.

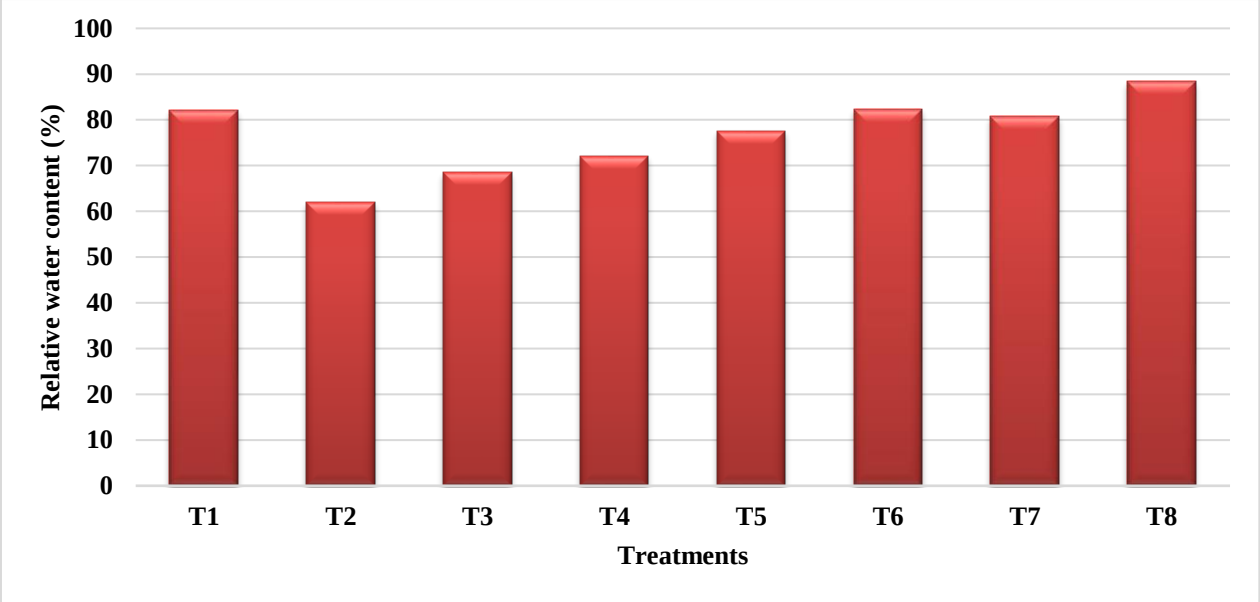
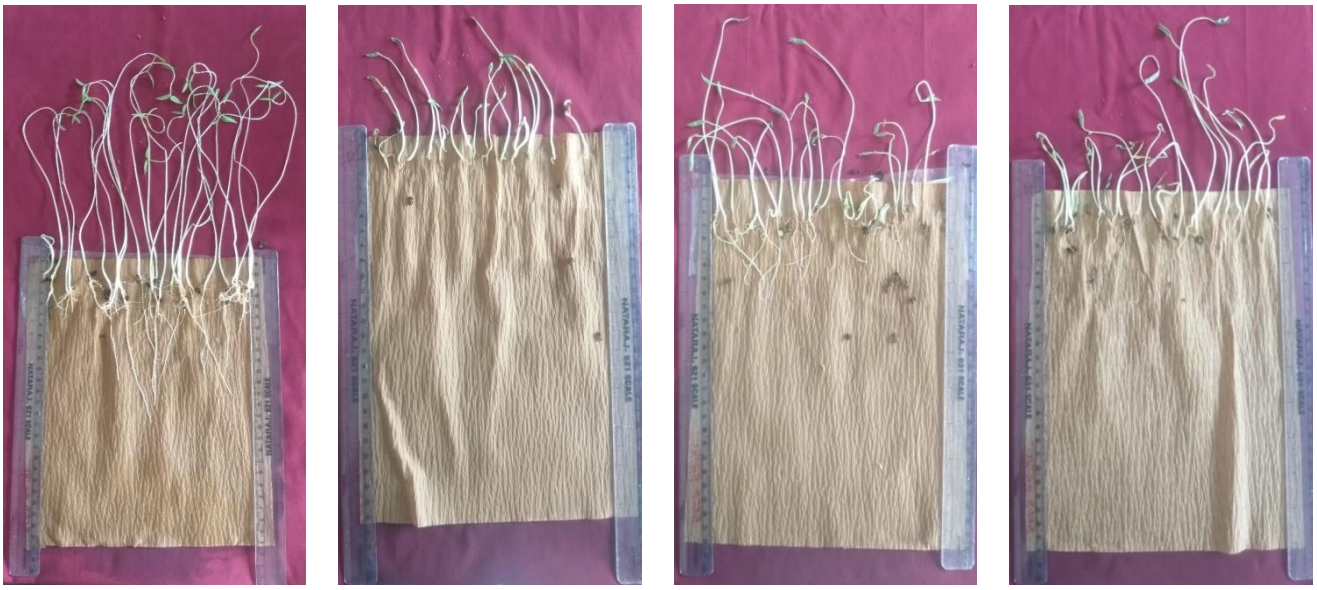


FIGURE 2: Effect of PGRs on RWC (%) of green gram under salinity

IV. SUMMARY AND CONCLUSION

The objectives of the study were to find the effects of salinity during seed germination of Greengram and to study the effect of the plant growth regulators in response to salinity stress during the seed germination in Greengram **(Figure 3)**. The NaCl solution was first standardized so that the resulting germination would be in the range of 50%. This is done with the trial of germinating the seeds with different concentrations NaCl solutions, such as 75 mM, 100 mM, 150 mM and 200 mM solutions. In these the 150 mM NaCl solution yielded the desired result and this solution was used for the study going forward. The seeds are treated by soaking the seeds with the 100 ppm (T3) and 200 ppm (T4) of NAA, 50 ppm (T5) and 100 ppm (T6) of Kinetin and 100 ppm (T7) and 200 ppm (T8) of GA3, for 8 hours. Then the treated seeds are places in the germination papers and are regularly saturated with the 150 mM NaCl solution except for the Absolute control which is saturated with normal irrigation water.



T1:Absolute control (Without salinity) **T5:Kinetin 50 ppm** **T7:GA3 100 ppm** **T8:GA3 200 ppm**

FIGURE 3: Effect of plant growth regulators on seedling growth of Greengran CO8 under salinity

Stress tolerance index (STI) indicates the tolerant potential of the plants during stress. In this present investigation the STI of GA3 200ppm (T8) treatment noticed up to (70%) with high stress tolerance index compared to control followed by GA3 100ppm (T7) (6.8%). This increment may be due to GA3 200ppm (T8) induced germination, vigour index, shoot and root length under saline environment.

There was a decrease in the total chlorophyll content upto 92%, chlorophyll a upto 90% and chlorophyll b upto 93% compared to the control in response to the 150 mM NaCl treatment. The seeds treated with the Kinetin 100 ppm (T6) had a high chlorophyll content of 92%. Relative water content was decreased up to 26.59% by salinity stress. This might be due to high level of sodium chloride changed the water potential and decrease the water absorption of the plant, hence Relative water content is decreased. Reduction in relative water content was a common effect of salinity stress. GA3 200 ppm (T8) had increased the relative water content upto 26.59%.

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Hydroxyl Radical Oxidation Processes in Vegetables: Review and Safety Criteria

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Abstract— *Ethylene is an invisible, colorless and odorless gas, which has no known dangerous effect in humans, on concentrations found within the storage chain and sale of fruits and vegetables. The ethylene molecule is relatively small and simple: it consists of two carbon atoms associated with four hydrogen atoms. The molecular weight of ethylene is 28.05 gmol^{-1} and fruits and vegetables produce different amounts of ethylene as they mature. Usually, ethylene cannot be detected by humans, although sometimes only people with well-developed olfactory ability can smell large amounts, but to this fact other volatile organic compounds of fruits and vegetables also contribute. Since its specific weight (1,178 kgm^{-3} to 15ºC) is similar to that of air (1,225 kgm^{-3} to 15ºC), ethylene freely diffuses to any other adjacent fruit or vegetables and to the spaces in which they are stored.*

In addition to CO_2 and O_2, ethylene is the most important gas to be monitored and controlled in the fruit and vegetable supply chain. Less than one part per million (1 ppm) in volume of ethylene gas, is enough to trigger the maturation process of the climatic fruit (which can continue to mature, once collected). Ethylene is considered a plant hormone that controls a wide range of physiological processes. During storage, after harvesting fruits and vegetables, ethylene may induce effects including senescence, over-maturation, accelerated quality loss, increased susceptibility to fruit pathogens and affecting different physiological processes. In addition to the endogenous production of ethylene by plant tissues, there are also external sources such as contaminants and the own metabolism of plants and fungi.

Keywords— ethylene, hydroxyl radical, volatil organic compounds (VOCs), advanced oxidation processes (AOP), Oxidative stress.

I. INTRODUCTION

It is known that ethylene concentration in of ethylene in fruit and vegetable storage areas and the presence of harmful elements for products stored in cold rooms is a problem for production and consumer companies as fruits and vegetables produce different amounts of ethylene as they ripen (1). Among the harmful elements are volatile organic compounds (VOCs) (2), as a result of the metabolism of stored products the same, fungi, dispersed by the air through their spores and ethylene which, although encompassed in VOCs, deserves attention apart, given its character as a vegetable hormone, triggering the ripening processes in fruits.

II. MATERIAL AND METHODS

Ethylene is the most important gas to be monitored and controlled in the fruit and vegetable supply chain (3,4). Solutions based on advanced oxidation processes (AOP) have been routinely used through photocatalysis, preferably with titanium dioxide (TiO_2) and ultraviolet (UV) radiation that, unlike those made with ozone (O_3), are safer by directly producing hydroxyl radicals (OH•) responsible for oxidation processes, preventing the accumulation of toxic gases (5,6).

OH• production occurs naturally in organisms as well as in the atmosphere. OH• is formed in biological systems by oxidative metabolism in which, predominantly in mitochondria, superoxide radicals (O_2-•) are formed as an unwanted byproduct. Superoxide radical can be removed by the enzyme superoxide dismutase. The product, H_2O_2, can be disposed of by other enzymes (catalase), or may undergo a Fenton reaction in the presence of transitional metal ions to generate OH•.

In the oxidation of ethylene up to CO_2 and H_2O it is necessary a series of steps that produce intermediate products, which can be even worse than the original product. These intermediate products are ethylene oxide, formaldehyde, or methanol depending on the oxidative characteristics of the medium, all with hygienic hazards. This situation is complicated as the largest is the size of the molecule to be destroyed, increasing the chances of intermediate products. VOC's measurement allows you to determine whether the scrubber process reduces the levels present and whether secondary products resulting from oxidation appear.

III. RESULTS

In a study to determine the concentrations of ethylene, VOC's and fungal colony forming units (CFUs) in products stored in cold rooms exposed to AOP(7), the samples were analyzed using different methodologies and eventually analyzed by thermal desorption and gas chromatography with mass spectrometer (CGMS) for the identification of different compounds. All results showed a significant reduction in study variables. In the specific case of the chamber where melons were stored (Cucumis melo) the concentrations of ethylene before and after the AOP went from 1.5 - 3.4 ppm to 0.6 - 1.3 ppm respectively and in the chamber where peppers were stored (Capsicum annuum), the CFU went from an average of 149, AOP to 25 CFU's after treatment was applied. The choice of study parameters was determined, in the case of ethylene, by their importance in fruit aging processes limiting their duration in cold rooms; in the case of CFUs due to disease problems in stored fruits and VOC because they are generally compounds from the metabolism of stored products that produce the odors of stored products and because they can worsen the quality of these, being absorbed by other fruits or vegetables.

The OH• radical is the most reactive free radical interacting with virtually any molecule it finds, reacting almost instantly at the site of its formation due to its high reactivity and short half-life. The distribution of an OH• radical attack depends on the density of electrons at the site of the attacked molecule. Due to its electrophilic nature, the OH• radical is preferably added to the site with the highest electron density. Other reactive oxygen species (ROS) that are not present in a radical form, such as O_3 cross biological membranes expanding their field of action and toxicity for longer periods of time (8,9).

Much of the VOC in the atmosphere comes from plant emissions, which is more evidence that the composition of the Earth's atmosphere is largely determined by biological activity. OH•/VOC's reactions lead to the formation of alkyl radicals (R•), alcoxi radicals (RO•), peroxi radicals (RO2•) and other species, which are transformed by decomposition, isomerization or hydrolysis, leading to the formation of oxygenated compounds, such as alcohols, carbonyls (aldehydes or ketones), carboxylic acids and hydroxycarbonyls (10). Both OH• and O_3 contribute more or less equally to the oxidation of d-limonene (11).

3.1 Reactions involving OH•

There are 3 main types of reactions involving OH• (see fig.1): hydrogen abstraction, electron addition and transfer. All these reactions predict the effect caused by OH•. Basically, all reactions lead to the formation of new radicals and therefore propagate chain reactions. An example of abstraction is OH•'s reaction to alcohols. OH• extracts H• and forms water, leaving an unpaired electron in the alcohol carbon atom. Easily, OH• can be added to double bonds to form an oxygenated intermediate derivative that can also participate in electron transfer reactions(12). In the case of an aromatic compound, OH• follows the mechanism of an **Electrophilic Aromatic Substitution** (EAS), during the process of its incorporation into the aromatic ring.

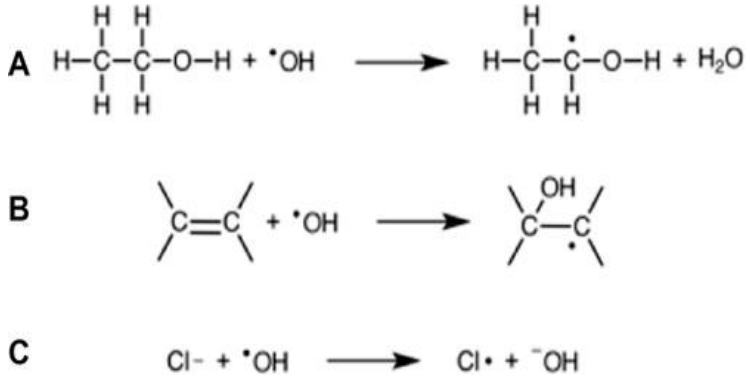

FIGURE 1: Three Main Types of Oh• Reactions: (A) Hydrogen Abstraction; (B) Addition to the Double Bond; (C) Electron Transfer. Taken from Treml & Šmejka, 2016: Flavonoids as Potent Scavengers of Hydroxyl Radicals (https://doi.org/10.1111/1541-4337.12204).

There is a difference between OH• getters and antioxidants. The mechanism of action of OH• removers is direct scanning, while antioxidants include OH• removers, transforming oxidation precursor compounds (such as O_3), ions that participate in the chelation of metals and increase the activity and production of antioxidant enzymes. An imbalance of this process leads to a mismatch in the control of ROS, resulting in effects on plant cellular functionality. Thus, the production of ethylene in tangerine epicarpium tissues with dark spots has been shown to be lower than in spotless tissues and these cells showed significant increases in membrane permeability, hydroxyl radical production and lipid peroxidation (13).

On the other hand, it is known that OH• can cause oxidation damage that leads to damage to the cell wall and deterioration of the quality of banana fruit during storage(14). The metabolism of ROS may also depend on the action of ethylene (15) and therefore influences the maturation and senescence and shelf life of the fruit. Ren et al. (16) suggest that improving the quality and prolonging life of mango fruits can be achieved by reducing oxidative damage caused by ROS during maturation. Other authors consider that, given the role of OH• in modifying cell wall polysaccharides (17), inhibition of OH• could contribute to maintaining the firmness of these fruits. On the other hand, it has also been proven, as the biosynthesis of flavonoids in plants is improved almost exclusively by oxidative stress (18), in fact, the reducing functions of flavonoids are of key importance in plants, under severe stress conditions.

3.2 Oxidative stress in vegetables

Plants exhibit greater synthesis of polyphenols such as phenolic acids and flavonoids under abiotic stress conditions, helping the plant cope with environmental constraints. An organism's exposure to unbalanced oxidative stress has many biological consequences. Oxidative stress mediated by free radicals ends up being beneficial to the plant, as it promotes the production of secondary metabolites and adaptation of the plant defense system thus contributing to a greater nutritional benefit. It has been suggested that a threshold level of free radicals is necessary for the normal physiological functioning of plants (19).

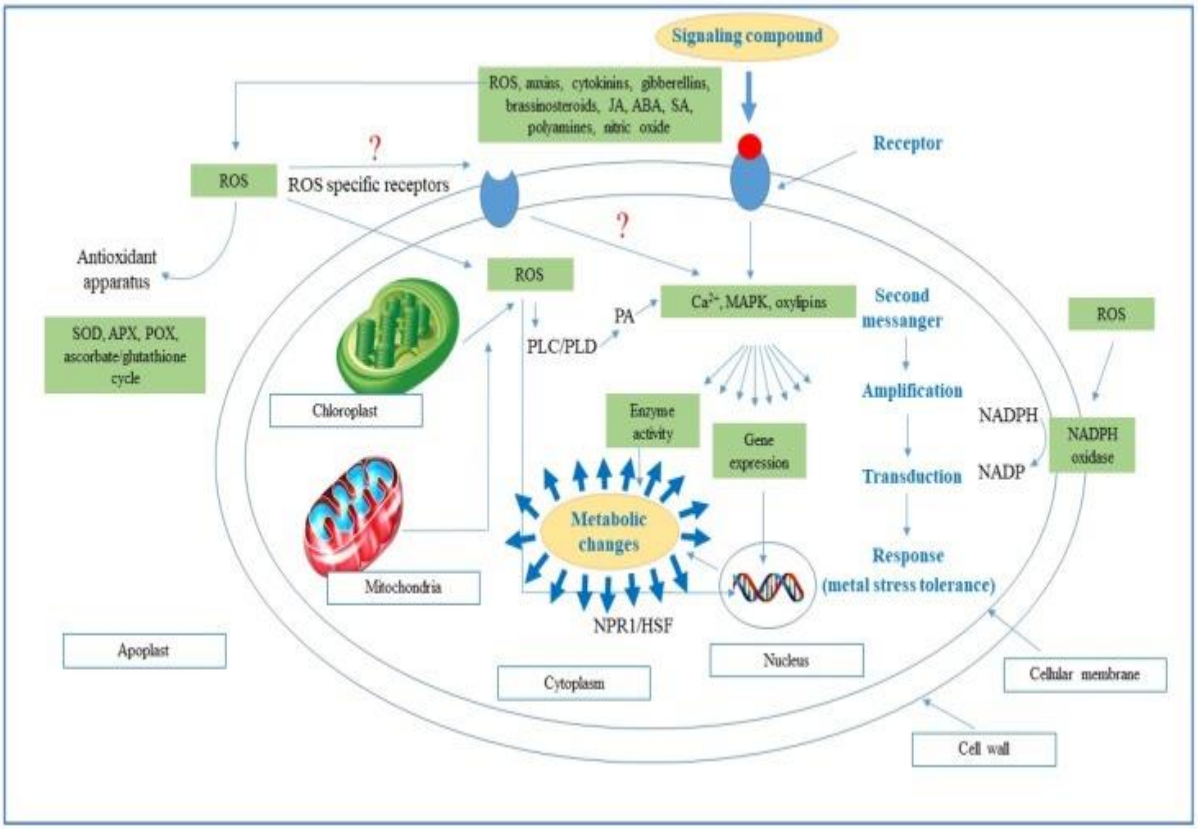

FIGURE 2: Outlining the transmission and transduction of signals in plant cells.

Abbreviation: *ABA, abscisic acid; APX, ascorbate peroxidase; HSF, redox sensitive transcription factor; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; NADP, oxidized dinucleotated adenine nicotinamide; NADPH, reduced dinucleotated adenine nicotinamide; NPR1, redox sensitive transcription factor; OXI1, seine / threonine kinase; PA, phosphatidic acid; PLC / PLD, class C and D phospholips; POX, peroxidas; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase. Taken from: Farooq et al.,2019*

Flavonoids remove hydroxyl radicals (OH•) generated by UV photolysis of hydrogen peroxide(20). Twenty-five vegetables (artichoke, asparagus, beetroot, beans, broccoli, Brussels sprouts, carrot, cauliflower, celery, chicory, cucumber, eggplant, escarole, garlic, green beans, leek, lettuce, corn, onion, pea, pepper, radish, spinach, chard and zucchini) were used to evaluate their antioxidant activity. All the fresh vegetables studied were able to remove the radical lipoperoxyl and radical hydroxyl. All vegetables also had a good antioxidant capacity, except cucumber, endive, carrot and zucchini. Vegetables stored (7 days) in a household refrigerator recorded the same antioxidant activity as fresh samples, except cucumber and zucchini (lipid peroxidation) and broccoli, Brussels sprouts and leek. Canned vegetables showed a more pronounced loss of antioxidant activity than frozen vegetables compared to fresh vegetables. Over the life of processed vegetables (8 months for frozen vegetables and 18 months for canned vegetables), some products showed losses (19-48%) lipoperxyl radical removal ability and total antioxidant activity (21).

Plants that grow in stressful environments have the ability to biosynthesize more phenolic compounds compared to plants that grow under normal conditions. These compounds have antioxidant properties and are able to eliminate free radicals, resulting in reduced cell membrane peroxidation (22).

Flavonoids can improve the process of metal processing, which helps reduce levels of harmful hydroxyl radicals in plant cells (23, 24) and this fits with the observation that flavonoid levels in plants have been increased by excess metals (25). Low metal toxicity, the accumulation of specific flavonoids that are involved in assisting the plant's defense mechanism also increases, including anthocyanins and flavonals.

IV. CONCLUSION

Based on the results of this review, we could suggest that the preservation of fruits and vegetables in cold rooms is dependent on the accumulation of VOC, the proliferation of microorganisms, the conditions of the chambers and the characteristics of each vegetable. The elimination of VOCs (including ethylene) can be used as an indicator of the operation of oxidizing processes as environmental scrubbers of the storage and storage chambers.

The previously determined results of this review, it allows to affirm that the radical OH• is the oxidizing element responsible for the AOP used and, consequently, presents a potential action on the modulation of ethylene levels that favourably affect fruits and vegetables, as well as its microbiological control.

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Vector and Non-Vector Infection up to Nano-Vector in Association with RNAI Transfection

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Abstract— *Transfection is a translocation of DNA or RNA pieces, which could occur naturally or from human effort through laboratory/pharma results, that is so advanced now. Still the same incidence and prevalence in natural-huge laboratory/ incubator tropical rainforest is neglected as the pathogenesis mechanism on the laden from nano-infection. Nano-vector could deliver 3 different payloads incl. plasmid, mRNA, and RNP for CRISPR/Cas9 genome editing. They were knowing the benefit and disadvantages of transfection in association with the high prevalence of transfection diseases such as gen silencing/ failure in gene expression/ gene blocking/ gene mutation and gene polymorphism. This review article manually using a search engine. First, confirm the difference between the host and vector. Then digging non-vector transfection, nano-carriers, and nanoparticles, in drugs delivery using 'binahong' (Anredera cordifolia) leaves which are already used in pharmacies and imaging diagnostics. Lipid nanoparticle, the first siRNA drug deliver. Ferrite Nickel (NiFe2O4) synthesis, and CoFe2O4 nanoparticle synthesis using 'binahong, kelor, salam', etc. leaves extraction, also are nowadays nano-carriers. Meanwhile, the main class of vector viruses which had been tested for clinical application, incl. retroviruses (RV), adenoviruses (AV), adeno-associated viruses (AAV), lentivirus (LV), and herpes simplex viruses (HSV). Vector viruses, plasmid, mRNA, RNAi, and Lipid, polymer, or inorganic composition, which are used for drugs, could occur in nature. This study concluded that RNAi for nowadays drugs and Next Generation (NG) Pharma, occurrences, exist in the laboratory, at home, and in the garden, and naturally.*

Keywords— Transfection; Payload; Nano-vector; CRISPR/Cas9; RNAi; Silencing.

I. INTRODUCTION

The high prevalence of transposon (nanomolecule) transfection in tropical rainforests incl. Indonesia and India are not watching out for the vector aspect in the prevention and promotion application so specific character is neglected. Singapura and Japan has solved DHF by making big water tunnels so that there is no puddle at the beginning and the end of rainy season. Indonesia solves DHF by buying vaccines and doing fogging which is vulnerable to becoming pesticide resistant. This study aims to look over the information about vector dan non-vector transmission of transposon/ virus to humans.

Efficient and effective prevention and promotion with parsimony spirit.

Focus on diseases caused by CGG-repeat[1,2,3,4] and sepsis.[5] is now a usual occurance so that almost cases in primary care and ICU could find these patients. Macro- (parasite), micro- (bacteria) and nano- (virus) infection in meter measurement have patients which are not known as infectious diseases, but as proteomic and metabolomic failures. Abnormality such as Parkinson Syndrome, Autism, Bipolar, and other brain diseases, also mental and behavioral, and social disorders, are far away from infectious and are known as non-communicable diseases, which are separated from infectious diseases. Several cases are broadly known caused by pesticide use.[4] This study appoints these cases as transposon transfection from the vector and non-vector aspect.

The aims of this study is the prevention and promotion of vector and non-vector chains related to countermeasures nano-infection in the tropical rainforest areas.

II. MATERIAL & METHOD

The review article was done manually using a search engine. Firstly, writing the Problem, Interference, Comparison, and Outcome (PICO) from the topic, that is vector transfection. Secondly, consolidation of the definition from keywords and

similarity which related especially with nano infection. Thirdly, aims and Topic which discuss such as vector and non-vector in the nanometer infection, which is mRNA/RNAi vector e.g. virus, lipid/polymer, and semiconductor, etc. Aims and topic become the title of this review article.

III. RESULT

Nano transfection patients, with and without vectors, up to nano-vectors, nano-carriers, semiconductor nano-particles are found as follows:

3.1 Patients and what kind of transfection vector is needs

The transfection method could be through chemical or physical manner. With mRNA vectors (nanometer), the delivery of mRNA used transfection reagents used for optimizing lentivirus vectors.[6]

Transfer mRNA into cells after treatment with the high electric field. Also with nonelectromagnetic field, but with the help of ultrasound is used in mRNA delivery. 'Gene gun' is also used for mRNA transfection delivery. Injection mRNA delivery also occur. Besides all, reporting of Bactofection and mycofection for delivering mRNA. Following the samples' DNA/RNA delivery with the vector. Plasmid DNA,[7] ultrasound/microbubbles for mRNA delivery,[8] GLP-1AR, and FGF-21 is safely delivered *in vitro* and *in vivo* with silica nanoparticle,[9] non-contact delivery of encoding EGFP plasmid in electromagnetic frequent with high intensity,[10] bioelectrochemistry,[10] RNAi with vector-mediated viral,[11] as nanometer-transfection which occur in laboratory and Pharma.

Meanwhile, the case information of those non-communicable disorders in nature are not identified. The real information lack is specific covered by taboo but easily found in foundations and many clubs with the same kind of cases. Domestic culture, religiousness, and social stigmatization, make the patients consider have normal physic, but more often known as LGBTQA behavior, or mentally bipolar.[1]

3.2 Transfection patients and are they need a host or not to transmit

The jargon of host and vector refers to the transmission track of some infectious diseases from human to animal. Host is a living creature which bacteria, viruses, protozoa, and other microorganisms which usually cause diseases, stay inside their body.

The host is larger and incl. human which give nutrition and a place for the growing of other organisms such as a parasite, mutualistic guest organism and symbiont commensal, where vectors are small living creatures that transmitted infectious agents from an infectious animal to a human or other animals. A known example usually is a biological vector, such as mosquitos, and bugs, which may bring pathogens that multiplicated in the body and than could be transmitted to a new host, usually through bites. Anopheles is a definitive host and human is an intermediate host for malaria diseases.

DHF has Aedes Aegypti as a vector, malaria has also a specific vector Anopheles. Cattle is an intermediate host of in human. Bats and birds has been mentioned as vectors of avian flu, and SARS virus and SARS-CoV-2 in COVID-19, which the last is transmitted from human to human, also, SARS. *Severe acute respiratory syndrome* (SARS) is in the Coronavirus family.

Vector virus mediated by RNAi used the power of RNAi which is a strong mechanism for gene silencer, incl. signal delivery to a target cell, immune response, and change the endogen microRNA (miRNA).[11] Avian flu is a flu transmitted from bird to human, caused by the H5N1 or H7N9 virus. Swine flu a kind of flu transmitted from pig to human, is caused by the H1N1 virus, which in 2009 then transmitted between humans. A newer type of Virus H1N1, the R4N1 variant, developed far faster then the parent.

3.3 Transfection of mRNA, long before occur naturally, as genesis on biodiversity in wet-warm climate area

But nowadays, not only natural as the transfection on the genesis of biodiversity mechanism, because transgenic in tropical rainforest areas, at night, where CO2 level is high by CO2 loss from leaves, the same condition in the laboratory of technology progression for diagnosis and treatment. A prestige presentation in the economy of the biomolecular pharmaceutical companies, and also in superior seed production for agriculture and agribusiness.[12] The side effect of using ARMGs of superior seed, give Antibiotic Resistance monoculture. Become the source in increase of high prevalence of *Multi-Drug Resistance* (MDRs), sepsis, and new antibiotics are born. Nowadays, sepsis treatment even gets around the use of old antibiotics in a rural and remote areas.[13]

IV. DISCUSSION

Various vector viruses from mRNA/RNAi could be in the form of Viruses, lipids, polymers, inorganic composition, plasmids, etc. In this discussion, trying to open step by step with grouping, as RNAi or its' vector, either for silencer or to increase gene expression.[14]

4.1 Vector virus from mRNA/ RNAi

Various viruses are recorded as vectors, such as Lentivirus,[14,15] herpes simplex virus,[16] adenovirus DNA- and V-2 mRNA-Covid-19,[17] Nano-vector for editing gene CRISPR/Cas9,[18] and also for combating against vectors mechanism and mechanism of vector-borne viruses (RNAi).[19] Lentivirus vectors are broadly known used for delivering alien genes for long-term expression. The possibility to integrate into human genome is shown by vector vaccines or adenovirus vaccine of COVID-19.

Is the expression of adenovirus genes expressed in vaccines based on vectors? Each of them has its specificity, for silencing, increase gene expression, or editing.[18] Non-viral nano-vector deliver *payloads* CRISPR/Cas9 genome editing.[18] Non-viral vector carrier is also known as nanocarriers or nanoparticles. Moreover, Agarwal reported new molecules approach to combat vectors and *vector-born viruses*: focus specific on interference RNA (RNAi) mechanisms.[19]

4.2 Lipids, polymers, or inorganic composition

Three of them, are samples specific from nano-vector.[18] In open-air tropical rainforests, camouflage insects similar of color and form, to plant habitat, has reported as RNAi indigenous.[20]

Microemulsions (μEs) and in the form of Solid Lipid Nanoparticles (SLNs Solin TM), Lipid nanocarriers are used for drug delivery, also active stuff which is for used in cosmetic or in nutritional fields. Also used for cardiovascular disease imaging (contrast, phospholipids, liposomes and micelles). Lipid nanovector also find nontoxic and noninduced the form of *Reactive Oxygen Species*/ROS, and also not give response to stress, and give pro-survival signal line.[21,22] Stimulate-respond lipid-based magnetic nanovectors based on lipid increasing apoptosis.[23] Cationic lipid-nanoceria hybrids, is nonviral vectors used clathrin-caveolae-mediated endocytosis and loss subsequent release of endosomes.[21] Lipid nanoparticle is a siRNA delivery for drugs. Then, knowing Nickel Ferrite (NiFe2O4) synthesis,[24] and also CoFe2O4 nanoparticle synthesis using binahong (*Anredera cordifolia*) leaves extract which has the potency for medical diagnosis.[25] Cobalt Ferrite is also found in *salam (Syzygium polyanthum), papaya (Carica papaya), kelor (Moringa oleifera*) leaves besides *binahong*. Synthesis and having magnetic characteristics from nanoparticles cobalt ferrite (CoFe2O4) is prepared in the way of wet chemical, is reported in Magnetism and Magnetic Materials journal.[26]

4.3 Nano-vector could deliver 3 different payloads incl. plasmid, mRNA and RNP for CRISPR/Cas9 genome editing.[18]

Appearance researches of CRISPR using Ribonucleoprotein (RNP) is a variance delivery method of Cas9-gRNA RNP. CRISPR-Cas9 plasmids is used in human patients, often is not optimal due to effects which is not wanted such as cytotoxic and unpredicted situation. Integrated DNA Technologies. CeO2/DODAB nanovector could do genes transfection in vivo without cause toxic sign. Nano-vector has potensial using in genes delivery in biomedic aplication.[21]

4.4 RNAi is a new class therapy strategy for silencing endogen and viral gene of mosquito.[19]

Double stranded dsRNA long chain for RNAi (RNA *Interference*) application that is for silencing a gene from an organism, which blocking gene expression. Long dsRNA synthesis for plants, insects and RNA Virus for agriculture and aquaculture Blocking mechanism on gene expression (gen *silencing*) in post transcription. Through dsRNA induction into target cells so that stick to mRNA sequence. Technology application of RNA *Interference* (RNAi) in Aquaculture.[4,20,27,28]

Although remind by medical faculty about high relative humidity in tropical rainforest area increase the insertion of RNAi in global warming action, about separation of risk laden that have to bear such as LGBT crisis, hypospadia crisis, sepsis crisis etc., it is need to promote and prevent to industrial countries so that this effect is not continuously neglected. Meanwhile, the effort to empowering seashore and rice field, forest, mountains, river, in tropical rainforest area, consider potential to be main player of sea grasses, fishery, agriculture for industry 4.0 raw material and food/energy crisis.[28,29]

This condition is important occur around one month toward the sign of G-20 Indonesia presidential Nov 2022 summit, 15-16 November, with the material of discussion about green activity, blue carbon, food crisis, energy crisis, climate crisis, economy crisis as the consequence of Rusia-Ukraina war VS. The sound of development countries with LGBT, hypospadias, sepsis

laden problem which steadily blame on using pesticide and wild use of antibiotic. Meanwhile the use of RNAi and Antibiotic Resistance Markers Genes (ARMGs) for superior seed constantly still hidden.[12]

V. LIMITATION

This review article has limitation on:

1) Large cases transfection of GMEs/ RNAi/ dsRNA /ssRNA in wet and warm climate area is seldom occur in dry-hot climate area. The difference of steam-bath vs. Sauna, industrial countries which not have wet, moisturized climate is difficult to accept that bulk of problem in healthy proteomic and metabolomics in tropical rainforest countries such as Indonesia and Thailand in SEA. Moreover, the artificial nano-transfection, associated with industry 4.0 is now depends on collagen and cellulose as the main raw material. This situation is known as industry 4.0 without society 5.0, where nano-vector is still often imagine as macroform such as bats and microform such as adenovirus. In the other hand, RNAi is considered as editing and silencing gen for therapy, not as a gen silencer in agriculture and aquaculture in tropical rainforest climate countries.
2) Many comparisons do not associate vector without connected with climate, such as dry-hot and dry-cold VS. wet-warm and wet-cold, which has to be given a needed large attention
3) Observational design seldom consider far away from physics law and chemical law which is has always to be a remembered.
4) Confounding risk are weak because widely broadly known that pesticide is the cause, not RNAi
5) This review article offers important information about the advantage and the harm of the using RNAi in the laboratory and in the garden of tropical rainforest area.

VI. CONCLUSION

Prevention and promotion of vector chain and non-vector chain associated the countermeasures nano-transfection in tropical rainforest with the effect silencing/blocking the genes, has already reported and described for the first time opportunity. RNAi for nowadays treatment Next Generation (NG) pharma could occur in the laboratory, at home, and in the garden in nature.

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Morphological heterogeneity in the giant African River Prawn, *Macrobrachium vollenhovenii* from three rivers systems in the Niger Delta Region, Nigeria

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Abstract— *The study investigated morphological variations among samples of Macrobrachium vollenhovenii captured from Taylor Creek, Calabar Estuary and New Calabar River in the Niger Delta Region. A total of 76 individuals were caught using cone-shaped bamboo basket traps. The prawns were identified and 18 morphological characters were measured on each individual. All the data generated were analyzed using the PAST3 and JASP statistical software. The results of the study indicated that the morphometric measurements among the populations of M. vollenhovenii total length, rostrum length, third segment length, fifth segment length and abdominal length were significant different ($p < .05$) while telson length, telson width, caudal length, merus length and palm length were not different ($p > 0.05$). The coefficient of regression in the length-weight relationship were positively allometric ($p < 0.05$) for all populations with values of 3.5402, 4.6686 and 4.420. The condition factor varied from 0.668 to 2.729 averaging 1.498, 1.628 and 1.630 for New Calabar River, Calabar Estuary and Taylor Creek, respectively.*

Keywords— African River prawn, Macrobrachium vollenhovenii, morphological heterogeneity, length-weight relationship, condition factor.

I. INTRODUCTION

The genus *Macrobrachium* (Bate, 1868) belongs to a group of freshwater prawns (Crustacea, Decapoda, and Palaemonidae). They constitute one of the most diverse, abundant, and widespread crustacean genera (Murphy and Austin, 2005), occurring throughout the tropical and subtropical zones of the world with the exception of Europe (Holthuis, 1980; Fossati et al., 2002; March et al., 2002). Several studies have reported the existence of about 74 species (Chen et al., 2009; Holthuis and Ng, 2010; de Grave and Fransen, 2011). However, the number of species in the Niger Delta Region (NDR) of Nigeria are not well-understood and poorly known. The species that have been documented so far in the NDR include *M. macrobrachion*, *M. vollenhovenii*, *M. dux*, *M. felicinum* and the invasive species of *M. equidens*.

The most economically important species for NDR are *M. macrobrachion* and *M. vollenhovenii*. The African river prawn, *M. vollenhovenii* is endemic to the west coast of Africa stretching from the Senegal River in the north to Angola in the south (Holthuis, 1980; Willfuhr-Nast *et al*., 1993; Paterson, 2007). In Nigeria, It does not, however, occur as plentiful in nature as *M. macrobrachion* even though it has a higher fecundity (Lawal-Are and Owolabi, 2012; George *et al*., 2013; Nwosu and Holzlöhner, 2016). In the Niger Delta, *M. vollenhovenii* is an important economic resource in the rural areas, supporting and sustaining viable artisanal fisheries in some rivers and estuaries within the region. Because it is the biggest of all the *Macrobrachium* species in West Africa (Konan *et al*., 2008) and grows at relatively fast rates in addition to a number of other favourable culture characteristics, it is considered a good candidate for aquaculture (Marioghae, 1987; Willfuhr-Nast *et al*., 1993; Niass and Fall, 2015). The African river prawn is also hardy in many parameters, it thrives in murky waters with dissolved oxygen as low as one part per million (Jimoh *et al*., 2011)

To develop the culture of the species, knowledge of the degree of genetic variation is crucial and must be understood because it will provide information on the condition or status of a population with respect to long-term survival of a species and the fitness of the population to adapt to environmental dynamics (Dunham, 2002). Also, natural population are perhaps the best

gene bank, a critical resource for genetic variation for current and future application in genetic improvement for farmed species and specialized sports fish application (Dunham, 2011).

One of the major requirements for shrimp aquaculture is the initial selection of breeders which involves analyses of external morphology. Morphological variability is not only used to explore differences among geographically distinct populations but also to ascribe distinct genetic structures or environmental conditions to each geographic location (Kinsey *et al*., 1994). In order to effectively manage fishery resources and exploit the aquaculture potentials, identification of the population structure of an explored species is necessary (Grimes *et al*., 1987).

At the present, scant information is available on the morphological variations of *M. vollenhoveniii* from the Niger Delta Region of Nigeria thereby creating a knowledge gap. Most importantly, no study has compared morphological traits of populations of the animal in the Niger Delta. Hence, the purpose of this study was to determine the morphological variations of *M. vollenhovenii* caught in three locations from the Niger Delta Region.

II. MATERIALS AND METHODS

2.1 Study location

The study was conducted with specimen of *M. vollenhovenii* caught during 2019 in three river or drainage systems in the Niger Delta Region (NDR), namely; Taylor creek in Bayelsa State, New Calabar River in Rivers State and the Calabar estuary in Cross River State (Fig. 1). Taylor Creek (BAY), Calabar Estuary (CAL) and New Calabar River (NCR) are tributaries of the Niger Delta Basin. The Niger Delta is characterized by extensive coastline (approximately 450 km) and 21 estuaries that drain the inland waters into the Atlantic Ocean in the Gulf of Guinea.

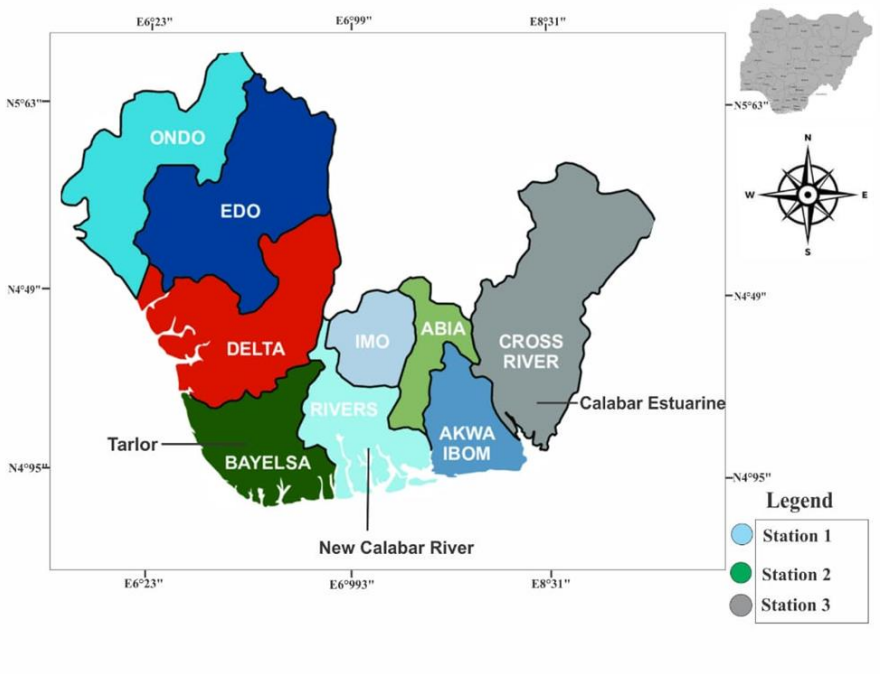

FIGURE 1: Sampling Stations in Niger Delta, Nigeria

2.2 Collection of Samples

The fishing was done with cone-shaped bamboo basket traps described by Solarin *et al*. (2003) and Jimoh *et al.* (2011). The basket trap had a two non-return valve mechanism at the center of the trap. It had a total length and opening aperture of about 1.0 m and 0.3 m, respectively. Fresh oil palm fruits were used as baits for the prawns. The prawns were preserved in ice-chest with ice-blocks and later fixed in 75% ethanol. *Macrobrachium vollenhovenii* was identified using morphological characters reported by Holthius (1980) and Powell (1982).

2.3 Morphometric measurements and analyses

Each specimen was weighed using an electronic balance, coded, and preserved in 75% ethanol. Morphometric measurements were taken on the following traits including total length (TL), carapace length (CL), rostral length (RL), abdominal length

(AL), first segment (FSL), second segment (2SL), third segment (3SL), fourth segment (4SL), fifth segment (5SL) and sixth segment (6SL), telson length (TEL), eye diameter (ED), and carapace height (CH) using dial calipers to the nearest 0.01 cm.

2.4 Statistical Analyses

The relationships between total length and wet weight (W) was established by calculating Log W=Log a+b Log L, Where, W=weight of prawn in g, L=total length in cm, a=regression constant and b=regression coefficient. The association degree between TL and W was calculated by the determination coefficient (r^2). Fulton's condition factor (K) was estimated from the individual length and individual weight in the sample estimated from this equation:

$K=100\ W/SL^b$,

Where K=condition factor, W=mean weight (g), SL=body length (cm), and *b* value was derived from the $W=a\times SL^b$. Morphometric variables were evaluated by one-way analysis of variance (ANOVA). Variables presented in this study were expressed as mean ± SD, and one-way ANOVA was used to compare the differences in morphometric traits and Fulton's condition factor between the three populations of the shrimp (PAST 3.26). When a significant family effect was found, Tukey's test was performed for multiple range comparisons ($P<0.05$). The correlation coefficients obtained from the various linear regression analyses were tested for significance using the Student t-test. The analysis were tested for significance at 5% level of significance.

III. RESULTS

TABLE 1
MORPHOMETRIC CHARACTERS OF *M. VOLLENHOVENII*

Trait	CAL (N=28)				NDR ARAC (N=22)				BAY (N=26)				P-Value
	Min	Max	$\bar{X}$ ±SE	CV%	Min	Max	$\bar{X}$ ±SE	CV%	Min	Max	$\bar{X}$ ±SE	CV%	
Wt (g)	4.6	41.5	15.16±1.42	49.69	02.0	33.50	12.47±1.73	65.10	2.1	45.0	18.18±2.26	59.99	*
TL cm	6.8	12.0	9.55±0.21	11.89	6.60	12.20	9.04±0.28	14.47	6.8	12.2	10.16±0.27	13.56	*
RL cm	3.0	4.8	4.05±0.08	10.33	2.6	4.8	3.85±0;11	13.57	3.2	6.2	4.60±0.15	16.43	**
CL cm	1	3.7	1.49±0.11	40.67	0.9	3.2	1.25±0.11	41.43	1	3.8	1.84±0.16	45.29	**
CH cm	1	3.5	1.92±0.10	26.48	1	3.7	1.54±0.14	41.80	1	3.7	1.93±0.13	34.41	*
AL cm	3	5.8	4.21±0.12	14.58	2.3	6	3.73±0.19	24.08	2.5	6	4.41±0.17	20.19	**
FSL cm	0.3	1	0.55±0.04	35.04	0.3	0.7	0.47±0.02	22.34	0.4	1	0.67±0.03	26.43	**
2SL cm	0.3	0.9	0.61±0.03	24.93	0.4	0.7	0.56±0.02	15.73	0.5	1.6	0.73±0.05	38.03	**
3SL cm	0.3	0.8	0.55±0.03	24.99	0.4	0.8	0.57±0.02	18.33	0.4	0.9	0.64±0.02	19.78	*
4SL cm	1	1.6	1.34±0.03	11.92	0.5	1.4	0.9±0.08	40.57	0.5	1.8	1.29±0.08	32.21	**
5SL cm	1	1.4	1.23±0.02	10.09	0.5	1.4	0.90±0.07	37.91	0.5	1.4	1.10±0.06	26.32	**
6SL cm	0.4	0.8	0.59±0.02	16.57	0.4	0.8	0.65±0.02	17.71	0.4	0.8	0.64±0.03	21.65	NS
TELcm	1	2	1.29±0.04	15.51	0.8	1.8	1.28±0.05	17.91	0.9	2	1.38±0.05	19.67	NS
TEWcm	2	3.7	2.60±0.08	16.65	1.2	3.6	2.25±0.13	26.25	1.2	3.6	2.33±0.11	24.80	*
CaL cm	1	3	1.41±0.07	25.14	1.2	3.4	1.58±0.13	39.44	1	3.4	1.72±0.13	39.52	NS
ML	1	2.6	1.39±0.06	21.75	1	2.5	1.50±0.08	24.31	1	2.5	1.52±0.08	25.64	NS
PL	1.1	6	1.9±0.17	46.22	1.4	2.5	1.75±0.07	20.03	1	7.1	2.38±0.30	63.26	NS
ED	1.2	1.7	1.42±0.03	11.04	0.5	1.7	1.30±0.06	23.36	0.5	1.7	1.39±0.05	17.23	NS

3.1 Morphometric variation

Morphometric characters of *M. vollenhovenii* from three locations of the NDR are shown in Table 1. Weight varied from 4.5-41.5g, 2.0-33.5g and 2.1-45 g with corresponding means of 15.16±1.42g, 12.47±1.73g and 18.18±2.26 g for CAL, NCR and BAY, respectively. One-way ANOVA showed significant differences in mean weight of the prawn ($P\leq0.05$). The range of TL were 6.8-12 cm in CAL (X = 9.55±0.21cm), 6.8 cm – 12.2 cm in BAY (X=10.16±0.27cm) and 6.8 cm – 12.2 cm (X= 9.04±0.28cm) in NCR. The TL showed statistically significant difference between NCR and BAY/CAL ($P<0.05$). However, there was no difference in TL between BAY and CAL ($P>0.05$). The mean RL of 3.85±0.11cm, 4.60±0.15cm and 4.05±0.08cm

for NCR, BAY and CAL, respectively were significantly different (P<0.05). The ANOVA further showed that populations displayed marked difference differences in ED, Table 1: Spatial variation in weight and length of Macrobrachium volenhovenii from the Niger Delta 6SL, CaL, TEL, TEW, ML, PL and CH (P>0.05).

*mean across rows with p<0.05, ** across means with p<0.01 were significantly different; NS means across row not significant (p>0.05)

3.2 Fulton condition factor and growth pattern distribution of *M. vollenhovenii*

The Fulton's condition Factor varied from 1.08 – 2.729 (X=1.628 ±0.310), 0.668 -2.318 (X=1.498±0.465) and 0.668 – 2.670 (X=1.630 ±0.481) for CAL, NCR and BAY, respectively. One-way ANOVA showed that the condition factor was the same for all three populations (p>0.05) and the Welch test for unequal variances failed also to detect any significant difference (p>0.05).

The Log length/Log weight relationships are shown in Figures 2 to 4. The calculated log of the length/weight relationship of the *M. vollenhovenii* showed a linear relationship between the length and weight of the species with R^2 of 0.877, 0.894 and 0.881 and corresponding R of 0.936, 0.945 and 0.939, respectively for CAL, NCR and BAY.

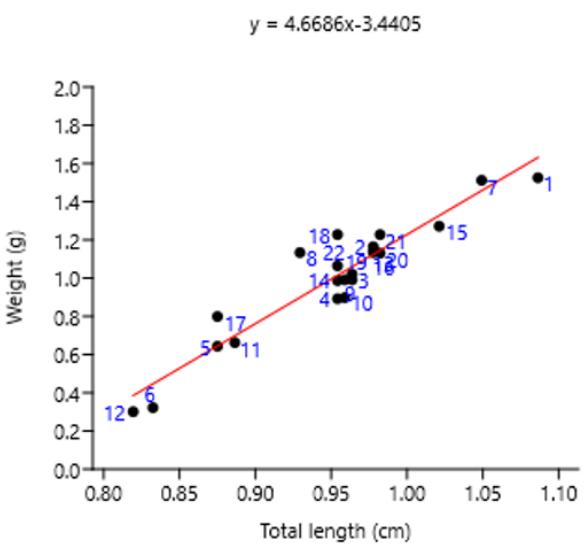

FIGURE 2: Length Weight relation of M. vollenhovenii from New Calabar River

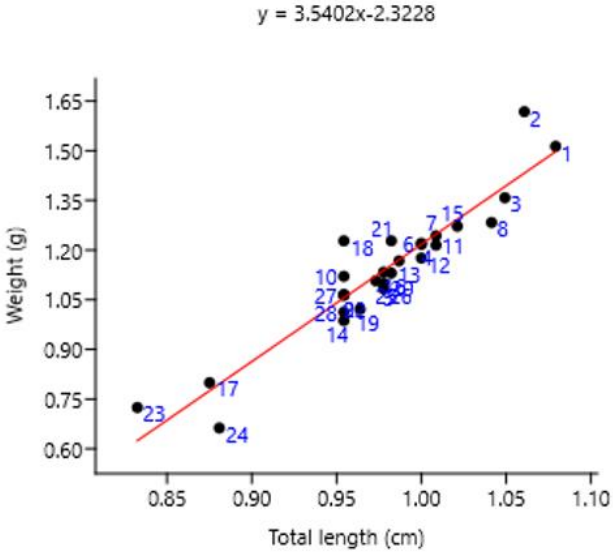

FIGURE 3: Length-weight relationship of *M. vollehovenii* from Calabar Estuary

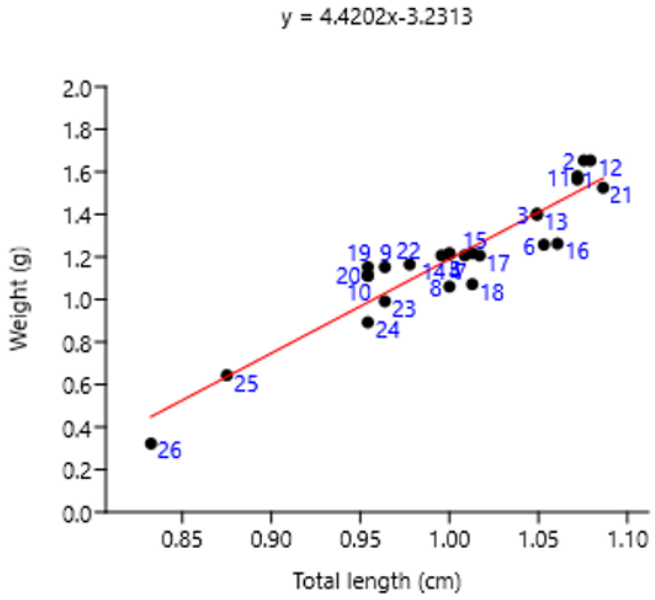

FIGURE 4: Length-weight relationship of *M. vollenhovenii* from Taylor Creek

IV. DISCUSSION

The Niger Delta Region supports major artisanal fishery that have been sustained for hundreds of years. The region is the richest in shrimp shellfish resources in Nigeria. The decapod crustaceans *Macrobrachium* species have sustained local fishery across the region, serving as source of income and rich animal protein for the communities and commercial traders. The rich organic sediment characteristic of the NDR makes it the ideal environment for shrimps to thrive and grow. In fish ecological studies, the condition index is the common denominator used in monitoring how populations respond to changes in the environmental over a period and in the assessment of the overall health and productivity (Richter, 2007). Fulton's condition index is generally used to estimate populations health and growth rate (Rochet, 2000), assess adaptation to culture systems (Araneda et al., 2008) and the overall biotic and abiotic conditions for growth (Gopalakrishnan et al., 2014). The high condition factor ranging from 1.478 to 1.630 of the species from the three rivers indicates, perhaps, similar prevailing environmental conditions that supports growth and provide adequate nutrition. The rich organic debris input arising from runoff due to frequent rains that characterize the delta basin support rich shrimp resources in and off the coast of the Niger Delta (Zabbey et al., 2010).

The Length–weight relationship (LWR) has important implications for fisheries management. LWR is used in determining growth rates, age structure and other aspects of shrimp population dynamics (Anastasiadou and Leonardos, 2008; Nahavandi et al., 2010). In selective breeding, LWR is a useful measure for body condition and comparison of morphological differences between populations in different regions (Nie et al., 2013). The condition of *M. vollenhovenii* assessed from the value of the slope in the length-weight relationship in the present study show that for all populations the slope was above 3, indicating increasing growth rate in relation to length. The relationship between length and weight *M. vollenhovenii* from the New Calabar River, Taylor Creek and Calabar Estuary were linear relationship. Lawal-Are and Owolabi (2012) reported that the prawn obtained from Lekki and Lagos Lagoons showed a linear relationship between length and weight, however, growth was allometrically negative with values of regression coefficient (b) ranging between 2.2788 and 2.7117. However, in the present study the prawn exhibited positive allometric growth with values of regression coefficient ranging from 3.54 to 4.67 (Fig 2-4). Variability in the LWR is a feature that can reflect fluctuations in the uptake and allocation of energy. Biswas (1993) suggested that differences in b values could be a result of gear selective influence and other factors such as sex, gonadal development, nutritive conditions in the environment of fish, physiological conditions of the fish at the time of collection and seasonal fluctuations in environmental parameters.

The phenotype of individuals including morphological traits within a species may be underlined by genetic and environmental effects or the interaction between genetic and environment, and selection (Cadrin, 2000; Poulet, 2004). Thus, explaining morphological differences between populations present some difficulties. However, it has been known that the morphometric characters can describe level of plasticity in response to environmental (Wimberger 2008). This study identified significant differences ($p< 0.05$) in the weight, total length, rostrum length, carapace length, abdominal length, carapace height and TEW of *M. vollenhovenii* from the three selected drainage. We suggest that morphological differences displayed by populations may be due to adaptation to prevailing local environmental conditions and limited larval dispersal. Environmental conditions, food availability and state of maturity may be responsible for the variations in weight and total length of the species. Elsewhere, studies have showed that environmental variation could lead to morphological heterogeneity (Tzeng et al., 1998; Begg et al., 1999; Giri and Collins, 2004; Collins et al., 2007; Giri and Loy, 2008; Torres et al., 2014). Mashiko and Numachi (2000) reported that populations of the oriental river prawn from fresh-water were differentiated from estuarine populations in morphometric traits. The high level of variations observed in this study could have been facilitated by limited larval dispersal of species with life histories associated with alternating freshwater and brackish-water environment creates room for wide morphological divergence due to lack of gene flow which homogenizes populations. We align with Begg et al. (1999) who suggested that morphometric variation could be more applicable for understanding short-term environmentally induced variation. Morphological characters are phenotypically plastic being influenced by the physical environment during different stages of growth.

V. CONCLUSION

There is a high level of morphological traits heterogeneity in *M. vollenhovenii* in the three selected drainage systems in the Niger Delta. The data in this study is valuable for selecting broodstock and establishing a monitoring and management system of the species in view of the environmental degradation of the Niger Delta. The current study provided the first baseline data comparing populations of *M. vollenhovenii* from different drainage systems of the Niger Delta Region of Nigeria and should be expanded to capture other river systems with corresponding genetic studies.

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Reproductive Performance and Short-Term Growth Pattern of The Progenies of the Reciprocal Hybrids of *Clarias gariepinus* and *Heterobranchus bidorsalis*

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Abstract— *The hybrids of Clarias gariepinus with Heterobranchus species are economically very important. The reciprocal interspecific progenies of Clarias gariepinus♀ x Heterobranchus bidorsalis♂ (Cgf x Hbm) and H. bidorsalis♀ x C. gariepinus♂ (Hbf x Cgm) were produced in the Fish Farm Demonstration Unit of the University of Port Harcourt. At Two weeks, mean weights were 7.0mg±0.0008 and 6.5mg±0.0006 for CGm x HBf and HBm x CGf, respectively with the corresponding total lengths of 1.01cm ±0.011 and 0.97cm±0.008. At the end of the experiment, mean weight of 10.77±1.65/11.04±1.79 and total length of 11.08±0.38/ 10.96±0.54 were observed for Cgm x Hbf/Hbm x Cgf, respectively. The total length was significantly different (P< 0.05) at the commencement of the experiment, which leveled off by the third week. All the parameters including the Head length, Head width, Dorsal fin length and adipose fin length did not differ significantly (p>0.05) for the reciprocal progenies. The condition factor was 1.27 for Cgm x Hbf and 1.28 for Cgf x Hbm*.

Keywords— Heterobranchus dorsalis, Clarias gariepinus, progenies, allometric growth.

I. INTRODUCTION

Global food-fish production based on capture from the wild, natural resource is now known to be limited in its capacity in meeting the demands for fish protein. This is evidentially due to degradation of the aquatic environment, increasing global population and resultant pressure on aquatic resources and increased dietary advices recommending the consumption of more fish visa-vis livestock flesh (Thustean and Roberts, 2014) and aquaculture is the viable alternative. Aquaculture production in Nigeria has witnessed increased production in the last two decades, growing 14.24 times from 1998 to 291,233 tons in 2018 (FAO, 2018; Adeleke et al., 2021). This growth is driven by the declining capacity of the nations' natural fishery resource to meet the demand for fish and destruction of critical habitats, discovery of candidate aquaculture species, population growth, changing dietary patterns, removal of regional barriers to movement of goods and services, development of new technologies and innovations resulting in intensification among others. From year 2000, Nigeria's contribution to global aquaculture production increased marginally from 0.07% to 0.44% in 2015. Currently, Nigeria is the leading producer of farmed fish in sub-saharan Africa and second to Egypt in Africa (Adeleke *et al*., 2021).

To improve growth and sustain the successes recorded in the industry, production of fast growing strains are needed. Hybridization is a breeding technique used to generate genetic diversity from different individuals of different species, genus or populations of the same species. The aims of hybridzation include improving growth performance, flesh quality and tolerance to adverse rearing environmental conditions; increasing disease resistance, producing sterile animals, and manipulating sex ratios as well as various other traits. The technique is also a viable alternative to selective breeding when there is little additive genetic variation in the desired traits of pure stocks to be exploited. Intergeneric hybridization is the crossing of different species belonging to different genus with the aim of producing offspring that will exploit heterosis and combine useful characteristics of both species. However, hybrids must be properly characterized, identified and classified (Akinwande et al., 2013). Part of the phases in intergenerc hybridization is the morphological description of the resulting hybrids. The detailed characteristics of the morphometric features of the hybrids between C. gariepinus and Heterobranchus species may be needed to distinguish it from the different species within the clariidae. The aim of this study is to compare the short-term growth, survival, and morphological characteristics of the progenies of the early stage of reciprocal hybrids of

Clarias gariepinus x *Heterobranchus bidorsalis*. It is expected that the present study will generate information that will contribute to the development of Nigerian aquaculture.

II. MATERIALS AND METHODS

Collection of broodstock: Sexually matured males (♂) and Females (♀) of *Heterobranus bidorsalis* and *Clarias gariepinus* weighing 1000-2000g were procured from two commercial farms in Port Harcourt, Rivers state. The history of the broodstock could not be traced.

To enable release of eggs, the females were injected with Ovaprim® hormone at a dosage of 0.5 ml per kg weight and the males sacrificed to obtain the milt. The reciprocal hybrids were produced by fertilizing female *H. bidorsalis* with milt from male *C. gariepinus* (♂CGm x ♀HBf) and male H. bidorsalis fertilized with milt from female *C. gariepinus* (♂HBm x ♀CGf). The larvae were reared for two weeks in two tanks before separation into experimental tanks. Thus a total of 600 two-weeks old hybrid catfish (♀HBf x ♂CGm and ♀CGf x ♂HBm) fry were used. Two weeks after hatching, 50 fry of each cross in 5 replicates were were randomly chosen and reared for 8weeks in transparent rectangular tanks of 44 x 29 x 24 cm.

2.1 Reproductive performance parameter

The numbers of eggs released in each experimental unit was determined by subtracting the weight of the brood stock after stripping (Wb) in grams from the weight of the broodstock before stripping (Wa) in grams and multiplying the difference by 700(1g=700eggs) (Viveen et al.,1985).

In determining fertilization rate, 150eggs were taken from each experimental unit about 20 minutes after fertilization in a container containing water and translucent eggs containing embryonic eyes were counted as fertilized; while opaque eggs were considered as unfertilized. This was then calculated according to Adebayo and Popoola (2008) as follows:

$$\text{Percent fertilization} = \frac{Number\ of\ fertilized\ aggs}{Total\ number\ of\ Eggs\ counted} \times 100$$ (Adebayo and Popoola, 2008)

2.2 Determination of Hatchability

Hatchability was determined by direct counting of fry in each experimental unit according to Akinwande et al (2013) as follows:

$$\text{Hatchability} = \frac{Number\ of\ hatchlings\ (two-day\ old)}{Total\ number\ of\ fertilized\ eggs} \times 100$$

Survival rate was calculated as follows:

$$\text{Percentage Survival} = \frac{Number\ of\ fish\ at\ the\ endof\ the\ experiment}{Initial\ number\ of\ fish} \times 100$$

Specific growth rate,

$$\text{SGR} = \frac{Ln\ W2 - Ln\ W1}{T2 - T1} \times 100$$

Where

Ln W2 : Natural log of final Weight, W2

Ln W1: Natural log of initial weight, W1

T1 and T2: Duration of experiment in Days

2.3 Condition factor

The condition factor (K) was estimated for each genetic cross to determine the state of wellbeing of the fish according to the equation:

$$\text{Fulton condition factor (K)} = \frac{W}{L^3} \times 100$$ (Lagler, 1956)

Where: W= weight and L= length

Paired data were analysed by Student's t-test and when necessary, analyses were performed after logarithmic transformation for weights or angular transformation for survival rates in order to stabilize residual variance. In text and tables, means are given with the confidence interval at 5% probability. To reduce variation, shooters or jumpers were culled to eliminate outliers that may influence the results.

III. RESULTS

3.1 Fertilization, Hatchability and Survival

The percentage fertilization and hatching rate of eggs and survival rate of fry differed significantly ($P<0.05$) in each of the genetic crosses (Table 1). The highest percentage fertilization, hatchability and survival rate of 85.00%, 91.07% and 98.67%, respectively occurred in ♂HBm x ♀CGf cross.

TABLE 1
GROWTH CHARACTERISTICS OF RECIPROCAL HYBRIDS BETWEEN CLARIAS GARIEPINUS AND HETEROBRANCHUS BIDORSALIS AT 82 DAYS

Characteristics	♂CGm x ♀HBf	♂HBm x ♀CGf	Level of Significance
Condition Factor	1.27	1.28	NS
Initial weight	0.00708±0.0008	0.00625±0.0006	NS
Final Weight	10.77±1.65	11.04±1.79	NS
Initial length	1.01±0.011	0.967±0.008	**
Final length	11.08±0.376	10.96±0.54	NS
Head length at 84 days	3.15±0.116	3.32±0.172	NS
Head width at 84 days	2.22±0.086	2.25±0.129	NS
Dorsal fin length at 84 days	5.68±0.278	5.48±0.304	NS
Adipose Fin length at 84 days	1.03±0.066	0.978±0.098	NS
Fertilization Rate	73.70±1.66	85.00±2.94	**
Hatchability %	67.33±3.60	91.07±4.83	**
Survival rate %	89.00±8.04	98.67±8.70	**
Specific growth Rate (%day^{-1})	2.45	2.47	NS

3.2 Body weight

The hybrid ♂CBm x ♀HBf recorded non-significantly higher mean weights ($p > 0.05$) compared to the alternative genetic type up to the 70th day and during the last weeks the mean weight of ♂HBm x ♀CGf cross was non-significantly higher than ♂CBm x ♀HBf (Fig. 1).

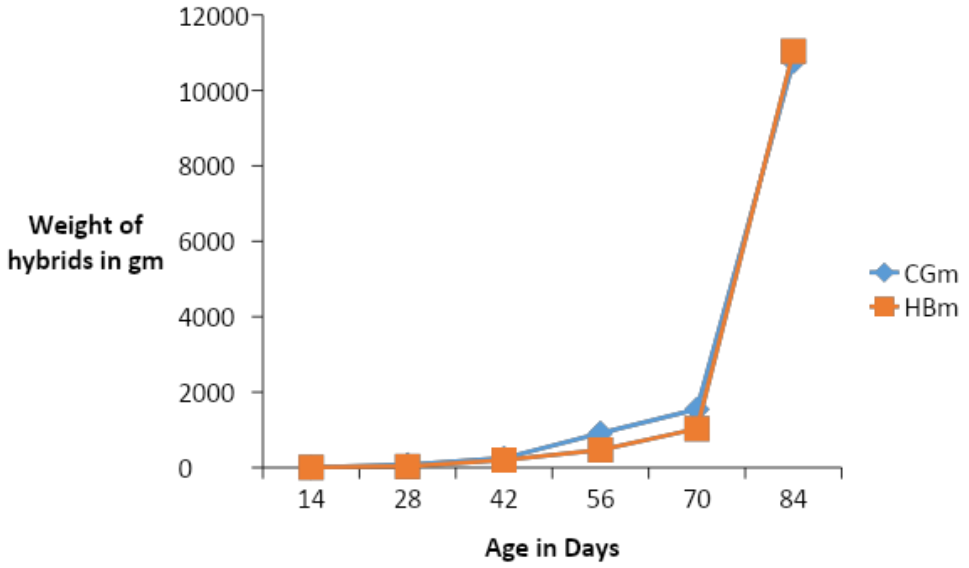

FIGURE 1: Growth in weight of reciprocal hybrids of *Heterobranchus bidorsalis* and *Clarias gariepinus* reared for 84 days in transparent rectangular plastic tanks

3.3 Specific growth rate

The corresponding Specific growth rates were 2.45 and 2.47% per day for ♂CBm x ♀HBf and ♂HBm x ♀CGf cross, respectively. The CV associated with weight was very high especially during the last two weeks of the 84 day experiment.

3.4 Linear Morphometric characters

The total length differed significantly between the reciprocal hybrids at 2 weeks ($P<0.05$) but levelled off at the close of experiment ($P>0.05$), growing from 1.01±0.011 to 11.08±0.386 cm in ♂CGm x ♀HBf and 0.987±0.008 to 10.96±0.54 cm in ♂HBm x ♀CGf. The linear growth in total length continued to be higher until week 5 when TL for ♂HBm x ♀CGf was 6,41±0.48 cm compared to 5.94±0.41cm ($P>0.05$). The trend in linear growth of total length of the hybrids is as shown in Fig. 2. The ♀HBf x ♂CGm hybrids displayed an even faster growth in linear terms than HBm x ♀CGf though insignificant ($P>0.05$).

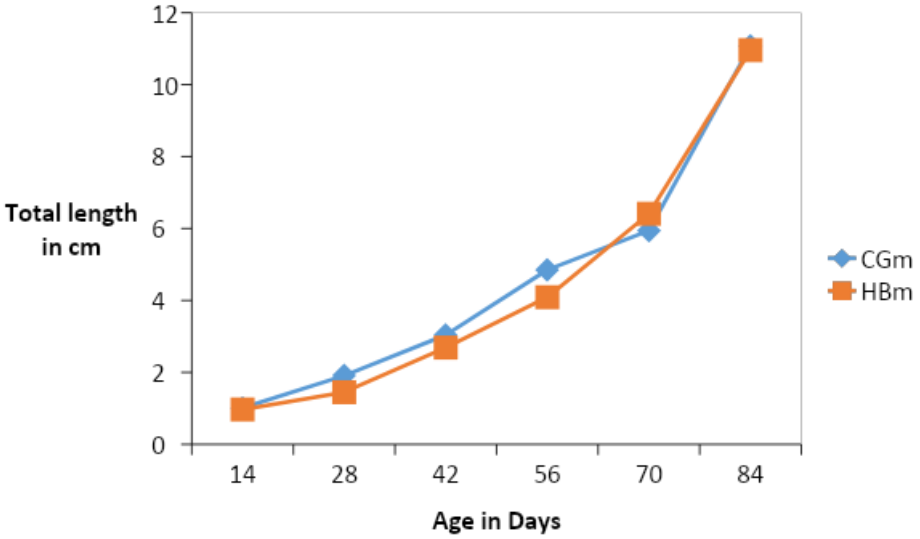

FIGURE 2: Growth in total length of genetic cross of ♂CGm x ♀HBf and reverse cross reared for 82 days in transparent rectangular plastic tanks.

The head length increased linearly from 0.246 ±0.002 cm in ♂CGm x ♀HBf and 0.248 ±0.005 in ♂HBm x ♀CGf to 3.15 cm and 3.32 cm, respectively ($P>0.05$).

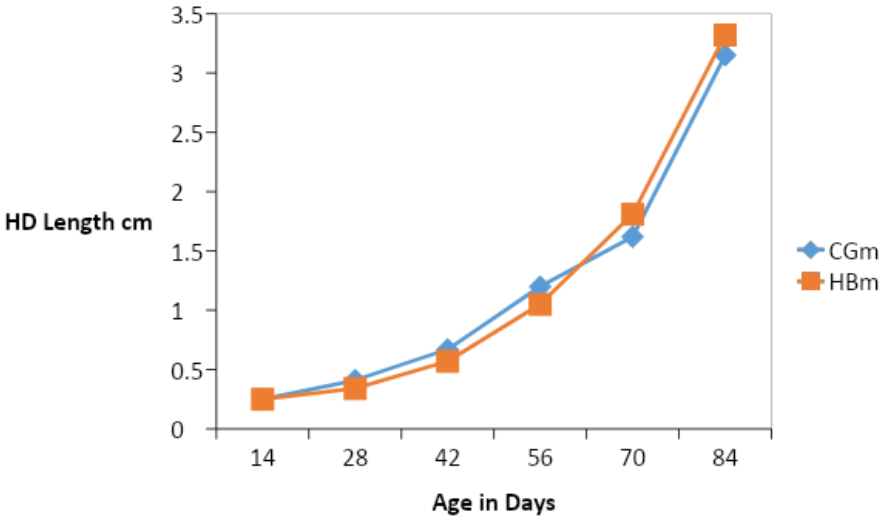

FIGURE 3: Growth in Head length of genetic cross of ♂CGm x ♀HBf and reversed cross reared for 82 days in transparent rectangular plastic tanks.

The dorsal fin length, DFL and adipose fin length, AFL and head width, HDW were measured from the 56th day. Table 2 shows the variations in the DFL, AFL and HDW, which were non-significantly different in ages. The AFL in this study represented 9.30 and 8.92% of the total length for ♂CGM x ♀HGF and ♂HBM x ♀CGF, respectively.

TABLE 2
MEAN DORSAL FIN LENGTH (DFL), ADIPOSE FIN LENGTH (AFL) AND HEAD WIDTH (HDW) OF GENETIC CROSS OF *HETEROBRANCUS BIDORSALIS* AND *CLARIAS GARIEPINUS*.

Age in Days	Character	♂CGm x ♀HBf			♂HBm x ♀CGf			Level of sign.
		X±SE	Variance	SD	X±SE	Variance	SD	
56	DFL	2.37±0.09	0.05	0.22	2.47±0.22	0.30	0.55	NS
70		2.94±0.22	0.28	0.53	3.29±0.22	0.30	0.55	NS
84		5.68±0.28	3.17	1.78	5.48±0.30	2.97	1.72	NS
56	AFL	0.49±0.04	0.01	0.12	0.23±0.04	0.01	0.09	NS
70		0.67±0.06	0.02	0.14	0.53±0.06	0.02	0.15	NS
84		1.03±0.07	0.18	0.42	0.98±0.10	0.30	0.55	NS
56	HDW	0.9±0.04	0.01	0.10	0.77±0.09	0.04	0.21	NS
70		1.25±0.14	0.11	0.33	1.25±0.12	0.08	0.29	NS
84		2.22±0.09	0.31	0.55	2.25±0.13	0.53	0.73	NS

NS means Not significant at 5% confidence level.

3.5 Condition Factor

The Condition factor of 1.27 for CGm and 1.28 for HBm were recorded for the genetic types.

IV. DISCUSSION

The reciprocal interspecific progenies of ♀CGf x ♂HBm and ♀HBf x ♂CGm were produced in the Demonstration Fish Farm Unit of the University of Port Harcourt to determine reproductive performance and growth pattern of different body parts. The high rate of fertilization of about 85% for ♂HBm x ♀CGf in this study is comparable to 87.5% obtained by Ipinjolu et al. (2013) and Ola-Oladimeji (2015) in similar hyridisation studies between *H. bidorsalis* (male) and exotic *Clarias gariepinus* (female) in a different ecological zone. This suggests a maternal influence on hatchability and fertilization. The hybrid fry produced using the eggs of *C. gariepinus* had significantly higher survival (85.00±0.00%) than the fry of hybrid derived from the eggs of *H. bidorsalis* (75.50±2.12%), thus indicating maternal inheritance of survival (Ola-Oladimeji (2015) Nwadukwe (1995) made similar conclusion for the hybrid of *C. gariepinus* and *Hetrobranchus longifilis.* Maternal inheritance could result from innate differences in egg quality due to age, size, and condition at the time of spawning (Butts et al., 2014; Dunham, 2011).

The mean length of fry at the start of measurement was close to those of *C. gariepins* at 10 days in Verreth et al. (1993). The mean length and mean weight obtained for ♂CGm x ♀HBf and ♂HBm x ♀CGf, respectively at 84 days were close to values obtained by Okeke (2014) at 112 days for the cross between *Clarias gariepinus* female and *Heterobranchus spp*. male. Sanda et al (2015) reported superior growth of the hybrids *Clarias anguillaris*♀x *Heterobranchus bidorsalis*♂ to *Heterobranchus bidorsalis*♀ x *Clarias anguillaris*♂. However, Ola-Oladimeji (2015) observed that growth in total length of ♀ *C. gariepinus* x ♂ *H. bidorsalis* cross was not significantly different from ♀*H. bidorsalis* x ♂*C. gariepinus* cross .

The SGR recorded in this work concurs with 2.44 observed by Angahar (2017) for hybrids reared under similar conditions and falls within the range of 2.12%.day^{-1} to 3.96%.day^{-1} obtained for various strains of *H. longifilis* by Nguenga *et al* (2000). Akinwande *et al* (2009) obtained highest SGR of 2.11% for reciprocal hybrids of *H. longifilis* (♀) and *H. bidorsalis*(♂). It appears that there is a paternal effect on SGR because the Cross between ♂ *H. bidorsalis* and (♀) *C. gariepinus* produced the highest SGR in the present study. Olaniyi and Omitogun (2018) showed the the superiority of the hybrid ♀ *C. gariepinus* × ♂ *H. bidorsalis* with respect to SGR and other parameters. However, the influence of broodstock size cannot be dismissed (Odedeyi, 2007; Uedeme-Naa and Nwafili, 2017), The CVs for the various characters were high. Fleuren (2007) confirms large variation in body weight of hybrids between *C. gariepinus* and *H. longifilis* in agreement with this s Ponzoni study. High CVs are usually associated with various fish species in the range of 20-35% (Gjedrem, 1997). The emergence of shooters or jumpers

have been identified in clariid hatcheries (Abdulraheem et al 2019). The sharp increase in weight (Fig. I) was due to the emergence of shooters in both genetic types. To deal with these problems, shooters were culled during the initial selection of individuals and thereafter on weekly basis until the end of the experiment. Young fish exhibit allometric growth patterns, high growth potentials than the older ones, the intensity of cannibalism would reach a maximum in the early weeks or months of the history when the variability of individual growth would be maximum (David et al., 2010).

Onyekwelu et al (2021) in their 6 weeks study of fingerlings of *Clarias gariepinus* obtained HDL 1.05-1.50 cm. The HDL represented 28.42% of of TL in CGm x ♀HBf and 30.29% in HBm x CGf. Olaniyi et al (2017) reported that the HDL was 29.9% of the standard length in adult H. bidorsalis captured from Kainji Lake while the AFL was 23.4%. The HDW was 20.04% and 20.53%, respectively for CGm x ♀HBf and HBm x ♀CGf. In *C. gariepinus* from Lake Nubia, the HDL corresponded to 24.9% of the standard length (Hamad, 2014).

The HDW obtained in this study is close to one-half reported by Akinwande *et al.* (2013) for hybrids of *C. gariepinus* and *H. longifilis*. These values are close to midpoint values reported by Agbebi et al. (2009) for diploid and triploid progenies of *H. bidorsalis*. Intermediate values have been reported for some morphometric characters between hybrids of *C. gariepinus* and *Heterobrancus* species (Nwadukwe, 1995; Legendre et al., 2006).

The condition factor is a quantitative parameter the state of well-being and it reflects recent feeding condition of the fish. The result implies that the two genetic types were in the same physiological condition. Therefore, many factors such as sex, age, state of maturity, size, state of stomach fullness, sampling methods and sample sizes and environmental conditions affect fish condition and parameters of length-weight relationships in fish (Khan et al., 1991; Anene, 2005; Yem et al., 2007).

V. CONCLUSION

In the selection of breeding pairs, considering maternal influence on fertilization, hachability and growth rate, hybridization between *male heterobranchus* mating and female *Clarias gariepinus* would be desirable.

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Proximate and Physicochemical Properties of Flours from Improved Sorghum and Cassava Varieties Grown in Awka, South Eastern Nigeria

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Abstract— *Flours are among the essential raw materials used in industries such as food, pharmaceutical and other industries for the production of different products. Scarcity and undesirable qualities of flours now lead to search for plant materials of improved qualities from which flours of high value can be isolated. Improved variety of sorghum and cassava from the Anambra State Agricultural Development Program (ADP) were employed in this research. The proximate and physicochemical analyses were done using standard methods. Results showed that the sorghum and cassava yielded 44.8% and 68.7% quantities of pale white and white colour respectively with neutral pH. Sorghum flours had 4.35±0.11%, 2.6±0.23%, 0.26±0.03%, 3.28±0.06%, 2.16±0.10% and 25.02±1.02% respectively for moisture, ash content, fat content, crude protein, crude fibre and carbohydrate content. Cassava flour gave 5.24±0.58% (moisture), 3.35±1.02% (ash), 0.18±0.45% (fat), 1.02±0.33% (crude protein), 3.14±2.02% (crude fibre) and 28.06±0.54% (carbohydrate). Sorghum flour isolated also had 240.6±1.3%, 123.1±0.8%, 800.5±2.0%, 3.5±3.1%, 22.5±0.5%, 67.0±0.7 and 0.78±0.2% for water absorption capacity, oil absorption capacity, swelling power, solubility index, amylose content, amylopectin content and bulk density respectively while cassava flour showed 260.4±2.0% (water absorption capacity), 128.0±1.1% (oil absorption capacity), 744.3±2.2% (swelling power), 4.3±2.0% (solubility index), 32.0±1.2% (amylose), 78.0±0.2% (amylopectin) and 0.89±0.1% (bulk density). The two isolated flours showed good pasting properties with peak viscosity and final viscosity of 2215±2RVU and 1126±2 RVU for sorghum flour and 3204±5 RVU and 1422±3 RVU for cassava flour. It is therefore deduced that flours isolated from the improved sorghum and cassava varieties will be suitable and cheap source of raw material for food, pharmaceutical and other industries due to their high qualities.*

Keywords— Cassava, Sorghum, Flour, Industries, Improved variety.

I. INTRODUCTION

Guinea corn (*Sorghum bicolar*) is a grass specie cultivated for its grain which is used as energy food. It belongs to the grass family poaceae (Al-Suwaiegh *et al.,* 2002). It originated in Africa about 3000 to 5000 years ago (Odibo *et al.,* 2002; Sophina *et al.,* 2017). The crop is environmental friendly, water-efficient, requires little or no fertilizer, can resist pest attack and its refuse is biodegradable (FAO, 1995, Al-Suwaiegh *et al.,* 2002). The grains are consumed as food as well as being used in industries like brewing industries for malt production (Ogbonna and Okolo, 2005; Sophina *et al.,* 2017).

Cassava (*Manihot esculenta* Crantz) is a perennial shrub with an edible starchy root, which grows in the tropics and sub-tropical areas of the world (Burrell, 2003; Umeh, 2011).

Food and Agriculture Organisation, FAO, (FAOSTAT, 2011) estimates the world production of cassava at more than 230 million metric tonnes annually with major producing countries to

include Nigeria, which produced 37.5 million tonnes per annum (Hasmadi *et al.,* 2020). Cassava ranks second only to cereal grains as the chief source of energy in Nigerian diet

(Umeh, 2011). By this, cassava plays an important role in alleviating African food crises, though poor in protein (about 1.2%) and rich in cyanide (>10mg/100g fresh weight) in some varieties (Nwabueze and Odunsi, 2006; Cumbana *et al.,* 2007; Umeh, 2011). Cassava varieties cultivated in different regions differ in their nutritional, proximate and other physicochemical properties (Cumbana *et al.,* 2007; Hasmadi *et al.,* 2020). In the tropics, cassava is the most important root crop and as source of energy, the calorific value is high compared to most starchy crops (Hasmadi *et al.,* 2020). Cassava root contains a number of mineral elements, in appreciable amounts, that are useful in the human diet. The root contains significant amounts of iron, phosphorus and calcium, and is relatively rich in vitamin C (Enidiok *et al.,* 2008; Hasmadi *et al.,* 2020).

It is an important component in the diets of many people around the world (FAO, 2007) and is the third-largest carbohydrate food source within the tropical regions, after rice and corn (Ceballos *et al.,* 2006; Hasmadi *et al.,* 2020). Cassava is a food security crop (Barratt *et al.,* 2006) and the roots can be left inside in the soil for up to two years without spoilage. Cassava cannot be consumed as a fresh food item due to its cyanide content but can be processed into various food and non-food products, such as starch, flour, beverages, animal feeds, biofuels and textiles (Tewe and Lutaladio, 2004; Hasmadi *et al.,* 2020). The nutritional value of cassava roots is important because they are the main part of the plant consumed in developing countries. However, there is much variation in the nutrient quality of the cassava root depending on several factors, such as geographic location, variety, age of the plant, and environmental conditions (Benesi *et al.,* 2007; Sanni *et al.,* 2008; Montagnac *et al.,* 2009).

Sorghum grains and cassava tubers can be preserved by processing them into flours. The flours are employed in baking, pharmaceuticals, food and other industries for production of different products. This work therefore determined the proximate and physicochemical properties of flours of improved cassava variety (TMS 30555), called *Onuanwuru* and improved sorghum variety (SC 114) cultivated by the Anambra State Agricultural Development Program (ADP).

II. MATERIALS AND METHODS

2.1 Collection of materials

Dry grains of sorghum (Guinea corn) variety (SC 114) and 9 months old freshly harvested cassava tubers, (*Manihot esculenta* Crantz), (TMS 30555) called '*Onuanwuru*' were collected from the Anambra State Agricultural Development Program (ADP) office in Awka and taken to the Laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka for the research.

Chemical and reagents used were purchased from the Onitsha Bridge Head Drug market and they were of high analytical grade.

2.2 Processing the cassava tubers into flour

The method of Kaur *et al.,* (2016) was modified and used to process the cassava tubers into flour. Three kilogram (3kg) of the fresh tubers were peeled, washed, cut in smaller pieces and soaked in 5 litres of water using a plastic bucket. The tubers were steeped for 2 days at room temperature (28±2^{o}C), washed, hard fibres removed and dried in the oven at 60^{o}C for 10 hrs and allowed to cool. They were ground with a blender and sieved with 0.1 mm sieve to obtain the cassava flour which was oven dried again in a tray for another 3 hrs at 50^{o}C and stored in an airtight container.

2.3 Processing the sorghum grains into flour

The method of Umerie and Umeh, (2016) was used in processing the sorghum flour. One hundred kilograms (100Kg) of the dried grains were weighed into a flat tray. The grains were sorted to remove stones, sand and other unwanted substances therein. They were soaked in 2 litres of water containing 0.813g of Potassium metabisulphite for 48 hrs at 30±1^{o}C. The grains were removed from the steeping water and milled with a Moulinex type electric blender to slurry. The slurry was re-suspended in distilled water, stirred and allowed to stand for 5 minutes. It was then filtered through a 100-mesh sieve cloth for coarse sieving. The sediment was re-suspended in distilled water, stirred and allowed to stand for another 12 hrs. The supernatant was again decanted and the sediment passed through a 200-mesh sieve for fine sieving. The suspension was allowed to settle for another 8 hrs and excess solution decanted. The resulting slurry/sediment was slurried in 200 ml of distilled water, sediment and decanted twice. The recovered wet flour was dried for 24 hrs in a tray in the oven set at 40^{o}C. The resulting flour was crushed using a blender and oven dried again in a tray at 50^{o}C for 3 hrs and stored in an airtight container.

III. ANALYSIS OF THE FLOURS

3.1 Proximate analysis of the flours

Total moisture content, total ash, crude fat, crude protein, crude fibre and total carbohydrates were determined using AOAC (2000).

3.2 Physicochemical analysis of the flours

3.2.1 Water Absorption Capacity (WAC)

WAC of flours was determined using the method used by Raphael *et al.,* (2022). One gram (1g) of the flour sample was mixed with 10 ml distilled water in a 15 ml graduated centrifuge tubes and stirred on a vortex mixer for 5 min. The mixture was allowed to stand for 30 min at room temperature, centrifuged at 3000 rpm for 15 minutes and the supernatant carefully decanted. The weight of the wet flour in the tubes was determined and the Water absorption capacity of the flours was calculated using the equation:

$$\%\text{Water Absorption Capacity (\%WAC)} = \frac{\text{Weight of absorbed water}}{\text{Weight of flour}} \times 100$$

3.2.2 Oil Absorption Capacity (OAC)

OAC of the flours was determined using the method of Chandra and Samsher (2013) as modified by Raphael *et al.*, (2022). One gram (1g) of each sample was dissolved in 10 ml of vegetable oil in a 15 ml graduated centrifuge tubes and then stirred on a vortex mixer for 5 minutes. The mixture was allowed to stand for 30 min at room temperature, centrifuged at 3000 rpm for 15 minutes and the supernatant carefully decanted. The oily flour mixture in the tubes was weighed and Oil Absorption Capacity was calculated using the formula:

$$\%\text{Oil Absorption Capacity (\%OAC)} = \frac{\text{Weight of oil absorbed}}{\text{Weight of flour}} \times 100$$

3.2.3 Swelling power and solubility index

Swelling power and solubility index of the flours were determined using the method used by Raphael *et al.,* (2022). Approximately one gram (1g) of the flours was mixed with 10 ml of distilled water in a 15 ml graduated centrifuge tube and heated at 85°C for 30 minutes in a water bath. The resulting slurries were allowed to cool to room temperature and centrifuged at 3000 rpm for 15 minutes Decanted supernatants were evaporated in an electric hot air oven at 105°C for 30 min. The dried supernatants and sediments were weighed. Swelling power and solubility index were calculated using the equations:

$$\%\text{ Swelling Power} = \frac{\text{Weight of paste}}{\text{Weight of flour}} \times 100$$

$$\%\text{ Solubility Index} = \frac{\text{Weight of soluble fraction}}{\text{Weight of flour}} \times 100$$

3.3 Determination of the colour of the flours

The colour of sorghum and cassava flours was determined using Hunter Colour Meter.

3.4 Determination of the bulk density of the flours

Bulk density of the flours was determined according to the method used by Raphael *et al.,* (2022). A one hundred gram (100g) of the different flours was weighed and gently filled in 250 ml measuring cylinder. The bottom of the cylinder was gently tapped until there was no further diminution of the sample level. The final volume of the flour in the measuring cylinder was noted and Bulk density of the flour was expressed as:

$$\text{Bulk density} = \frac{\text{Weight of sample (g/m)}}{\text{Unit volume of sample}} \times 100$$

3.5 Determination of the pasting properties of the flours

Viscosity of the flours was determined using the method of Williams *et al.,* (2019) as described by Rapheal *et al.,* (2022). A paste was formed in pre-weighed canister from each sample using sample mass and volume of water calculated from the

moisture content of the respective samples. The canister with the formed paste was fixed into the Rapid-Visco Analyzer. Each suspension was kept at 50°C for 1 min and then heated to 95°C in 7 minutes with a holding time of 5 minutes followed by cooling to 50°C in 7 minutes with a 1-min holding time. The pasting parameters (Peak viscosity, Trough viscosity, Breakdown viscosity, Final viscosity and Setback viscosity) were read from the screen of the analyzer and recorded as Rapid-Visco Analyzer unit (RVU).

3.6 Determination of *pH* of the flour samples

The pH of the cassava flour was determined using Hanna Model pH-meter as described by Umeh (2011). One gram of each flours were thoroughly mixed in 10 ml of distilled water in centrifuge tubes and the electrode of the pH-meter, already standardized to pH of 7 was immersed and the readings on the screen recorded.

3.7 Determination of Amylose and Amylopectin content of the flours

Amylose and Amylopectin content of the isolated flours were done as described by Raphael *et al.,* (2020).

3.8 Statistical analysis

All the experiments were carried out in triplicates and the data analysed were the mean values of the results obtained. Data analysis was done using T-test performed using SPSS software version 23.0.

IV. RESULTS AND DISCUSSION

Appreciable quantities of flour were isolated from the sorghum grains and cassava tubers as presented in Table 1. Sorghum grains gave flour yield of 44.8% while cassava tubers yielded 68.7 % flour. This compared well with the findings of Umerie and Umeh (2016) that found starch yield from another *Digitaria exilis*, a related grain to be 35.8%. The results also

confirmed the findings of Chandra and Samsher (2013) who found flour yield from rice grain to be up to 45.4%. The results showed that grains can yield a good quantity of flour. Moisture

content of the flours was 7.35±0.11% and 9.24±0.58% for sorghum and cassava flours respectively. Based on the study conducted by Chandra and Samsher (2013), moisture content of wheat, rice, green gram and potato flours were 13%, 11%, 8% and 9% respectively. This shows that the sorghum and cassavas flour have very low moisture content compared to other types of flour. Low moisture content in sorghum and cassava flours is desirable since this will improve the palatability of the flours (Cumbana *et al.,* 2007). The research done by Hsu *et al.* (2003) reported that high quality flour usually contains moisture content range from 9.0% to 12.0%. Moisture is an important factor in the storage of flours, very high levels greater than 12% can allow microbial growth and the flour will deteriorate in a short time. Low moisture content in flour samples are favourable and confer longer shelf life to the flour. All the samples had good moisture levels and hence have the potential for better shelf life.

The values for ash contents were higher compared to the results obtained by Rodríguez-Sandoval *et al.* (2008) who postulated that ash content of flours range from 0.1% to 0.7% (Hasmadi *et al.,* 2020). The high ash contents in this work suggested that the flours contain high minerals (Hasmadi *et al.,* 2020). Niba *et al.* (2001) postulated that the high ash composition of cassava flour can be attributed to the mineral content of the soil from where the tubers were cultivated. The high ash content may also be due to the fact that the crops used are improved varieties.

Fat content of flours was within the range found by Moorthy (2002) which is between 0.19% to 0.98% (Hasmadi *et al.,* 2020). Fat content of flours influence its pasting properties. The high ash and low fat contents in these flours are desirable attribute since too much fat will lead to the high possibility for rancidity and increase cloudiness in the flour (Mishra and Rai, 2006; Hasmadi *et al.,*2020). Researchers had found out that high fat content causes low swelling power and solubility in flour (Roa *et al.,* 2014).

Crude protein content of the flours in this research was 3.28±28% and 1.02±0.33% for sorghum and cassava respectively. This indicates that sorghum flour had a higher crude protein than the cassava flour. The finding supports the finding of other researchers who postulated that cassava had a low protein content range of 1-3% (Ceballos *et al.,* 2008; Umeh *et al.,* 2014; Hasmadi *et al.,* 2020).

Crude fibre of the isolated flours corresponds with the findings of other researchers who had found cassava flour to possess crude fibre in the range of 1.66 - 4.27% (Fakir *et al.,* 2012; Hasmadi *et al.,* 2020).

Total carbohydrate content of the flours was high. This confirms the findings of Umeh *et al.*, 2014; Hasmadi *et al.*, (2020) that the large composition of tubers and grain composition is made of starch. The findings in this work on the proximate composition of the flours showed that high grade flours were obtained from the sorghum and cassava varieties.

TABLE 1
PROXIMATE COMPOSITIONS ON THE ISOLATED FLOURS

Parameters (%)	Sorghum flour	Cassava flour
Moisture content	4.35±0.11	5.24±0.58
Ash content	2.16±0.23	3.35±1.02
Crude fat	0.26±0.03	0.18±0.45
Crude protein	3.28±0.06	1.02±0.33
Crude fibre	2.16±0.10	3.14±2.02
Total carbohydrate	25.02±1.02	28.06±0.54
Flour yield	44.8	68.7

Result of Physicochemical properties of the flours isolated from the sorghum grains and cassava tubers were shown in Table 2. The flour of Sorghum and cassava showed very high percentage of water absorption capacity, oil absorption capacity, swelling power and solubility index. Water absorption capacity (WAC) is an index of the maximum amount of water a food product absorbs and retains while oil absorption capacity is an index of the maximum amount of oil a product can absorb and retain. Oil absorption capacity (OAC) is a very important parameter for determining the flavour retaining ability of flours and starches (Raphael *et al.,* 2022). The high WAC seen in the work supported the findings of Hasmadi *et al.,* (2020) who also found very high WAC (1.12±0.05 ml/g and 1.30±0.11 ml/g) from two cassava samples from different locations in Malaysia. Raphael *et al.,* 2022 also determined very high WAC (263.9±19.3 and 263.3±7.3%) and OAC (121.0±13.3 and 119.0±63%) from the research on two cassava varieties in Ghana.

According to Aryee *et al.* (2006) as reported by Hasmadi *et al.,* (2020) the water absorption capacity of flours depends on the power of aggregation between starch molecules. Weak aggregation power between starch molecules causes the surface of its molecules to form a bond with water molecules become easier thus increase the rate of water absorption capacity. Water absorption capacity of flour is an important feature in order to increase its application in ready-to-eat food such as instant noodles, dough and soup (Singh, 2001; Hasmadi *et al.*, 2020).

Oil absorption capacity is highly related to lipophilic properties of the starch molecule in the sorghum and cassava flour. Flours with high OAC are used as raw materials in producing lipid-based products for help in flavour retention and organoleptic enhancement. It also helps in absorption of vitamins in food (Raphael *et al.,* 2022).

Swelling power determines the tendency of a substance to be hydrated and stands as one of the ways of measuring food quality. Swelling power of flour and starch is inversely proportional to solubility index. The high swelling power recorded resulted in a correspondingly low solubility index seen in the work.

TABLE 2
WAC, OAC, SWELLING POWER AND SOLUBILITY INDEX OF THE ISOLATED FLOURS

Parameters (%)	Sorghum flour	Cassava flour
WAC	240.6±1.3	260.4±2.0
OAC	123.1±0.8	128.0±1.1
Swelling power	800.5±2.0	744.3±2.2
Solubility index	3.5±3.1	4.3±2.0

Key: WAC – Water Absorption Capacity; OAC - Oil Absorption Capacity

Other physicochemical properties of the sorghum and cassava flours were recorded in Table 3. Amylose content of the flours were 22.5±0.5% and 32±1.2% for sorghum and cassava flours while their amylopectin contents were 67±0.7% and 78±0.2%

respectively. Amylose and amylopectin ratios affect swelling and solubility characters of flours. They also help in stronger intermolecular interaction and higher hydrophobicity and account greatly for greater swelling power and lower solubility (Raphael *et al.,* 2022). High values of amylose and amylopectin result in low solubility of flours. Flours with low solubility indices against high swelling powers are suitable for making dough with high elasticity. The flours isolated from this research can be suitable for dough moulding. The results of amylose and amylopectin is in line with the findings of Raphael *et al.,* (2022).

Bulk density was 0.78±0.2 and 89.01g/cm^3 for sorghum and cassava flour respectively. The value of the bulk density of the two samples were low and comparable with the values obtained by Hasmadi *et al.,* (2020) who recorded 0.57±0.05 and 0.79±0.13g/cm^3 for two different cassava varieties in Malaysia. Bulk density is a measure of the heaviness of a flour sample (Hasmadi *et al.,* 2020). Previous works reported that Bulk density is generally affected by the particle size and density of the flour and it is one of the essential parameters in determining the type of packaging material, handling and application in the food industry (Hasmadi *et al.,* 2020).

pH of the isolated flours were 6.6±0.05 for sorghum and 6.8±0.02 for cassava flours. The *pH* values were high tending towards neutrality. Sorghum grains are not acidic in nature and their flours will not in any way harbour acidity unless as a contaminant during the processing methods. Cassava tubers are known to be acidic and their flours had been found to have a *pH* range of 5.5 to 8.5 (Aryee *et al.,* (2006). Other researchers (Muzanila *et al.,* 2000; Apea-Bah *et al.,* 2011) had recorded *pH* values of 6.22 in Tanzania and 5.07 and 6.65 range in Ghana respectively. The value obtained for cassava in this work may be as a result of the improved variety used. Agricultural Development Programs are currently researching and improving the cassava varieties to contain lower levels of hydrogen cyanide (HCN) and acidity. *pH* is one of the important attributes in order to maximize the application of cassava flour in food industries especially in the making of bakery products (Aryee *et al.,* 2006; Hasmadi *et al.,* 2020). Colour of the isolated flours was pale white and white respectively for sorghum and cassava which are standard flour colours suitable for industrial application.

TABLE 3

OTHER PHYSICOCHEMICAL PROPERTIES OF THE SORGHUM AND CASSAVA FLOURS

Parameters	Sorghum flour	Cassava flour
Amylose content (%)	22.5±0.5	32.0±1.2
Amylopectin (%)	67.0±0.7	78.0±0.2
Bulk density (g/cm^3)	0.78±0.2	0.89±0.1
pH of flours	6.6±0.05	6.8±0.02
Colour	Pale white	White

Table 4 presents the pasting properties of the flours isolated in this work. Pasting property is one the vital properties which measures the ability of flour or starch to form a paste. It is a parameter that cannot be sidelined in the measurement of the quality of flour and starch since it dictates the textural integrity of products (Adebowale *et al.,* 2011; Raphael *et al.,* 2022). Peak viscosity is the measure of the highest viscosity a starch granule can attain before collapsing (Adebowale *et al.,* 2011). Higher peak viscosities lead to lower pasting times and temperatures. The peak viscosity in this research is high and makes them good as industrial raw materials. Breakdown viscosity of the flours was low. The breakdown viscosity of any material is its ability to withstand thermal treatment when incorporated in any manufacturing process. The breakdown viscosity shows the resistance of the paste to shear stress and the stability of the paste during thermal treatment. Lower breakdown viscosities dictate the tenacity of the paste to thermal and shear interruptions, which are very vital in determining the stability of pastes (Raphael *et al.,* 2022). Other pasting properties were in the range that confers durability and acceptance to the isolated flours.

TABLE 4
PASTING PROPERTIES OF THE ISOLATED FLOUR

Parameters	Sorghum flour	Cassava flour
Peak viscosity (RVU)	2215±2	3204±5
Trough viscosity (RVU)	1003±2	2115±1
Breakdown viscosity (RVU)	123±1	214±2
Setback viscosity (RVU)	240±3	406±3
Final viscosity (RVU)	1126±2	1422±3
Pasting temperature ^{o}C	68±2	72±2
Pasting time (minutes)	6.2±3	5.2±1

Key: RUV - Rapid-Visco Analyzer unit.

The findings in this research conform to most of the flour characteristics already in the market. The sorghum and cassava varieties used were improved varieties from the Anambra State Agricultural Development Program and this is the first institutional research performed on them. Sorghum and cassava flours isolated from them can make good industrial flours.

V. CONCLUSION

These improved sorghum and cassava varieties were able to yield high quantity of flours greater than other varieties from literature. Their physicochemical and functional properties show encouraging characteristics for them to be used in various industries. The values of their pasting properties also made them food flours for food as well as pharmaceutical industries.

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