Promotive effect of carrier based biofertilizers on *Oryza sativa*.

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Promotive effect of carrier based biofertilizers on *Oryza sativa*" is based on the results of investigations carried out by me in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Dr. Rupali Bhandari and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation. I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

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This is to certify that the dissertation report **"Promotive effect of carrier based biofertilizers on *Oryza sativa*"** is a bonafide work carried out by **Ms Sanjana Satish Arobekar** under my supervision in partial fulfilment of the requirements for the award of the degree of Masters in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University.

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Abstract

The present work was conducted to evaluate the response of the Jaya rice variety to carrier-based biofertilizers on morphological, physiological, and biochemical parameters. The biofertilizer used was *Azolla pinnata* and the carrier materials used were multani mitti, curry leaf powder and charcoal. The Jaya rice variety plants were raised in vermiculite under a controlled environment and supplemented with carrier-based biofertilizers and chemical fertilizers and Hoagland solution containing all nutrients and Hoagland solution with the absence of nitrates. It was observed that plants grown in Hoagland solution containing no nitrates and Hoagland solution containing all the nutrients treated with *Azolla*+mutani mitti showed greater biomass than other treatments. Plants grown in Hoagland solution containing no nitrates and Hoagland solution containing all the nutrients treated with *Azolla*+charcoal showed greater RWC than other treatments. The seed germination rate increased in seeds treated with *Azolla*+multani mitti in Hoagland solution containing all nutrients and Hoagland solution containing no nitrates. The photosynthetic efficiency increased in plants grown in Hoagland solution containing all nutrients and Hoagland solution containing no nitrates with *Azolla*+multani mitti and decreased in other treatments. Plants grown in Hoagland solution containing all nutrients with carrier based biofertilizers and chemical fertilizer showed an increase in Chlorophyll a and Chlorophyll b content, whereas the carotenoid content showed a reduction in all treated plants. Plants in the absence of nitrates treated with *Azolla*+multani mitti showed an increase in Chlorophyll a, whereas the Chlorophyll b and carotenoids concentration was reduced in all treated plants as compared to control. Plants grown in Hoagland solution containing all nutrients and Hoagland solution containing no nitrates with a combination of *Azolla*+charcoal showed an increase in total sugar content compared to control. Plants grown in Hoagland solution containing all nutrients and Hoagland solution

containing no nitrates with a combination of *Azolla*+curry showed an increase in protein content compared to control. The glycolipid content in plants treated with *Azolla*+charcoal was high compared to all the treatments grown in Hogland solution containing all nutrients. Whereas in plants in the absence of nitrate treated with *Azolla*+charcoal, the glycolipid content drastically increased compared to all the treatments. Biofertilizer treatment with carrier material multani mitti and charcoal increased nitrogen uptake and enhanced the yield of the Jaya rice variety with better physiological and biochemical attributes even in the absence of inorganic nitrogen fertilizer application. The results indicated that carrier-based biofertilizers would be an excellent substitute for inorganic fertilizer and can be used for eco-friendly yield boosts with low input costs reducing the continuous use of inorganic chemical fertilizers. However, the patterns observed in the results indicated that the application of carrier-based biofertilizers might be crucially important in small to medium input structures in cultivation. The outcomes can be practiced to provide better instruction for root-level farmers on biofertilizers.

Chapter - 1

INTRODUCTION

1. INTRODUCTION

The demand for agricultural products is rising worldwide due to the increasing human population. Currently, there are approximately 8.1 billion people residing on the planet, and this number is expected to increase significantly, with an estimated growth of almost 10 billion over the next 50 years. As the world's population continues to grow, the demand for food is also increasing. This means that feeding the current population, which will only continue to expand in the future, is a major challenge. To address the issue of food scarcity due to population growth, various agricultural methods have been employed, including the use of synthetic fertilizers, pesticides, and insecticides. These methods are aimed at increasing crop yields quickly and protecting crops from pests and insects during and after harvest. The use of fertilizers and insecticides has become a cause for concern due to their impact on the safety, sustainability, and security of our food supply. Studies have revealed that pesticide residue is present in food long after it leaves farms for human consumption. This highlights the need for alternatives, such as biofertilizers, to ensure the safety and security of our food supply. Synthetic fertilizers, which contain nutrients such as nitrogen, phosphorus, potassium, and sulfur, can also become harmful if overused. These harmful effects include weakened plant roots, a higher incidence of disease, soil acidification, and eutrophication of water bodies, causing nitrates to leach into groundwater and leading to "blue baby syndrome" or "acquired methemoglobinemia." The impact of these chemicals will not only affect the present but also future generations. Therefore, it is necessary to adopt eco-friendly approaches, such as biofertilizers, which play a significant role in sustainable agriculture.

1.1 Chemical Fertilizers

In the world of intensive agriculture, chemical fertilizers are frequently used. These fertilizers are made artificially from soil-essential macronutrients like nitrogen, phosphorous, and potassium, making them robust and powerful. They may contain ammonium sulfate, urea, potash, and ammonia, among other substances, depending on their structure and the crops and soils for which they are intended. These fertilizers can be applied and spread in a variety of methods, either mechanically or by hand. Chemical fertilizers are more resistant to the environment, reduce soil fertility, and actually cause a lot of degradation of soil and land (Liu et al., 2009). The use of chemical fertilizers reduces soil microorganisms and causes groundwater pollution. Nitrogen fertilizers break down in the soil and converts into nitrates that are soluble in water and easily pass through the soil and they can remain in that position for decades, and this accumulation causes problems. This accumulation of chemicals leads to surface and groundwater pollution (Uthirapandi et al., 2018).

1.2 Disadvantages of Chemical fertilizers

Chemical inorganic fertilizers contain set measured levels of minerals and they usually contain the big three minerals (nitrogen, phosphorus, potassium). They often lack diverse micronutrients that a plant would receive from naturally decomposing materials, instead of containing a lot of "filler." Research by the University of Vermont found that chemical contamination from synthetic fertilizers can cause nearby waterways to turn green or cloud with algae blooms, take on an unusual odor, and deplete oxygen for fish and other species, suffocating them. Because of the effects of this chemical run-off, artificial fertilizers are often not considered environmentally friendly. These inorganic substances are highly concentrated solutions, and if over-sprayed or over-applied can overwhelm the

landscape becoming too much of a good thing. The high mineral levels can cause instant damage like root burn and long-term chemical use can alter the pH balance of the soil and cause a toxic build-up of certain nutrients.

1.3 Biofertilizers

Biofertilizers are microorganisms that support the growth of plants by enhancing the nutrient supply to the host plant when given to seeds, plants, or the soil. Plant growth-promoting microorganisms colonize either the rhizosphere or the inside of plants. These microorganisms play an important role in the soil ecosystem by participating in various biotic activities that make it dynamic and sustainable for the growth of crops. Biofertilizers are widely used to accelerate microbial activities that increase the availability of nutrients that plants can easily absorb. They increase soil fertility by fixing atmospheric N2 and solubilizing insoluble phosphates in the soil, resulting in plant growth-promoting chemicals. These biofertilizers utilize naturally available biological systems to mobilize nutrients, improving soil fertility and crop productivity. It has been reported that the biofertilizer market is estimated to grow at a compound annual growth rate (CAGR) of 14.0% from 2015 to 2020 and is expected to reach USD 1.88 billion by 2025. Because of strict regulations on the use of chemical fertilizers, biofertilizers are the most widely used in Europe and Latin America.

Biofertilizers are organic products that contain specific microorganisms extracted from plant roots and their surrounding areas. They are known to enhance plant growth and yield by 10-40%. These bioinoculants are applied to the rhizosphere and the interior of the plant, where they colonize and aid in promoting plant growth. In addition to improving soil fertility and crop yield by adding nutrients to the soil, they also protect the plant

against pests and diseases. Biofertilizers have been proven to aid in seedling survival, prolong the root system's lifespan, eradicate harmful chemicals, and reduce flowering time. A further advantage of using biofertilizers is that they are no longer necessary after continuous use of 3-4 years, as the parental inocula are sufficient for growth and multiplication. Plants require 17 essential elements for effective growth and development, including N2, P, and K, which are required in significant amounts. Biofertilizers are commonly made up of various microorganisms such as nitrogen-fixing soil bacteria, cyanobacteria, phosphate-solubilizing bacteria, molds, and mushrooms. These microorganisms produce phytohormones which are essential growth-promoting compounds for plants. They contain amino acids, vitamins, and indole acetic acid (IAA), which help to improve the soil's fertility and productivity and conserve crop yield.

1.3.1 TYPES OF BIOFERTILIZERS

1. Nitrogen fixing: Nitrogen is most abundant and ubiquitous in the air, yet becomes a limiting nutrient due to the difficulty of its fixation and uptake by plants. However, certain microorganisms, some of which can form various associations with plants as well, are capable of considerable nitrogen fixation. These microbes can be:

Bacteria are of three types

• **Free-living**: Free living in the soil. Example: *Azotobacter*.

• **Associative**: Living in rhizosphere (associative/associated) without endophytic symbioses. Example: *Azospirillum*.

• **Symbiotic**: Having symbiotic and other endophytic associations with plants. Example: *Rhizobia*, *Frankia*.

Blue-green algae (Cyanobacteria): They have been reported to help enhance rice-field fertility for the cultivation of rice in many parts of the world. BGA can further provide natural growth hormones, 172 proteins, vitamins, and minerals to the soil.

Examples: *Anabaena, Nostoc, Tolypothrix, Cylindrospermum* etc.

Azolla: *Azolla* is a floating pteridophyte, which contains an endosymbiont the nitrogen-fixing cyanobacterium *Anabaena azollae*. *Azolla* is either incorporated into the soil before rice transplanting or grown as a dual crop along with rice.

2. Phosphate solubilizing:

The phosphorus-solubilizing bacteria (PSB) can increase phosphorus availability to plants by dissolution of bound phosphates in soil by secreting organic acids characterized by lower pH in their vicinity. Examples: *Bacillus* spp., *Paenibacillus* spp., *Pseudomonas* spp. etc.

3. Phosphate mobilizing:

The mycorrhizal fungi form obligate or facultative functional mutualistic symbioses with more than 80% of all land plants, in which the fungus is dependent on the host for photosynthates and energy and in return provides a plethora of benefits to its host. The mycelium of the fungus extends from host plant root surfaces into soil, thereby increasing the surface area for more efficient nutrient access and acquisition for the plant, especially from insoluble phosphorus sources and others like calcium, copper, zinc, etc, for examples: ectomycorrhiza (*Laccaria* spp., *Pisolithus* spp., *Boletus* spp., *Amanita* spp.), endomycorrhiza (examples: Arbuscular mycorrhiza- *Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp., and *Sclerocystis* sp.).

4. Mineral-Solubilizing Biofertilizers:

Potassium solubilizing: Certain rhizobacteria can solubilize insoluble potassium forms, which is another essential nutrient necessary for plant growth.

Examples: *Bacillus edaphicus, B. mucilaginosus, and Paenibacillus glucanolyticus*.

5. Silicate and zinc solubilizing:

Zinc is an important mineral that is present in low concentrations in the Earth's crust. As a result, it is often applied externally as the more expensive soluble zinc sulfate to overcome deficiencies in plants. However, certain microbes can solubilize cheaper insoluble zinc compounds like zinc oxide, zinc carbonate, and zinc sulfide present in the soil. Additionally, microorganisms can hydrolyze silicates and aluminum silicates by supplying protons and organic acids, which causes hydrolysis.

Examples: *Bacillus subtilis, Thiobacillus thioxidans, and Saccharomyces* sp.

6. Plant growth-promoting rhizobacteria: Besides nitrogen-fixing, phosphorus, and minerals solubilizing microbes, some microbes are suitable to be used as biofertilizers as these enhance plant growth by synthesizing growth-promoting chemicals like growth hormones (auxins, gibberellin, etc.). These bacteria show more than one mechanism of plant growth promotion viz. nitrogen fixation, phosphorus solubilization, production of antibiotics, cytokinins, chitinase, and other hydrolytic enzymes, and enhancement of soil porosity.

Example: *Achromobacter, Alcaligenes, Arthrobacter, Actinoplanes, Azotobacter, Bacillus, Pseudomonas fluorescens, Rhizobium, Bradyrhizobium* etc.

7. Compost Biofertilizers: Compost is a mixture of decaying organic matter, microorganisms, and other nutrients that enrich the soil. The microbial organic solid

residue oxidation causes the formation of humus-containing material, which can be used as an organic fertilizer that sufficiently aerates, aggregates, buffers, and keeps the soil moist, besides providing beneficial minerals to the crops and increasing soil microbial diversity. Compost is produced from a wide variety of materials like straw, leaves, cattle shed bedding, fruit and vegetable wastes, biogas plant slurry, industrial wastes, city garbage, sewage sludge, factory waste, etc. The compost is formed from these materials by different decomposing microorganisms like *Trichoderma viridae, Aspergillus niger, A. terreus, Bacillus* spp., several gram-negative bacteria (*Pseudomonas, Serratia, Klebsiella, and Enterobacter*), etc. that have plant cell wall degrading cellulolytic or lignolytic and other activities, besides having proteolytic activity and antibiosis (by production of antibiotics) that suppresses other parasitic or pathogenic microorganisms .

Vermicompost is an important type of compost that comprises earthworm cocoons, excreta, microorganisms such as bacteria, actinomycetes, fungi, and various organic matter. This compost is an excellent source of nitrogen, phosphorus, potassium, and various micronutrients. Vermicompost is an efficient method to recycle animal wastes, agricultural residues, and industrial wastes cost-effectively while using low energy.

1.4 CYANOBACTERIA

Cyanobacteria are the most abundant organisms on Earth. They are autotrophic and can be found in a variety of environments, particularly in marine and freshwater habitats. Marine water is the richest source of nutrients for cultivating cyanobacteria. They are typically small and unicellular, often forming large colonies. Cyanobacteria are a diverse group of bacteria that come in various shapes and sizes. They encompass around 150 known genera. Cyanobacteria exhibit traits similar to the oldest known fossils, dating

back more than 3.5 billion years. Due to their photosynthetic properties, they have played a significant role in shaping the Earth's atmosphere into the oxygen-rich environment we have today.

Proposed in 1985, the classification of cyanobacteria identifies four orders Chroococcales, Nostocales, Oscillatoriales and Stigonematales, with their phyla being Chroococcales, Gloeobacterales, and Pleurocapsales. Cyanobacteria are associated with the periods of origin of plants. The cyanobacteria are immensely important in determining the path of evolution and ecological changes all over the earth's history. In the late Proterozoic or the early Cambrian period, cyanobacteria began to take up residence within certain eukaryote cells, this event is called endosymbiosis, for the origin of the eukaryotes. They have the potential to fix atmospheric nitrogen, so that could be used as a biofertilizers for the cultivation of economically important crops such as rice and beans. Cyanobacteria have three distinct layers that make up their outermost structure: a mucilaginous layer, a cell wall, and an innermost plasma membrane. Inside the cytoplasm, pigmented lamellae can be found, but they are not organized into a plastid. The pigments present in cyanobacteria include chlorophylls, carotenes, xanthophylls, c-phycoerythrin, and c-phycocyanin. The last two pigments are specific to blue-green algae.

Cyanobacteria are composed of various organic inclusions that perform specific functions. These inclusions consist of different structures, including the light-harvesting antennae, phycobilisomes, polyphosphate bodies, cyanophycin granules, polyhydroxyalkanoate (PHA) granules, carboxysomes/polyhedral bodies, lipid bodies, thylakoid centers, DNA-containing regions, and ribosomes. Cyanophycin granules are large structures that contain polypeptides rich in amino acids such as arginine and aspartic

acid. These granules are visible under a light microscope and store nitrogen more efficiently. Cyanophycin granules are large structures that contain polypeptides that are rich in amino acids, such as arginine and aspartic acid. These granules are easily visible under a light microscope and are used to store nitrogen more efficiently. Similarly, carboxysomes are also present in nitrifying bacteria and thiobacilli. These carboxysomes are about 100 nm in diameter and have a polyhedral shape. They are used to store ribulose-1,5-bisphosphate carboxylase (RuBisCo) in a paracrystalline arrangement, which is the site of CO2 fixation. *Halobacterium and Thiothrix* are purple and green photosynthetic bacteria; contain organic inclusion bodies such as gas vacuoles which provide buoyancy to the cyanobacteria to float over the surface. The nucleoplasm or the DNA enclosing region is present in the center of the cell and shows a fibrillar structure.

Cyanobacteria are unique prokaryotes that have an unorganized nucleus. Their DNA is clumped together without a nuclear boundary or a nucleolus. During cell division, the nucleoplasmic materials disperse throughout the cytoplasm without the participation of the spindle apparatus. Cyanobacteria have two important cell types: Heterocysts, which are responsible for nitrogen fixation and ammonia synthesis, and Vegetative cells, which exhibit normal photosynthesis and reproductive growth.

1.4.1 *Anabaena*

Anabaena is a type of filamentous cyanobacteria that exists in water as plankton. They are well-known for their ability to fix nitrogen and form symbiotic relationships with certain plants like the mosquito fern. *Anabaena* is one of the four genera of cyanobacteria that produce neurotoxins. This production of neurotoxins is believed to be a means of protecting the plant from grazing pressure and is an important factor in its symbiotic

relationships. In 1999, a project was initiated to sequence the entire genome of *Anabaena*, which is comprised of 7.2 million base pairs. The study was specifically focused on heterocysts, which are responsible for converting nitrogen into ammonia. *Anabaena* has been used for fertilizing rice paddy fields, and certain species have been found to be quite effective in this regard.

Under conditions of limited nitrogen, the vegetative cells in filaments differentiate into heterocysts at regular intervals. These heterocyst cells are specialized for nitrogen fixation. The interior of these cells has very little oxygen due to increased respiration, inactivation of photosystem (PS) II which produces oxygen, and formation of a thickened envelope outside the cell wall. Nitrogenase, which is contained within these cells, converts dinitrogen into ammonia using ATP and reductant, both generated by carbohydrate metabolism. This process is supplemented by the activity of PS I in the light. Glucose, likely in the form of carbohydrate, is synthesized in vegetative cells and transported to the heterocysts. In return, nitrogen fixed in the heterocysts is transported back to the vegetative cells, at least partially in the form of amino acids.

The fern *Azolla* forms a symbiotic relationship with a cyanobacterium known as *Anabaena azollae*. This bacterium fixes atmospheric nitrogen, which allows the plant to access this essential nutrient. Due to this unique relationship, the *Azolla* plant is often referred to as a "super-plant". It can easily colonize freshwater areas and grow at an astonishing rate - doubling its biomass in as little as 1.9 days.

1.5 *Azolla*

Azolla is a highly productive type of plant that can rapidly double its biomass in as little as 1.9 days, depending on the growing conditions. When cultivated in Asian rice fields, it can yield as much as 8-10 tonnes of fresh matter per hectare. There are reports of *Azolla pinnata* producing 37.8 tonnes of fresh weight (equivalent to 2.78 tonnes of dry weight) per hectare in India (Hasan et al., 2009).

Azolla is a type of floating aquatic fern that stays afloat by using its small, overlapping leaves, while its roots dangle in the water. It has a special relationship with a type of cyanobacterium called *Anabaena azollae*, which lives outside of its host's cells and helps fix atmospheric nitrogen. The growth of *Azolla* is usually limited by phosphorus, which means that an excess amount of phosphorus, caused by factors like eutrophication or chemical runoff, can lead to an overgrowth of *Azolla*. Unlike other plants, *Azolla's* symbiotic microorganism transfers directly from one generation of *Azolla* to the next. *A. azollae* is entirely reliant on its host, as several of its genes have either been lost or transferred to the nucleus in *Azolla'*s cells.

The ability of *Azolla* to fix nitrogen has made it a popular choice as a biofertilizer, particularly in Southeast Asia. For over a thousand years, the plant has been utilized in China to enhance agricultural productivity. During spring, when rice paddies are flooded, they can be seeded with *Azolla*, which quickly spreads to cover the water and suppresses weed growth. As the *Azolla* dies and decomposes, it releases nitrogen into the water which in turn fertilizes the rice plants, providing up to nine tonnes of protein per hectare per year.

1.6 Carrier based Biofertilizer

Various types of material are used as carrier for seed or soil inoculation. For preparation of seed inoculant, the carrier material is milled to fine powder with particle size of 10 -40 μm. According to the "Handbook for Rhizobia" (Somasegaran and Hoben, Springer, 1994), the properties of a good carrier material for seed inoculation are: (1) non-toxic to inoculant bacterial strain, (2) good moisture absorption capacity, (3) easy to process and free of lump-forming materials, (4) easy to sterilize by autoclaving or gamma-irradiation, (5) available in adequate amounts, (6) inexpensive, (7) good adhesion to seeds, (8) good pH buffering capacity and (9) non-toxic to plant, is another important property. Peat is the most frequently used carrier material for seed inoculation. Peat-based rhizobial inoculant is already used in many countries and a number of information is available on the properties and effect of the inoculant. For soil inoculation, carrier material with granular form (0.5 – 1.5 mm) is generally used. Granular forms of peat, perlite, charcoa, multani, curry leaves or soil aggregates are suitable for soil inoculation.

The biofertilizer industry is a rapidly advancing field with growing interest of researchers and agricultural society toward the development of potential bioformulations. The different bioformulations are available in the market claiming to be better over one another. The most accepted bioformulations consists live or latent bacterial culture mixed with a carrier molecule. The carrier material is crucial for the development of biofertilizers as it ensures optimum viable bacterial count during the storage period. Different carrier molecules are chosen while developing a biofertilizer of specific bacterial strain. The selection of carrier material should be based on certain other factors also. Firstly, the carrier material should contain a minimum amount of carbon source that ensures the survival of microbial inoculants during the storage period. Secondly, the

release of microbes to the plant roots and their survival in the presence of other soil microbes is based on the choice of carrier material. The fine powder and microporous structure of the carrier material is recommended for a larger population of bacteria. Different types of carrier molecules are available that can be categorized as soil-based, plant-based, and inert material. Peat, clay, and lignite are soil-based carrier molecules used for biofertilizer bioformulation. Paddy straw, rice bran/wheat bran, and bagasse are widely used for the bioformulation of *Rhizobium*-, *Burkholderia*-, and *Bacillus*-based biofertilizers. Inert materials such as vermiculite, alginate, perlite, ground rock phosphate, and polyacrylamide gels have been used for the development of *Rhizobium*-, *A. lipoferum*-, *B. megaterium*-, and *Pseudomonas*-based biofertilizers.

Types of carrier materials are:-

1) Multani mitti: It has absorbent property and clayey texture. It may exhibit antimicrobial property. Multani mitti comprises of hydrated aluminium silicates, magnesium chloride, and calcium bentonite and has a composition similar to bentonite clay.

2) Curry leaf powder: It has antimicrobial and absorbent property. Curry leaves also have nutrients including protein, fiber, calcium, vitamins and minerals.

3) Charcoal: Charcoal exists in a solid amorphous state. Charcoal is a black porous solid that consists of carbon. It is a low-density compound. Charcoal shows low mechanical strength properties. The structure of carbon charcoal shows a large surface area. Charcoal acts as a good absorbent. It readily absorbs moisture. The high surface area of charcoal and high porosity enhances the contamination of charcoal by incidental contact with dust and soil. Therefore, it requires precautions while storing.

Azolla holds great promise as a sustainable biofertilizer with the potential to address soil fertility issues, reduce chemical fertilizer usage, and promote environmental sustainability in agriculture. Despite certain challenges, ongoing research and technological advancements offer opportunities to optimize its utilization and maximize its benefits in diverse agricultural systems. Further interdisciplinary studies integrating agronomy, microbiology, and environmental science are necessary to unlock the full potential of *Azolla* as a biofertilizer and promote its widespread adoption in global agriculture. Further research is needed to optimize *Azolla* biofertilizers with various career-based inoculum will enhance its nitrogen-fixing efficiency, and address potential concerns related to its use, such as allelopathic effects on other plant species.

Chapter - 2

REVIEW OF LITERATURE

2.Review of literature

Research on *Azolla* as biofertilizers has been ongoing for several decades, with earlier studies laying the groundwork for understanding its potential and limitations. Here's a brief overview of some key findings from earlier research. One of the earliest and fundamental discoveries regarding *Azolla* is its ability to fix atmospheric nitrogen through its symbiotic relationship with the cyanobacterium *Anabaena azollae*. Initial research focused on understanding *Azolla's* nitrogen-fixing ability. Studies conducted during this period, such as those by Reis and Guimarães (1967) and Burris et al., (1975), identified the presence of the nitrogen-fixing cyanobacterium *Anabaena azollae* within *Azolla's* leaf cavities. These studies laid the foundation for understanding Azolla's role as a natural biofertilizer. Research expanded to evaluate *Azolla's* agronomic performance and its impact on crop yields. Studies by Natarajan and Rajendran (1979) and Rao et al., (1984) demonstrated *Azolla's* potential as a green manure in rice cultivation, showing improvements in soil fertility, nitrogen availability, and rice yield. Studies by Wagner and Hager (1980) and Howarth and Anderson (1990) elucidated *Azolla's* high nitrogen-fixing capacity and its ability to enhance soil microbial activity, promoting nutrient cycling and soil fertility. Mandal and Nandi (1998) and Rao et al., (2003) investigated *Azolla's* efficacy as a biofertilizer in crops such as maize, wheat, and vegetables, as well as its integration into agroforestry systems. Recent research has emphasized *Azolla's* role in sustainable agriculture and its environmental benefits. Sood et al., (2009) and Baruah et al., (2018) highlighted *Azolla's* potential to reduce chemical fertilizer usage, mitigate greenhouse gas emissions, and improve soil carbon sequestration, contributing to environmental sustainability. Studies evaluated different methods of *Azolla* cultivation, harvesting, processing, and application to optimize its efficacy and convenience for farmers. Research highlighted the environmental benefits of *Azolla* as a biofertilizer,

including its role in reducing greenhouse gas emissions, improving soil carbon sequestration, and mitigating nutrient runoff and water pollution.

Kulasooriya et al., (1984) observed that the ability of *Azolla pinnata* to grow and establish itself in monoculture in rice fields was examined in several locations, falling within different agro-ecological zones of Sri Lanka. These results provide encouraging evidence for the ability of *Azolla pinnata* to grow rapidly in several rice-growing areas in Sri Lanka and, the suitability of *Azolla* as a biofertilizer for rice that can replace a substantial amount of nitrogenous fertilizer. Widiastuti et al., (2017) reported that utilizing *Azolla* as a biofertilizer can mitigate CO2 emissions from fossil fuel that is used in producing inorganic fertilizers such as urea. The greenhouse study aimed to identify the optimum nutrient concentrations in the growing medium, inoculation rate, and combined nutrient solutions that can maximize the growth of *A. mexicana* and to identify the nutrient concentrations in *A. mexicana* as a biofertilizer. Hasan et al., (2020) reported that cyanobacteria exhibit a wide distributional spectrum, they are ubiquitous under different soil, water and agroclimatic conditions. Gupta et al., (2022) observed that seedling germination was better with the bioformulation made with charcoal and tea leaf powder and they believed it might be used to alleviate abiotic stresses in a cost-effective and environmentally friendly manner. Compared to synthetic N-fertilizers, *Azolla* has various positive impacts on lowland rice production, including improving soil fertility, minimizing weeds, increasing soil organic carbon, improving microbial biomass, and thus nutrient cycling and enhancing rice growth and yield (Marzouk et al., 2023). It is necessary to evaluate and develop a balanced fertilization strategy that combines the use of chemical, organic or biofertilizer (Patil et al., 2010).

Biofertilizers improved plant productivity and quality in sunflower seed (Akbari et al., 2011).The application of biofertilizer decreased the saturated fatty acids (palmitic and stearic) and increased unsaturated fatty acids (linoleic acid and oleic acid) and oil content, compared with untreated plants. There is a positive effect of PGPB on germination, as well as applied biofertilizer treatments stimulated the germination and growth of maize by reason of excreting phytohormones and enhancing the nutrient mobilization from the seed (Bakonyi et al., 2013). *Azolla* could be utilized as a sustainable biofertilizer for vegetable production in dryland acidic tropical soils, in order to promote higher yields and maintain soil fertility (Widiastuti et al., 2017). Moreover, *Azolla* biofertilizer and manure can be used to enhance yields and nutrient concentrations in radish and spinach crops, improve soil fertility in the alluvial and peat soils, and enhance soil microbial communities and reduce abiotic microbial stress. Chilton et al., (2018) study showed positive effects of seeds bio-primed with cyanobacteria on germination and seedling growth of two species, *Senna notabilis* and *Acacia hilliana,* respectively. The potential benefits of applying indigenous bacteria via bio-priming seeds would not inhibit plant establishment, and indeed may be beneficial for some species used in dryland restoration. There is research on inoculants of heterocystous cyanobacteria genera, which are used as biofertilizers in crops by enhancing the plant shoot/root length, dry weight, and yield (Hasan et al., 2020). Khair et al., (2021) showed that *Azolla* could be used as an alternative fertilizer on rice fields because the soil treated with *Azolla* shows a comparable result with soil treated with inorganic fertilizer without *Azolla* on the total yield. *Azolla* application has tremendous potential to improve soil health and boost yield sustainability (Thapa et al., 2021).

Gupta et al., (2022) showed that seedling germination was better with the bioformulation made with charcoal and tea leaf powder, as a result, it is believed that it might be used to alleviate abiotic stresses in a cost-effective and environmentally friendly manner. Biofertilizers have quantifiable effects on soil microbial communities in a crop system setting, which underscores the opportunities for biofertilizers to promote N use efficiency and the circular N economy (Qiu et al., 2022). Liquid biofertilizers have been proven to perform better than the other forms in lasting for longer periods of time, improving crop quality, and requiring less amounts for application (Allouzi et al., 2022). Peat is a good carrier material for biofertilizer production as it not only enhances crop production but also the microbial number, in addition to improving soil quality (Safdar et al., 2022). The viability of cyanobacterial cells was studied by measuring the chlorophyll content of the formulation on monthly basis and highlights the possibility that neem leaves powder can be a suitable carrier for cyanobacterial bioformulation that can be used to enhance the agriculture production (Uniyal et al., 2023). Therefore, understanding mechanism of spore production, educating farmers on cheaper alternative ways of *Azolla* application, and testing different species of *Azolla* over different agroecological zones will help in maintaining *Azolla* biomass and applying it at low cost for further environmental conservation.

Overall, earlier research laid the groundwork for understanding the agronomic, ecological, and environmental implications of using *Azolla* as a biofertilizer. While subsequent studies have built upon these findings and addressed emerging challenges and opportunities, the foundational knowledge generated by earlier research remains invaluable in guiding current and future efforts to harness *Azolla's* potential for sustainable agriculture.

However, *Azolla* has not been accepted globally by rice farmers for field use and so far, farmers are relying on increasing rates of synthetic N fertilizers instead of taking advantage of *Azolla* which will improve long-term soil fertility and health. This systematic literature review and scientific evidence could help policymakers, scientists and researchers to understand the benefits, limitations, and innovative ways of utilizing *Azolla* as a cost-effective and eco-friendly amendment in rice production. Literature indicated that the use of *Azolla* as green manure incorporated before rice transplanting or grown together with rice and left until a few days of harvest alone or in combinations with other synthetic fertilizers in the lowland rice production saved the nitrogen requirement of rice up to 60 kg N ha−1, it enhances the availability of nutrients, improves physiochemical properties of soils, minimizes soil salinity, reduces the soil pH, and minimize weed germination. However, it was observed that incorporating *Azolla* as green manure is labor-intensive, and maintaining the *Azolla* inocula and phosphorous requirement are major restrictions for farmers. Therefore, understanding mechanism of spore production, educating farmers on cheaper alternative ways of *Azolla* application, and testing different species of *Azolla* over different agro ecological zones will help in maintaining *Azolla* biomass and applying it at low cost for further environmental conservation.

Overall, earlier research laid the groundwork for understanding the agronomic, ecological, and environmental implications of using *Azolla* as a biofertilizer. While subsequent studies have built upon these findings and addressed emerging challenges and opportunities, the foundational knowledge generated by earlier research remains invaluable in guiding current and future efforts to harness *Azolla's* potential for sustainable agriculture.

Research Gaps:

Research on carrier-based *Azolla* biofertilizers has made significant progress, but there are still several gaps that warrant further investigation. Some potential research gaps include:
1. There is a need to explore and identify the most suitable carrier materials for *Azolla* biofertilizers in terms of their ability to sustain *Azolla* growth, protect *Azolla* from environmental stressors, and enhance nutrient release.

1. Research could focus on assessing the long-term stability of carrier-based *Azolla* biofertilizers under various storage conditions, including temperature, humidity, and exposure to light, to ensure their efficacy over extended periods.

2. Investigating the kinetics of nutrient release from carrier-based *Azolla* biofertilizers can provide insights into the release patterns of essential nutrients such as nitrogen, phosphorus, and potassium, and how they correlate with plant uptake dynamics.

3. Conducting extensive field trials across different agro-climatic zones and soil types to evaluate the efficacy of carrier-based *Azolla* biofertilizers in enhancing crop productivity, nutrient uptake, and soil health under real-world agricultural conditions.

4. Understanding the interactions between carrier-based *Azolla* biofertilizers and soil microbiota, including rhizosphere microbial communities, can provide insights into their impact on soil microbial diversity, activity, and nutrient cycling processes

5. Assessing the environmental implications of large-scale application of carrier-based *Azolla* biofertilizers, including their effects on soil ecology, water quality, greenhouse gas emissions, and overall sustainability compared to conventional fertilization practices.

6. Investigating the economic feasibility of producing and using carrier-based *Azolla* biofertilizers at scale, including cost-benefit analyses, market demand assessment, and farmer adoption studies to overcome potential barriers to adoption

7. Research into genetic improvement strategies for *Azolla* strains to enhance their nitrogen-fixing capacity, nutrient uptake efficiency, and stress tolerance, thereby maximizing the effectiveness of carrier-based biofertilizers.

8. Exploring synergies between carrier-based *Azolla* biofertilizers and other sustainable agricultural practices such as conservation agriculture, organic farming, and integrated nutrient management to develop holistic and environmentally friendly cropping systems.

Addressing these research gaps can contribute to the refinement and optimization of carrier-ased *Azolla* biofertilizers, thereby promoting their wider adoption as a sustainable solution or improving soil fertility and crop productivity while reducing reliance on synthetic fertilizers.

OBJECTIVES

The present study aimed to assess the comparative effect of carrier-based biofertilizers (*Azolla pinnata*) and chemical fertilizers on morphological, physiological and biochemical parameters in Jaya rice variety. This work is important to layout the response of Jaya rice variety to carrier-based biofertilizers and chemical fertilizers, by analyzing below mentioned parameters:

- Percent germination (% germination).
- Leaf turgor
- Biomass.
- Photosynthetic efficiency (Fv/Fm ratio).
- Photosynthetic pigments (Chlorophylls, Carotenoids)
- Total sugar content.
- Protein content.
- Lipids content.

Chapter - 3

MATERIALS AND METHODS

3. Material and Methods

3.1. Collection of sample

Azolla pinnata was used as biofertilizers for this study. *Azolla* was collected from rice fields in Taleigao and Pilar. After collection, the specimen was washed with running tap water to remove micro-organisms and other extraneous matter. They were stored at -20^{o}C deep freezer.

3.2. Preparation of sample and biofertilizer

Azolla pinnata was ground using a mortor and pestle. The carriers were dried at 40^{o}C for 24 h and ground with mortar and pestle. Carriers used were Multani mitti, Charcoal and Curry leaves. *Azolla pinnata* and each carrier were mixed together (1:1 ratio).

3.3. Plant material and growth conditions

Jaya rice seeds were surface sterilized with 0.2% sodium hypochlorite for 5 min and repeatedly washed with distilled water to remove all the traces of the sterilizing agent. The seeds were soaked for 3 days before sowing. The seeds were sown in plastic pots containing vermiculite. Seedlings were grown in a plant growth room with 16 h of photoperiod at the temperature of 25^{o}C ±2°C with a light intensity of ≈ 200 µmol $m^{-2}s^{-1}$.

3.4 Treatment Conditions

The biofertilizer treatment was given as follows: -

AZOLLA+CARRIER+H.S+N	AZOLLA+CARRIER+H.S-N
CONTROL	CONTROL-N
CURRY LEAVES	CURRY LEAVES-N
MULTANI MITTI	MULTANI MITTI-N
CHARCOAL	CHARCOAL-N
CHEMICAL FERTILIZER	CHEMICAL FERTILIZER-N

- H.S - Hoagland solution
- N - Nitrates

The biofertilizer application was imposed right from germination, and the plants were allowed to grow for 15 days, and watering was done every day. For analysis, plants were harvested on the 16th day.

3.5. Physiological and Biochemical analysis

3.5.1 Estimation of Relative Water Content

The relative water content (RWC) of Jaya leaf was determined according to Barrs and Weatherley (1962). The first leaf of randomly selected plants was used for analysis. The fresh weight (FW) of the leaf was immediately recorded. The leaf samples were then soaked in distilled water containing a few drops of tween 20 for 4 h at room temperature, under constant light conditions to obtain the Turgid Weight (TW). On placing the leaves in the oven at 80°C for 24 h, the Dry Weight (DW) of the leaves was recorded. On obtaining the above values of FW, TW and DW, RWC was calculated according to the following formula:

$$RWC = [(FW-DW) / (TW-DW)] \times 100$$

3.5.2. Estimation of Total Biomass

Biomass analysis was carried out according to Chen et al., (2014) using ten random plantlets harvested and weighed to obtain the shoot and root's fresh weight (FW). The samples were then dried at 80℃ for 48 h and weighed to record their dry weight (DW). The total biomass was determined using the following formula:

$$\text{Total biomass} = (FW-DW)$$

3.5.3. Pigment analysis

3.5.3. a. Extraction of photosynthetic pigments

The extraction of photosynthetic pigments was carried out according to the method described by Sharma and Hall (1996). 0.5 g of leaf tissue was homogenized in 2 mL of 100% acetone containing Butylated Hydroxytoluene (BHT) using mortar and pestle at 4℃ in dim light, followed by centrifugation at 7000-8000 rpm for 10 min at 4℃. The supernatant was used for pigment analysis.

3.5.3.b. Pigment analysis by spectrophotometry

Chlorophyll a, Chlorophyll b and Carotenoids content were measured according to Arnon (1949). 0.2 g of tissue was homogenized with 2 mL of 80% acetone containing a few crystals of BHT, making the final volume 2 mL. The extract was kept overnight for incubation at 4℃. After 24 h the homogenate was centrifuged at 7000-8000 rpm for 10 min at 4℃. The supernatant was used to measure the absorbance at 663 nm, 645 nm, and 470 nm using a UV-visible spectrophotometer (UV-2450, Shimadzu).

Chlorophyll a (Chl a) (mg/g FW) = 12.27 × A663 - 2.69 × A645

Chlorophyll b (Chl b) (mg/g FW) = 22.9 × A645 - 4.86 × A663

Carotenoids (mg/g FW) = 4.7 × A443 – 0.27 × (20.2 × A665 + 8.02 × A663)

3.5.3.c. Measurements of photosynthetic efficiency

According to Sharma et al., (1997), Photosynthetic efficiency measurements were done using a chlorophyll fluorescence monitoring system. Jaya leaves were adapted to dark for 5 min to inhibit light-dependent reactions by oxidizing PSII electron acceptor molecules. Initial fluorescence (Fo) was measured by focusing on weak light beam modulation with an intensity of 3-4 μmol m^{-2} s^{-1}. The maximum fluorescence (Fm) was measured by exposing the sample to a saturation light pulse (≈ 4000 μmol m-2s-1 for 0.06s). Variable fluorescence (Fv) was calculated as Fv = Fm – Fo and the maximum quantum yield (Fv/Fm) ratio. Actinic light of ≈ 600 μmol m-2s-1 was allowed to reach the steady fluorescence yield (Fs), followed by a far-red pulse for 5 s.

3.5.4. Determination of seed germination

Seed germination was conducted according to Mazhar et al. (2016). The seeds were surface sterilized using 0.2% sodium hypochlorite, washed with distilled water, and soaked for 2 h. The treatment was given according to those mentioned above in 2.4, and the measurements were taken after the emergence of the radicle (2 mm). The growth function and germination rate (%) were calculated using the formula:

Germination rate (%) = Number of seeds germinated / Total number of seeds

3.5.5. Total sugars content

3.5.5.a. Extraction of total sugars

The total sugars were estimated according to Dubois et al., (1956), with slight modifications. 0.5 g of leaf tissue was weighed, cut into small pieces and hydrolyzed in 5 mL of 2.5 N Hydrochloric acid by placing it in a boiling water bath for 3 h and cooled at room temperature. The solution was neutralized with sodium carbonate until the effervescence ceased. The final volume was made to 15 mL and centrifuged at 5000 rpm for 10 min. The supernatant was used to estimate total carbohydrates.

3.5.5.b. Estimation of total sugars

0.5 mL of sample was taken and the final volume was made to 1 mL using double distilled water. 1 mL of 5% phenol solution was added, followed by 5 mL of concentrated sulphuric acid by gentle mixing. The test tubes were allowed to cool down for 10 min at room temperature. Further, the tubes were placed in the hot water bath for 20 minutes at 30 ℃ and allowed to cool down at room temperature. A tube without the sample served as blank. The absorbance of the orange colour formed was recorded at 490 nm against a reagent blank. The amount of sugar in the unknown sample was read from a calibration curve using D- glucose as the standard solution (1mg/1mL).

3.5.6. Protein Content

3.5.6.a. Extraction of Proteins

Proteins were measured according to Lowry et al., (1951). 0.5g of leaf tissue was homogenized in phosphate buffer saline (pH 7.4) using mortar and pestle. The final volume was made to 10 mL, and the extract was centrifuged at 5000 rpm for 20 min at 4℃. The supernatant was used to estimate protein content.

3.5.6.b. Estimation of proteins

0.5 mL of the sample was used, making up the final volume to 1 mL using double distilled water. 5 mL of alkaline copper sulphate reagent was added, including the blank, with proper mixing. The solution was incubated at room temperature for 10 min and 0.5 mL of Folin-Ciocalteau reagent was added with appropriate mixing. The reagent mixture was further incubated for 30 min at room temperature. A tube without the sample served as blank. The absorbance of the blue-coloured complex was recorded at 750 nm. The protein content in the unknown sample was calculated from a calibration curve using Bovine serum albumin (BSA) (1mg/1mL) as standard.

3.5.7. Total lipid content

3.5.7.a. Extraction of total lipids

Total lipids were extracted according to Turnham and Northcote (1984). 2 g of leaf tissue was cut into small pieces and boiled in a sufficient amount of isopropanol for 10 mins to inhibit lipase activity. The excess isopropanol was drained, and the tissue was dried using tissue paper. Further, the samples were homogenized in Chloroform: Methanol (1:2v/v) containing 0.01% BHT and making the final volume 10 mL. The mixture was transferred into a separating funnel and was kept undisturbed for 1 h at 4°C. The supernatant was collected, and the residue was washed with Chloroform: Methanol (1:1 v/v). The same was repeated, and the supernatant was pooled. Extracted lipids were purified as described by Folch e al., (1957). The lipid extract was centrifuged for 5 min at 2000-3000 rpm to get rid of cell debris. Further, the supernatant was transferred into a separating funnel, followed by the addition of 2 mL double distilled water and 2.5 mL chloroform. The mixture was shaken for 2 min, and 2.5 mL of 0.88% potassium chloride was added. On vigorous shaking for 5 min, the extract was kept for separation for 30 min. The lower

phase contains appreciable amounts of lipids. The extract was stored at -20℃ until further use. The entire extraction and purification process was carried out in diffused light to protect lipids from photo-oxidation.

3.5.7.b. Quantitative Estimation of glycolipids

Glycolipids were determined using phenol-sulphuric acid, according to Kushawa and Kates (1981). 0.1 mL of lipid sample was used, making the final volume 2 mL using double distilled water. 1 mL of 5% phenol solution was added to the solution, followed by gentle mixing, making sure that the film of lipids at the bottom of the tube was undisturbed. To this, 5 mL of concentrated sulphuric acid was added, followed by heating in a boiling water bath for 5 min and later allowed to cool for 15 min at room temperature. The orange colour absorbance was read at 490 nm against a reagent blank. The amount of sugar in the unknown sample was read from a calibration curve using D-glucose as the standard solution (1mg/ml).

Chapter - 4

Result

4. RESULT

3.5.1. Determination of Relative Water Content (RWC)

Relative water content is a stress indicator that indicates the plant's water use efficiency, i.e., it reflects on the water uptake and transpiration (Lugojan and Ciulca, 2011). In this study, the effect of carrier-based biofertilizers on relative water content was measured in Jaya rice variety leaves (**Fig.15, 16 and Tables 1,2**). RWC was increased in plants grown in Hoagland solution containing all nutrients treated with *Azolla* (1.2%), multani (3.7%), a combination of *Azolla*+ multani (4%), curry leaves (6%), a combination of *Azolla*+curry leaves (6.6%), charcoal (14%), a combination of *Azolla*+charcoal (21.2%) and chemical fertilizer by 1.1% as compared to untreated plants. Plants treated with *Azolla*+charcaol showed slightly higher RWC than plants treated with other combinations. Individually charcoal-treated plants showed an increase in RWC compared to the other treatments except the *Azolla*+charcoal treatment.

RWC was increased in plants grown in Hoagland solution (absence of nitrates) treated with *Azolla (*3%*)*, multani (4%), a combination of *Azolla*+ multani (4.4%), curry leaves (6%), a combination of *Azolla*+curry leaves (9.4%), charcoal (12.3%), a combination of *Azolla*+charcoal (16%) and chemical fertilizer by 1.2% as compared to untreated plants. Plants grown in the *Azolla*+charcoal combination showed greater RWC than other treatments. Individually charcoal-treated plants showed an increase in RWC compared to the other treatments except the *Azolla*+charcoal treatment.

3.5.4. Determination of seed germination

The effect of fertilizers on seed germination rate was measured in control and treated plants (**Fig.13,14,17,18 and Tables 1, 2**). Carrier based biofertilizers promoted seed

germination. Seeds treated with Hoagland solution containing all nutrients with *Azolla*, multani, a combination of *Azolla*+multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed an increase in germination rate by 26%, 43%, 63%, 16%, 35%, 17%, 27% and 8%, respectively, as compared to the control plants. Seeds treated with multani and a combination of *Azolla*+multani showed a slightly higher rate in comparison to the other treatments.

Seeds treated with Hoagland solution (absence of nitrates) with *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed an increase in germination rates by 24%, 38%, 55%, 13%, 29%, 19%, 22% and 7%, respectively, as compared to control plants (absence of nitrates). Seeds treated with multani and a combination of *Azolla*+multani showed a slightly higher rate in comparison to the other treatments.

3.5.2. Determination of Biomass

Shoot and root biomass was determined from plants treated with carrier-based biofertilizers and chemical fertilizers grown in Hoagland and Hoagland solutions without nitrates (**Fig.7,8,9,10,11,12,19,20 and Tables 3,4**). Plants grown in Hoagland solution containing all nutrients treated with *Azolla* showed an increase in the shoot biomass by 33%, multani (76%), a combination of *Azolla* + multani (107%), curry leaves (20.8%), a combination of *Azolla*+curry leaves (64%), charcoal (20%), a combination of *Azolla*+charcoal (28%) and chemical fertilizer by 6% as compared to untreated plants. The root biomass of plants treated with *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer increased by 2.3%, 55%, 122%, 23%, 41%, 11%, 2%, and 13% respectively, as compared to the control plants. A combination of *Azolla*+multani showed slightly higher root and shoot biomass compared to the other treatments.

Plants grown in Hoagland solution (absence of nitrates) with *Azolla* showed an increase in the shoot biomass by 12%, multani (55%), a combination of *Azolla*+ multani (72%), curry leaves (16%), a combination of *Azolla*+curry leaves (37%), charcoal (27%), a combination of *Azolla*+charcoal (58%) and chemical fertilizer by 3% as compared to untreated plants. The root biomass of plants treated with *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer increased by 12%, 23%, 39%, 2%, 9%, 13%, 19%, and 2% respectively, as compared to the control plants. A combination of *Azolla*+multani showed higher root and shoot biomass compared to the other treatments.

3.5.3.c. Determination of Photosynthetic efficiency (Fv/Fm ratio)

The Fv/Fm ratio, which is indicative of photosynthetic efficiency, was measured in control and treated plants (**Fig.21,22 and Tables 5,6)**. The Fv/Fm ratio increased in plants grown in Hoagland solution containing all nutrients with *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer by 3%, 15%, 19%, 6.4%, 16%, 6.3%, 9% and 0.3% as compared to control plants. Plants treated with chemical fertilizer showed decrease in Fv/Fm values as compared to control plants. Plants treated with multani and a combination of *Azolla*+multani showed increase in Fv/Fm values as compared to control plants.

Plants grown in Hoagland solution (absence of nitrates) with multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, and a combination of *Azolla*+charcoal, the photosynthetic efficiency increased by 15%, 19%, 6%, 16%, 6%, and 9%, respectively, as compared to control plants (absence of nitrates). However, *Azolla* alone and chemical fertilizer treated plants showed a slight decrease in photosynthetic efficiency by 3% and 0.4% as compared to control plants (absence of nitrates).

35.3.b. Estimation of Photosynthetic pigments

Various photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoids were measured in control and treated plants (**Fig.23,24,25,26 and Tables 7,8).** Plants grown in Hoagland solution containing all nutrients with *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed an increase in Chl a concentration by 9%, 26%, 50%, 20%, 35%, 17%, 2%, and 19%, respectively, compared to control plants. A similar trend was observed in the amount of Chl b. The concentration of chlorophyll b increased in *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer by 3%, 13%, 62%, 18%, 27%, 15%, 17%, and 4%, respectively, as compared to control plants. A combination of *Azolla*+multani showed increase in chlorophyll a and chlorophyll b as compared to the other treatments. The carotenoid concentration was decreased in all the treated plants as compared to the chlorophyll a and chlorophyll b.

Plants grown in Hoagland solution (absence of nitrates) with *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed an increase in chlorophyll a concentration by 23%, 67%, 97%, 23%, 25%, 16%, 19%, and 6%, respectively, compared to control plants. A similar trend was observed in the amount of chlorophyll b. The concentration of chlorophyll b increased in *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer by 13.5%, 13.3%, 61%, 51.4%, 51.8%, 56%, 61%, and 0.4%, respectively, as compared to control plants. A

combination of *Azolla*+multani showed increase in chlorophyll a and chlorophyll b as compared to the other treatments. There was a drecrease in carotenoid concentration in all the treated plants as compared to the chlorophyll a and chlorophyll b.

3.5.5.b. Estimation of total sugar content

Total sugar content was determined in plants grown in Hoagland solution and Hoagland solution containing no nitrates along with carrier based biofertilizers and chemical fertilizer (**Fig.27,28 and Tables 9,10)**. Plants grown in Hoagland solution containing all nutrients with *Azolla,* multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed higher total sugar content by 31%, 44%, 57%, 63%, 86%, 87%, 116%, 16%, respectively. A combination of *Azolla*+charcoal showed maximum total sugar content as compared to the other treatments.

Plants grown in Hoagland solution (absence of nitrates) with *Azolla,* multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed increased total sugar content by 15%, 19%, 58%, 82%, 104%, 90%, 113%, 29% respectively. A combination of *Azolla and charcoal* showed maximum total sugar content as compared to the other treatments.

3.5.6.b. Estimation of protein content

Protein content was measured in control and treated plants in Hoagland solution and Hoagland solution containing no nitrates along with carrier-based biofertilizers and chemical fertilizer (**Fig.29,30 and Table 9,10).** The plants grown in Hoagland solution containing all nutrients treated with *Azolla,* multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed an increase in protein content by 20%, 23%, 37%, 41%, 62%, 39%, 43% and 17% as compared to control plants. Plants treated with a combination of Azolla+curry leaves showed higher protein content as compared to the other treatments.

In plants grown in Hoagland solution (absence of nitrates) treated with *Azolla,* multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed an increase in protein content by 23%, 38%, 49%, 60%, 82%, 54%, 64% and 29% as compared to control plants. Plants treated with a combination of Azolla+curry leaves showed increased protein content as compared to the other treatments.

3.5.7.b. Estimation of glycolipid content

Glycolipid content was measured in control and treated plants in Hoagland solution and Hoagland solution containing no nitrates along with carrier based biofertilizers and chemical fertilizer **(Fig.31,32 and Table 11,12)**. Plants grown in Hoagland solution containing all nutrients treated with *Azolla+charcoal* showed higher glycolipid content by 197%. In contrast, plants treated with *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, and chemical fertilizer showed lesser content by 95%, 110%, 133%, 142%, 170%, 151% and 17%, respectively, as compared to control plants.

In plants grown in Hoagland solution (absence of nitrates) treated with *Azolla*, multani, a combination of *Azolla*+ multani, curry leaves, a combination of *Azolla*+curry leaves, charcoal, a combination of *Azolla*+charcoal and chemical fertilizer showed an increase in glycolipid by 77%, 86%, and 91%, 114%, 120%, 171%, 191%, and 1% respectively, as compared to control plants (absence of nitrates). The glycolipid content in plants treated with a combination of *Azolla*+charcoal increased as compared to the other treatments.

Chapter - 5

DISCUSSION

5. DISCUSSION

Our results showed that carrier-based biofertilizers increased biomass and RWC as compared to control and plants treated with chemical fertilizers **(Fig.15,16 and Tables 1,2)**. This increase in biomass may be due to the increased nitrogen uptake being responsible for higher yield of crops (Hirel et al., 2011) and the increase in nitrogen content in plants was due to the sustained availability of nitrogen because of the nitrogen-fixing ability of the biofertilizers (Razie and Anas, 2008). We observed that treatment with the combination of biofertilizers with different carriers has a beneficial effect on Jaya's growth compared to the other treatments. Jama et al., (2023) reported manure treatment resulted in the highest spinach yields, and the *Azolla* treatment applied at the same N rate as the manure yielded the same as the manure treatment on the peat soil and had the highest leaf and branch numbers. *Azolla* showed promise as a biofertilizer for dryland vegetable crops. Sghir et al., (2014) reported that the application of different biofertilizers benefited plant growth mainly leaf number, shoot height, root length, leaf area, and total dry biomass production. It was observed by Khair et al., (2021) that *Azolla* could be used as an alternative fertilizer on rice fields because the soil treated with *Azolla* shows a comparable result with soil treated with inorganic fertilizer without *Azolla* on the total yield. The viability of cyanobacterial cells was studied by measuring the chlorophyll content of the formulation monthly by Uniyal et al., (2023). The present investigation highlights the possibility that neem leaf powder can be a suitable carrier for cyanobacterial bioformulation that can be used to enhance agriculture production.

Our results showed that the application of carrier-based biofertilizers promoted the seed germination in comparison to the control and chemical fertilizer treatment (**Fig.13,14,17,18 and Tables 1,2**). Gupta et al., (2022) reported that seedling germination

was better with the bioformulation made with charcoal and tea leaf powder. As a result, it can be used to alleviate abiotic stresses in a cost-effective and environmentally friendly manner. Chilton et al., (2018) reported positive effects of seeds bio-primed with cyanobacteria on germination and seedling growth of two species, *Senna notabilis and Acacia hilliana*, respectively. Importantly, no significant negative effects of cyanobacteria were found for any of the species studied. The potential benefits of applying indigenous bacteria via bio-priming seeds would not inhibit plant establishment and indeed may be beneficial for some species used in dryland restoration. Bákonyi et al., (2013) reported that the seed-and-filter paper treatments with biofertilizer significantly increased by more than 20% the numbers of the germinated seeds in comparison to the untreated control. The dry weight of the shoot and root was higher by more than 7% than the control in the case of treatments with biofertilizer. The applied biofertilizer treatments stimulated the germination and growth of maize because of the excreting of phytohormones and enhancing the nutrient mobilization from the seed.

We reported an increase in the photosynthetic efficiency and photosynthetic pigments in plants treated with carrier-based biofertilizers (**Fig.21,22,23,24,25,26 and Tables 5,6,7,8**). This positive effect of biofertilizers on the photosynthetic pigments may be due to the improvement of chlorophyll formation, and photochemical efficiency of leaves. The yield of a crop plant is related to the photosynthetic capacity of the plant. The alleviated effect of biofertilizers on the growth and chlorophyll content of plants is reported by AlAghabary et al., (2004). The high chlorophyll content indicates a better and healthy root system that functions properly leading to empowering the plants to conquer better performance in water and nutrient uptake (Thakur et al., 2010). Medani et al., (2000) recorded a significant increase in chlorophyll a, chlorophyll b and carotenoids of sugar

beet leaves due to nitrogen application with a mixture of *Azospirillum sp., Azotobacter sp.* and *Bacillus sp.* inoculation. These findings may prove that the beneficial effect of inoculation with these species was mainly in improving the fixation of atmospheric N, increasing the release of P in the soil which is reflected in increasing P activity and the growth-promoting substances produced by them.

A positive correlation between leaf nitrogen fertilization and rate of the chlorophyll content is well documented for several plant species and has been investigated for rapid nitrogen determination for most major crops including corn, rice, and wheat (Houles et al., 2007). The regulation of metabolic and developmental processes by photosynthetic pigments often depends on nitrogen supply, therefore, the assay of wheat photosynthetic pigment contents may serve to optimize wheat fertilization technologies (Tranavičienė et al., 2008). The results of the present study are in agreement with those reported by Ramakrishnan and Selvakumar (2012) who found that *Azotobacter-treated* plants had the highest chlorophyll and protein contents. Similarly, biofertilizers significantly improved chlorophyll concentration in chilli (Selvakumar and Thamizhiniyan, 2011) and in black gram (Selvakumar et al., 2012). Individual biofertilizer treatment positively affected the chlorophyll content as compared to its combination, allowing greater photosynthetic efficiency. This indicated that biofertilizer treatment improved the plant tolerance to less favorable edaphic conditions (absence of nitrates) (Ordog et al., 2021). Furthermore, the activity of *Trichoderma* inoculation at root rhizosphere to trigger the synthesis of hormones that have a significant role in leaf chlorophyll content and photosynthetic improvement has also been reported (Guler et al., 2016; Harman, 2011).

We also reported an increase in sugar content, protein content, and glycolipid content in Jaya rice variety due to the treatment with carrier-based biofertilizers **(Fig.27,28,29,30,31,32 and Tables 9,10,11,12)**. Qiu et al., (2022) reported that biofertilizer at the 60% N-rate generated promising results with significantly higher biomass and sugar yield than the no-N control, which matched the 100% mineral N treatment. A shift in microbial diversity and composition accompanied this yield difference. Correlation analysis confirmed that shifts in microbial communities were strongly linked to soil mineral N levels, as well as crop productivity and yield. Collectively, the results confirm that biofertilizers have quantifiable effects on soil microbial communities in a crop system setting, which underscores the opportunities for biofertilizers to promote N use efficiency and the circular N economy. Patil et al., (2010) reported a combination treatment of biofertilizer and chemical fertilizer increased chlorophyll, growth, carbohydrates, and protein content compared to the control. Therefore, it is necessary to evaluate and develop a balanced fertilization strategy that combines the use of chemical, organic, or biofertilizers. The effect of biofertilizers on carbohydrate biosynthesis, especially soluble sugars, is considered to be the principal organic osmotica in several glycophytes subjected to saline conditions (Hassanein, 2004). Biofertilizers significantly improved sugar concentration in chili plants (Selvakumar and Thamizhiniyan, 2011) and in black gram plants (Selvakumar et al., 2012).

Our results showed that plants treated with carrier-based biofertilizers compared with other treatments showed increased protein content. Patil et al., (2014) also reported that height, leaf area, and protein content were at maximum under vermicompost and *Rhizobium* treatment as compared to inorganic treatments and control. However, the average content of reducing sugars decreased at all treatments and also when compared to

control in *Trigonella foenum-graecum L.* Dekhane et al., (2011) reported that *Rhizobium* significantly increased protein and N, P content as well as uptake of N and P by grain and stover. The 100 % RDF recorded the highest protein content as well as content and uptake of N and P by grain and stover but was par with 75 % RDF. Significant improvement in available N and P status in soil was also reported due to *Rhizobium* inoculation. Stephen et al., (2010) stated that soybean inoculated with *Bacillus pumilus* had higher seed protein content. Rahmani et al., (2008) reported that nitrogen is the most important element in protein synthesis and its increase in optimum conditions increased the amount of protein. In addition, Shehata and Khawas (2003) showed that the application of biological fertilizer on sunflower increased seed protein. The increase in the total protein content could be attributed to the growth hormones produced by microbes (Khalil and Ismael 2010), direct stimulation of the synthesis of protein (Stino et al., 2009), providing plants with essential nutrient elements required for protein formation (Hayat 2007).

We reported that applied carrier-based biofertilizer treatments caused a marked increase in glycolipid content of the Jaya rice variety. Zarei et al., (2012) also observed that biofertilizer treatment caused the highest increase in total unsaturated fatty acid of three flax cultivars. (Sanavy et al., 2011) reported that biofertilizer improved plant productivity and quality in sunflower seeds. The application of biofertilizers decreased the saturated fatty acids (palmitic and stearic) and increased unsaturated fatty acids (linoleic acid and oleic acid) and oil content, compared with untreated plants I0. The highest linolenic acid (53.28%) and oleic acid (40.65%) were observed in F3 and F1 treatments respectively. Darzi et al., (2009) stated that using organic and biofertilizers leads to a change in the composition of essential oil in the different plant species.

The current result showed that the carrier-based biofertilizer inoculated plants had significantly higher biomass, photosynthetic pigments, photosynthetic efficiency, proteins, sugars, and glycolipids even in the absence of inorganic N application and hence inoculated plants have been reported to benefit Jaya rice variety with better photosynthetic rate, stomatal conductance, specific relative chlorophyll contents and crop yield. The carrier-based biofertilizers showed great effectiveness on nutrient uptake and increased the availability of nutrients in the soil, especially total N, nitrate-N, ammonium-N, and available P and K.

Biofertilizer treatment with carrier material multani mitti and charcoal increased nitrogen uptake and enhanced the yield of Jaya rice variety with better physiological and biochemical attributes even in the absence of inorganic nitrogen fertilizer application. The results indicated that use of carrier based biofertilizers would be a great substitute of the inorganic fertilizer and can be used to eco-friendly yield boost up with low input costs reducing the continuous use of chemical inorganic fertilizer. However, the patterns observed in the results indicated that the application of carrier based biofertilizers might be crucially important in small to medium input structures in cultivation and the outcomes can be practiced to provide a better instruction for root level farmers on the use of biofertilizers.

Chapter - 6

CONCLUSION

6.CONCLUSION

Our study suggests that all the applied carrier based biofertilizers and chemical fertilizers caused changes in the Jaya rice variety .plant's morphological, physiological and biochemical parameters. Plants treated with *Azolla*+charcaol showed higher RWC than plants treated with other combinations in Hoagland solution containing all nutrients and in absence of nitrates. Individually charcoal treated plants showed an increase in RWC compared to the other treatments except *Azolla*+charcoal treatment in Hoagland solution containing all nutrients and in absence of nitrates. The shoot biomass and root biomass increased in plants grown in Hoagland solution containing all nutrients and in absence of nitrates treated with a combination of *Azolla*+multani. The seed germination rate increased in seeds treated with multani and a combination of *Azolla*+multani in Hoagland solution containing all nutrients and in absence of nitrates in comparison to the other treatments. Plants treated with chemical fertilizer showed the lowest Fv/Fm values as compared to control plants in Hoagland solution contaning all nutrients. Plants treated with multani and a combination of *Azolla*+multani showed the highest Fv/Fm values as compared to control plants in Hoagland solution containing all nutrients. The photosynthetic efficiency decreased in plants grown in Hoagland solution in absence of nitrates with *Azolla* and chemical fertilizer compared to control plants. Plants grown in Hoagland solution containing all nutrients with a combination of *Azolla*+multani showed an increase in Chlorophyll a and Chlorophyll b concentration compared to control plants. Also, the carotenoid concentration was reduced in all treated plants as compared to control. Plants treated with a combination of *Azolla*+multani showed an increase in Chlorophyll a and Chlorophyll b compared to control in nitrate absence; however, the carotenoids concentration was reduced. Plants grown in Hoagland solution containing all

nutrients and treated with a combination of *Azolla*+charcoal showed an increase in total sugar content. Plants grown in Hoagland solution (absence of nitrates) treated with a combination of *Azolla*+charcoal showed an increase in total sugar content compared to its control. Plants treated with *Azolla*+curry leaves showed higher protein content than control plants containing complete nutrients. Plants treated with *Azolla*+curry leaves showed more protein content than control plants in Hoagland solution containing no nitrates. The glycolipid content in plants treated with *Azolla*+charcoal was high compared to all the treatments grown in Hogland solution containing all nutrients. Whereas in plants treated with a combination of *Azolla*+charcoal the glycolipid content drastically increased compared to all the treatments grown in Hoagland solution (absence of nitrates). Carrier based Biofertilizer treatments provided a significant increase in nitrogen uptake and enhanced the yield of Jaya rice variety plants with better physiological and biochemical attributes even in the absence of inorganic nitrogen fertilizer application. The results indicated that the use of carrier based biofertilizer would be a great substitute for inorganic fertilizer and can be used for eco-friendly yield boost with low input costs reducing the continuous use of inorganic chemical fertilizer. However, the patterns observed in the results indicated that the application of carrier based biofertilizers might be crucially important in small to medium input structures in cultivation, and the outcomes can be practised to provide better instruction for root-level farmers on the use of biofertilizers.

Chapter - 7

REFERENCES

7.REFERENCES

Abbasniayzare, S. K., Sedaghathoor, S., & Dahkaei, M. N. P. (2012). Effect of biofertilizer application on growth parameters of Spathiphyllum illusion. *Amer-Eurasian J Agric Environ Sci*, *12*(5), 669-673.

Abou El-Yazied, A., El-Gizawy, A. M., Ragab, M. I., & Hamed, E. S. (2012). Effect of seaweed extract and compost treatments on growth, yield and quality of snap bean. *Journal of American Science*, *8*(6), 1-20.

Adam MS (1999) The promotive effect of the cyanobacterium Nostoc muscorum on the growth of some crop plants. Acta Microbiol Pol 48:163–171.

Aghabary, A. L., Zhu, K., & Shi, Q. H. (2004). Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato under salt stress. *J. Plant Nutr*, *27*, 2101-2115.

Aghabary, A. L., Zhu, K., & Shi, Q. H. (2004). Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato under salt stress. *J. Plant Nutr*, *27*, 2101-2115.

Ahmed, M. A., Ahmed, A. G., Mohamed, M. H., & Tawfik, M. M. (2011). Integrated effect of organic and biofertilizers on wheat productivity in new reclaimed sandy soil. *Research Journal of Agriculture and Biological Sciences*, *7*(1), 105-114.

Akbari, P., Ghalavand, A., Sanavy, A., & Alikhani, M. A. (2011). The effect of biofertilizers, nitrogen fertilizer and farmyard manure on grain yield and seed quality of sunflower (Helianthus annus L.). International Journal of Agricultural Technology, 7(1), 173–184.

Alam, S., & Seth, R. K. (2014). Comparative study on Effect of Chemical and Biofertilizer on Growth, Development and Yield Production of Paddy crop (Oryza sativa). *International Journal of Science and Research*, *3*(9), 411-414.

Allouzi, M. M. A., Allouzi, S. M. A., Keng, Z. X., Supramaniam, C. V., Singh, A., & Chong, S. (2022). Liquid biofertilizers as a sustainable solution for agriculture. *Heliyon*, *8*(12), e12609.

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in microbiology*, *8*, 971.

Anand, K. U. M. A. R., Kumari, B. A. B. Y., & Mallick, M. A. (2016). Phosphate solubilizing microbes: an effective and alternative approach as biofertilizers. *Int J Pharm Sci*, *8*(2), 37-40.

Arafa, A. A., Farouk, S., & Mohamed, H. S. (2011). Effect of potassium fertilizer, biostimulants and effective micro-organisms as well as their interactions on potato growth, photosynthetic pigments and stem anatomy. *Journal of Plant Production*, *2*(8), 1017-1035.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology*, *24*(1), 1.

Atilgan, A. T. I. L. G. A. N., Coskan, A. L. İ., Saltuk, B., & Erkan, M. (2007). The level of chemical and organic fertilizer usage and potential environmental impacts in greenhouses in Antalya region. *Ekoloji*, *16*(62).

Bákonyi, N., Bott, S., Szabó, A., Jakab, A., Tóth, B., & Makleit, P. (2013). Using biofertilizer to improve seed germination and early development of maize. Polish Journal of Environmental Studies, 22(6).

Bargaz, A., Lyamlouli, K., Chtouki, M., Zeroual, Y., & Dhiba, D. (2018). Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. *Frontiers in microbiology*, *9*, 1606.

Barrs, H. D., & Weatherley, P. E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian journal of biological sciences*, *15*(3), 413-428.

Bashan, Y., & Holguin, G. (1997). Azospirillum–plant relationships: environmental and physiological advances (1990–1996). *Canadian Journal of Microbiology*, *43*(2), 103- 121.

Castro, P. H., Lilay, G. H., & Assunção, A. G. (2018). Regulation of micronutrient homeostasis and deficiency response in plants. In *Plant Micronutrient Use Efficiency* (pp. 1-15). Academic Press.

Chen, Z. L., Li, X. M., & Zhang, L. H. (2014). Effect of salicylic acid pretreatment on drought stress responses of zoysiagrass (*Zoysia japonica*). Russian Journal of Plant Physiology, 61(5), 619-625.

Choudhary, K., More, S. J., & Bhanderi, D. R. (2015). Impact of biofertilizers and chemical fertilizers on growth and yield of okra (Abelmoschus esculentus L. Moench). *The ecoscan*, *9*(1&2), 67-70.

De Freitas, J. R., Banerjee, M. R., & Germida, J. J. (1997). Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica

Debnath, S., Rawat, D., Mukherjee, A. K., Adhikary, S., & Kundu, R. (2019). Applications and constraints of plant beneficial micro-organisms in agriculture. In *Biostimulants in plant science*. IntechOpen.

Dekhane, S. S., Khafi, H. R., Raj, A. D., & Parmar, R. M. (2011). Effect of Bio Fertilizer and Fertility Levels on Yield, Protein Content and Nutrient Uptake of Cowpea [Vigna unguiculata (L.) Walp.]. Legume Research, 34(1), 51–54.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, *28*(3), 350-356.

Ferreira, C. M., Soares, H. M., & Soares, E. V. (2019). Promising bacterial genera for agricultural practices: An insight on plant growth-promoting properties and microbial safety aspects. *Science of the total environment*, *682*, 779-799.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J biol Chem*, *226*(1), 497- 509.

Garcha, S., & Maan, P. K. (2017). Biological nitrogen fixation in cereals crops: a bacterial perspective. In *Advances in Soil Microbiology: Recent Trends and Future Prospects* (pp. 127-151). Springer, Singapore.

Germida, J. J., & Janzen, H. H. (1993). Factors affecting the oxidation of elemental sulfur in soils. *Fertilizer research*, *35*(1), 101-114.

Glick, B. R. (2015). *Beneficial plant-bacterial interactions* (pp. 139-180). Heidelberg: Springer.

Goel, A. K., Laura, R. D., Pathak, D. V., & Goel, A. (1999). Use of biofertilizers: Potential, constraints and future strategies-a review. *International Journal of Tropical Agriculture*, *17*(1/4), 1-18.

Grayston, S. J., Nevell, W., & Wainwright, M. (1986). Sulphur oxidation by fungi. *Transactions of the British Mycological Society*, *87*(2), 193-198.

Gupta, A. B., & Lata, K. (1964). Effect of algal growth hormones on the germination of paddy seeds. *Hydrobiologia*, *24*(1), 430-434.

Haneef, I., Faizan, S., Perveen, R., & Kausar, S. (2014). Impact of bio-fertilizers and different levels of cadmium on the growth, biochemical contents and lipid peroxidation of Plantago ovata Forsk. *Saudi journal of biological sciences*, *21*(4), 305- 310.

Haneef, I., Faizan, S., Perveen, R., & Kausar, S. (2014). Impact of bio-fertilizers and different levels of cadmium on the growth, biochemical contents and lipid peroxidation of Plantago ovata Forsk. *Saudi journal of biological sciences*, *21*(4), 305- 310.

Harman, G. E. (2006). Overview of Mechanisms and Uses of Trichoderma spp. *Phytopathology*, *96*(2), 190-194.

Hassanein, M. S., Ahmed, A. G., & Zaki, N. M. (2018). Effect of nitrogen fertilizer and bio-fertilizer on yield and yield components of two wheat cultivars under sandy soil. *Middle East J. Appl. Sci*, *8*(1), 37-42.

Hirel, B., Martin, A., Tercé-Laforgue, T., Gonzalez-Moro, M. B., & Estavillo, J. M. (2005). Physiology of maize I: A comprehensive and integrated view of nitrogen metabolism in a C4 plant. *Physiologia Plantarum*, *124*(2), 167-177.

Hirel, B., Tétu, T., Lea, P. J., & Dubois, F. (2011). Improving nitrogen use efficiency in crops for sustainable agriculture. *Sustainability*, *3*(9), 1452-1485.

Hirel, B., Tétu, T., Lea, P. J., & Dubois, F. (2011). Improving nitrogen use efficiency in crops for sustainable agriculture. *Sustainability*, *3*(9), 1452-1485.

Horwath, W., & Paul, E. A. (2015). Carbon cycling: the dynamics and formation of organic matter. *Soil microbiology, ecology and biochemistry*, *4*, 339-82.

Houles, V., Guerif, M., & Mary, B. (2007). Elaboration of a nitrogen nutrition indicator for winter wheat based on leaf area index and chlorophyll content for making nitrogen recommendations. *European Journal of Agronomy*, *27*(1), 1-11.

Jaenudin, A., Surawinata, T., & Maryuliyanna, M. (2016). Pengaruh Kombinasi Kompos dan NPK (16:16:16) terhadap Pertumbuhan dan Hasil Tanaman Bunga Matahari (Hellianthus annuus L.). *Agroswagati : Jurnal Agronomi/Agrowasgati : Jurnal Agronomi*, *4*(2).

Jama, A., Widiastuti, D. P., Gafur, S., & Davis, J. G. (2023). Azolla Biofertilizer is an effective replacement for urea fertilizer in vegetable crops. *Sustainability*, *15*(7), 6045.

Khair, M. I., Azman, E. A., Ismail, R., & Rani, M. N. (2021). Biofertilizer: *Azolla Pinnata* in-Combination with Inorganic Fertilizer on Growth and Yield of Rice.

Kulasooriya, S. A., Hirimburegama, W. K., & Abeysekera, S. W. (1984). *Azolla* as a biofertilizer for rice in Sri Lanka. Practical Application of Azolla for Rice Production, 188–201.

Kuraganti, G., Sujatha, E., & Pallaval, V. B. (2020). Cyanobacteria as biofertilizers: current research, commercial aspects, and future challenges. In *Microorganisms for sustainability* (pp. 259–278).

Lu, H., Wu, Z., Wang, W., Xu, X., & Liu, X. (2020). Rs-198 Liquid Biofertilizers Affect Microbial Community Diversity and Enzyme Activities and Promote Vitis vinifera L. Growth. *BioMed research international*, *2020*.

Marzouk, S. H., Tindwa, H. J., Amuri, N. A., & Semoka, J. M. (2023). An overview of underutilized benefits derived from *Azolla* as a promising biofertilizer in lowland rice production. Heliyon, 9(1).

Muñoz-Rojas, M., Chilton, A. M., Liyanage, G. S., Erickson, T. E., Merritt, D. J., Neilan, B. A., & Ooi, M. K. J. (2018). Effects of indigenous soil cyanobacteria on seed germination and seedling growth of arid species used in restoration. *Plant and Soil*, *429*(1–2), 91–100.

Qiu, Z., Paungfoo-Lonhienne, C., Ye, J., Garcia, A. G., Petersen, I., Di Bella, L., Hobbs, R. J., Ibanez, M., Heenan, M., Wang, W., Reeves, S., & Schmidt, S. (2022). Biofertilizers can enhance nitrogen use efficiency of sugarcane. Environmental Microbiology, 24(8), 3655–3671.

Safdar, H., Jamil, M., Hussain, A., Albalawi, B. F. A., Ditta, A., Dar, A., Aimen, A.,Ahmad, H. T., Nazir, Q., & Ahmad, M. (2022). The Effect of Different Carrier

Materials on the Growth and Yield of Spinach under Pot and Field Experimental Conditions. *Sustainability*, *14*(19), 12255.

Selection of carrier materials to formulate bioinoculant package for promoting seed germination. (2022). *Letters in Applied NanoBioScience*, *12*(3), 65.

Selvakumar, G., & Thamizhiniyan, P. (2011). The effect of the arbuscular mycorrhizal (AM) fungus Glomus intraradices on the growth and yield of chilli (Capsicum annuum L.) under salinity stress. *World Appl Sci J*, *14*(8), 1209-1214.

Thapa, P., & Poudel, K. (2021). Azolla: Potential biofertilizer for increasing rice productivity, and government policy for implementation. Journal of wastes and biomass management, 3(2), 62–68.

Tranavičienė, T., Urbonavičiūtė, A., Samuolienė, G., Duchovskis, P., Vagusevičienė, I., & Sliesaravičius, A. (2008). The effect of differential nitrogen fertilization on photosynthetic pigment and carbohydrate contents in the two winter wheat varieties. *Agronomy Research*, *6*(2), 555-561.

Table 1. Effect of carrier based biofertilizer treatment on relative water content and percent germination of Jaya rice variety. (+N): presence of NO_3; where ± indicates standard deviation, n=3.

TREATMENT	RWC	% Change	SEED GERMINATION (%)	% Change
CONTROL + N	81.112 ± 0.000707	0	56.5 ± 2.12132	0
***AZOLLA* + N**	82.124± 0.001414	1.2	71.5 ± 2.12132	26.5
MULTANI + N	84.158± 0.002121	3.7	81 ± 1.41421	43.4
***AZOLLA*+ MULTANI +N**	84.435± 0.002828	4.0	92.5 ± 3.53553	63.7
CURRY LEAVES + N	86.046± 0.002878	6.0	66 ± 1.41421	16.8
***AZOLLA*+ CURRY LEAVES+N**	86.538± 0.002786	6.6	76.5 ± 2.12132	35.4
CHARCOAL + N	92.957± 0.002121	14.6	46.5 ± 2.12132	-17.7
***AZOLLA*+ CHARCOAL + N**	98.324± 0.000707	21.2	72 ± 2.82843	27.4
CHEMICAL + N	80.144± 0.000707	-1.1	61.5 ± 2.12132	8.8

Table 2. Effect of carrier based biofertilizer treatment on relative water content and percent germination of Jaya rice variety. (-N): absence of NO_3; where ± indicates standard deviation, n=3.

TREATMENT	RWC	% Change	SEED GERMINATION (%)	% Change
CONTROL - N	81.064 ± 0.0007071	0	60 ± 2.828427125	0
***AZOLLA* - N**	84.25 ± 0.0042426	3.9	74.5 ± 2.1213203	24.2
MULTANI - N	84.534 ± 0.0070710	4.2	83 ± 1.414213562	38.3
***AZOLLA*+ MULTANI - N**	84.678 ± 0.0028284	4.4	93 ± 2.828427125	55.0
CURRY LEAVES - N	86.6 ± 0.00070710	6.8	68 ± 1.414213562	13.3
***AZOLLA*+ CURRY LEAVES - N**	88.686 ± 0.0021213	9.4	77.5 ± 0.707106781	29.2
CHARCOAL - N	91.051 ± 0.0007071	12.3	48.5 ± 0.707106781	-19.2
***AZOLLA*+ CHARCOAL - N**	94.077 ± 0.0007071	16.0	73.5 ± 0.707106781	22.5
CHEMICAL - N	82.114 ± 0.0007071	1.2	64.5 ± 0.707106781	7.5

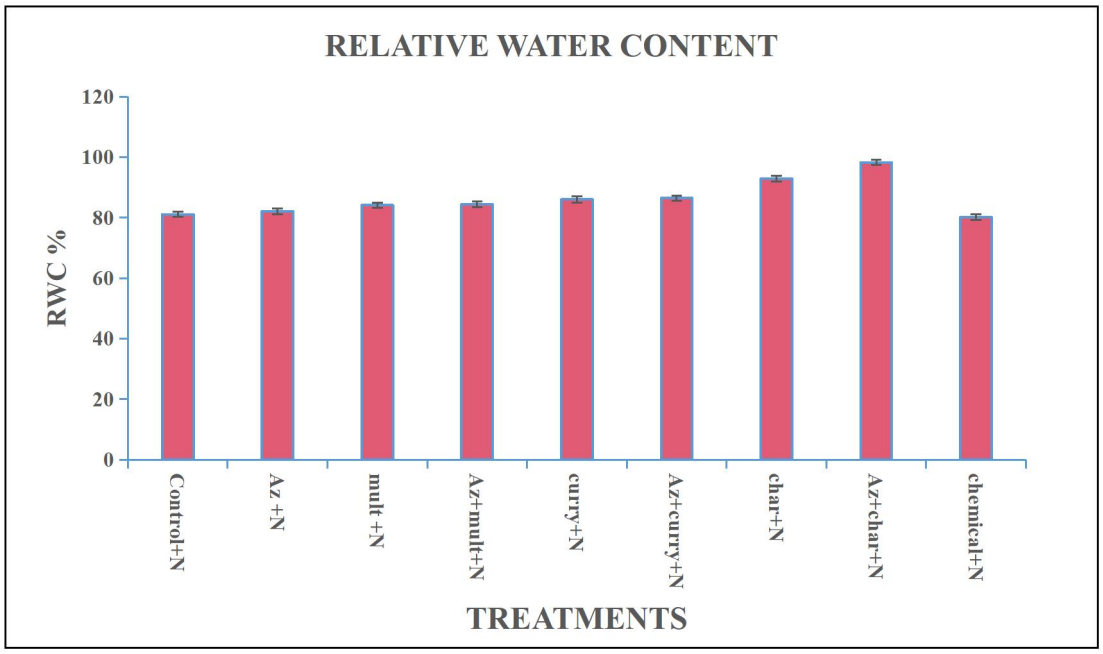

Fig.15. Effect of carrier based biofertilizer on RWC in Jaya rice variety. Az+N: *Azolla* (+NO_3), mult+N: Multani (+NO_3), Az+mult+N: *Azolla*+multani (+NO_3), curry+N: Curry leaves (+NO_3), Az+curry+N: *Azolla*+curry leaves (+NO_3), char+N: charcoal (+NO_3), Az+char+N:*Azolla*+charcoal (+NO_3), chemical+N: Chemical (+NO_3), control: Control (+NO_3). (+N): presence of NO_3; where ± indicates standard deviation, n=3.

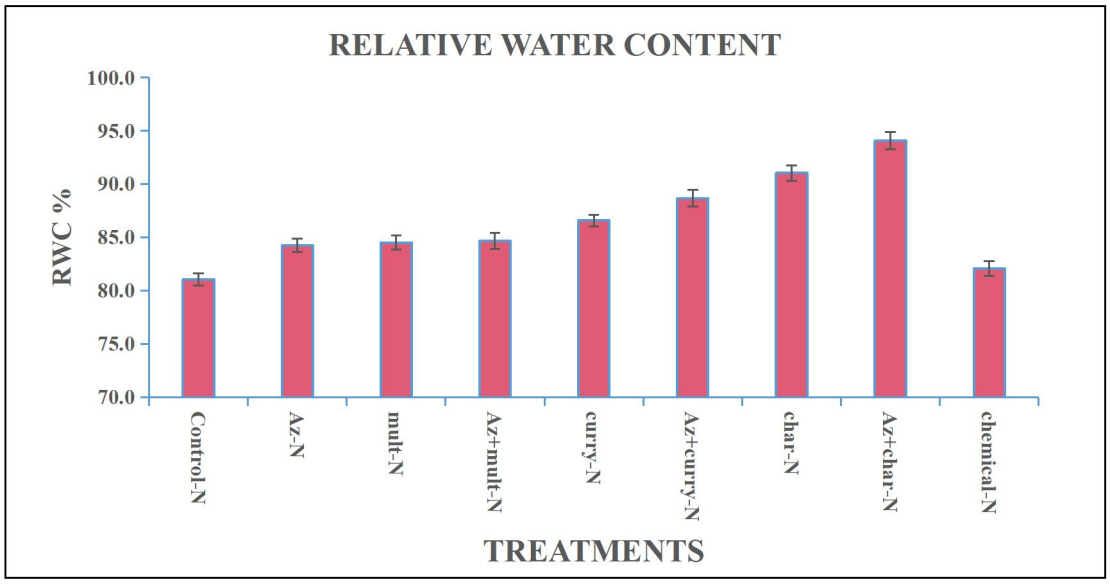

Fig.16. Effect of carrier based biofertilizer on RWC in Jaya rice variety. Az-N: *Azolla* (-NO_3), mult-N:Multani (-NO_3), Az+mult-N: *Azolla*+multani (-NO_3), curry-N: Curry leaves (-NO_3), Az+curry-N: *Azolla*+curry leaves(-NO_3), char+N: charcoal(-NO_3), Az+char-N: *Azolla*+charcoal(-NO_3), chemical-N: Chemical(-NO_3), control-N: Control(-NO_3). (-N): absence of NO_3; where ± indicates standard deviation, n=3.

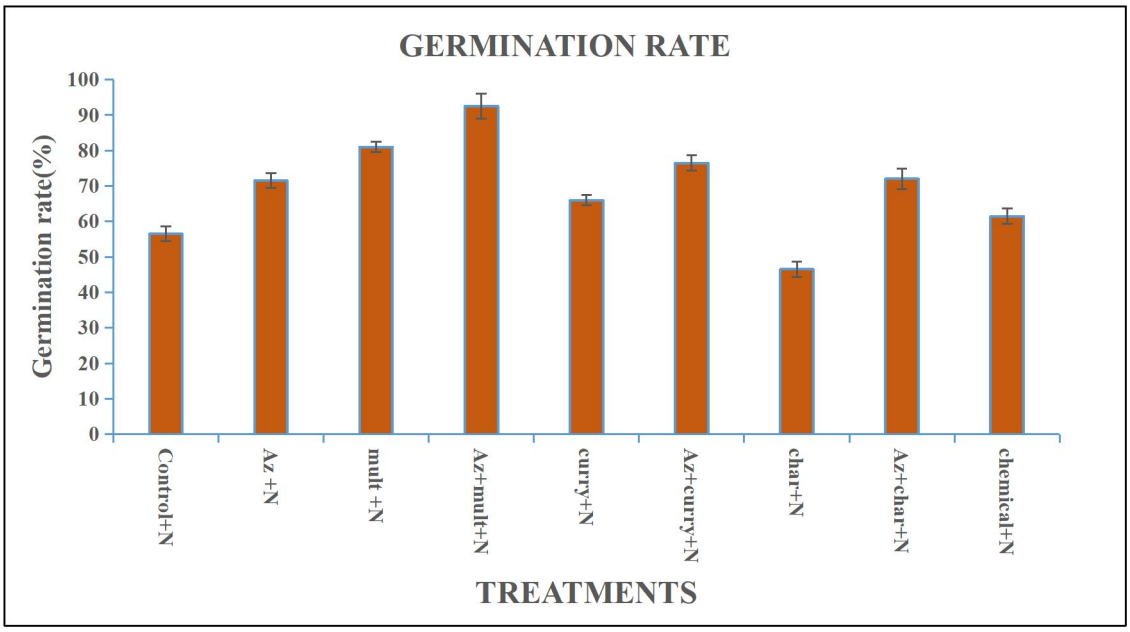

Fig.17. Effect of carrier based biofertilizer on percent Germination in Jaya rice variety. Az+N: *Azolla* (+NO_3), mult+N: Multani (+NO_3), Az+mult+N: *Azolla*+multani (+NO_3), curry+N: Curry leaves (+NO_3), Az+curry+N: *Azolla*+curry leaves (+NO_3), char+N: charcoal (+NO_3), Az+char+N: *Azolla*+charcoal (+NO_3), chemical+N: Chemical (+NO_3), control+N: Control (+NO_3). (+N): presence of NO_3; where ± indicates standard deviation, n=3.

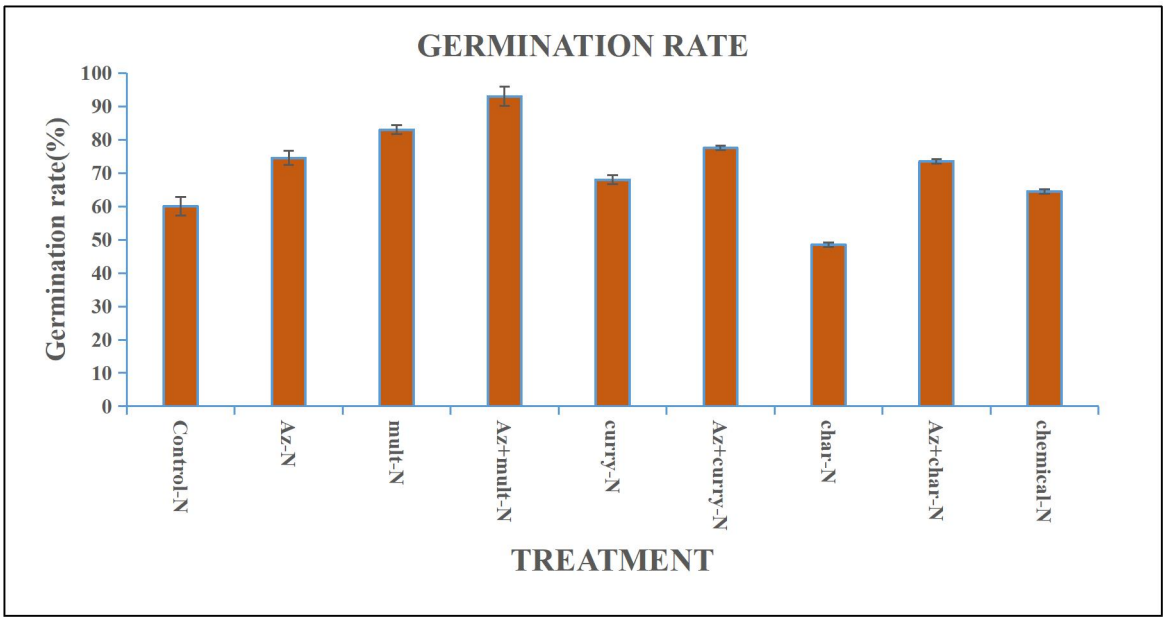

Fig.18. Effect of carrier based biofertilizer on percent Germination in Jaya rice variety. Az-N: *Azolla*(-NO_3), mult-N:Multani(-NO_3), Az+mult-N: *Azolla*+multani(-NO_3), curry+N: Curry leaves(-NO_3), Az+curry-N: *Azolla*+curry leaves(-NO_3), char+N: charcoal(-NO_3), Az+char-N: *Azolla*+charcoal(-NO_3), chemical-N: Chemical(-NO_3), control-N: Control(-NO_3). (-N): absence of NO_3; where ± indicates standard deviation, n=3.

Table 3. Effect of carrier based biofertilizer treatment on Biomass of Jaya rice variety. (+N): presence of NO_3 ; where ± indicates standard deviation, n=3.

TREATMENT	SHOOT BIOMASS	% Change	ROOT BIOMASS	% Change
CONTROL + N	0.0221 ± 0.0037	0	0.0197 ± 0.0093	0
***AZOLLA* + N**	0.0295 ± 0.0040	33.4	0.0202 ± 0.0009	2.3
MULTANI + N	0.0390 ± 0.0004	76.4	0.0307 ± 0.0024	55.8
***AZOLLA*+ MULTANI +N**	0.0458 ± 0.0073	107.3	0.0438 ± 0.0364	122.3
CURRY LEAVES + N	0.0267 ± 0.0007	20.8	0.0243 ± 0.0070	23.3
***AZOLLA*+ CURRY LEAVES+N**	0.0364 ± 0.0013	64.7	0.0278 ± 0.0087	41.1
CHARCOAL + N	0.0266 ± 0.0076	20.3	0.0174 ± 0.0269	-11.6
***AZOLLA*+ CHARCOAL + N**	0.0283 ± 0.0122	28	0.0201 ± 0.0346	2
CHEMICAL + N	0.0235 ± 0.0460	6.3	0.0224 ± 0.0024	13.7

Table.4. Effect of carrier based biofertilizer treatment on Biomass of Jaya rice variety. (-N): absence of NO_3 ; where ± indicates standard deviation, n=3.

TREATMENT	SHOOT BIOMASS	% Change	ROOT BIOMASS	% Change
CONTROL - N	0.0201± 0.0020	0	0.0198± 0.0009	0
***AZOLLA* - N**	0.0226 ± 0.0061	12.4	0.0173 ± 0.0101	-12.6
MULTANI - N	0.0313± 0.0091	55.7	0.0245 ± 0.0032	23.7
***AZOLLA*+ MULTANI - N**	0.0347 ± 0.0094	72.6	0.0277 ± 0.0045	39.8
CURRY LEAVES - N	0.0234 ± 0.0006	16.4	0.0193 ± 0.0024	-2.4
***AZOLLA*+ CURRY LEAVES - N**	0.0277 ± 0.0001	37.7	0.0216 ± 0.0043	9
CHARCOAL - N	0.0256 ± 0.0001	27.1	0.0224 ± 0.0034	13.1
***AZOLLA*+ CHARCOAL - N**	0.0319 ± 0.0012	58.7	0.0237 ± 0.0001	19.6
CHEMICAL - N	0.0208 ± 0.0024	3.4	0.0203 ± 0.0011	2.5

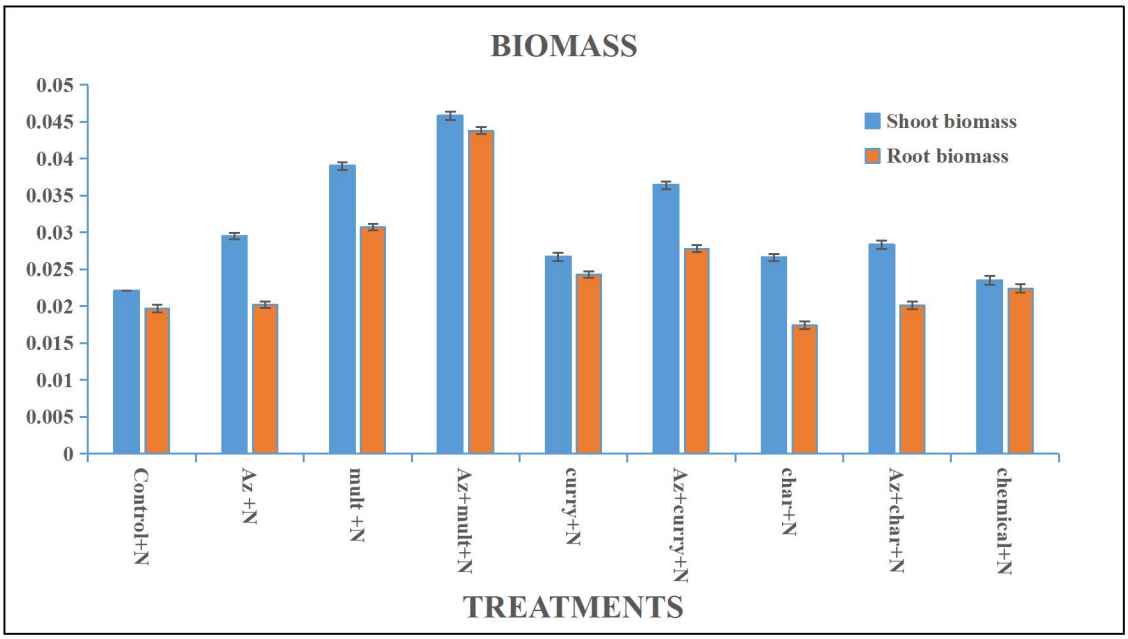

Fig.19. Effect of carrier based biofertilizer on Biomass (shoot and root) in Jaya rice variety. Az+N: *Azolla* (+NO_3), mult+N: Multani (+NO_3), Az+mult+N: *Azolla*+multani (+NO_3), curry+N: Curry leaves (+NO_3), Az+curry+N: *Azolla*+curry leaves (+NO_3), char+N: charcoal (+NO_3), Az+char+N: *Azolla*+charcoal (+NO_3), chemical+N: Chemical (+NO_3), control+N: Control (+NO_3). (+N): presence of NO_3; where ± indicates standard deviation, n=3.

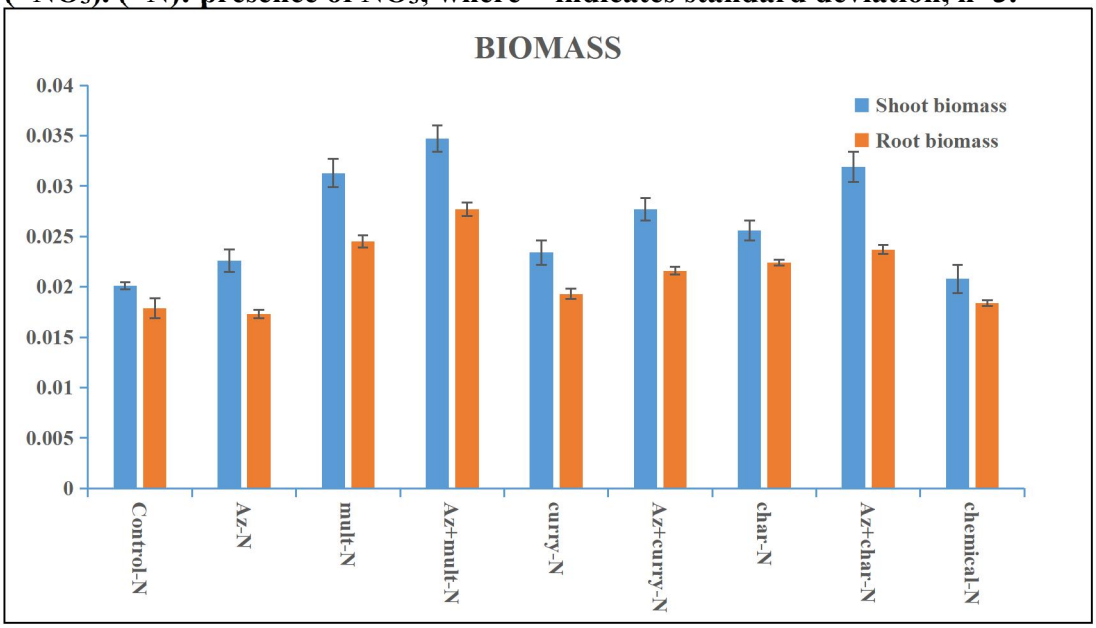

Fig.20. Effect of carrier based biofertilizer on Biomass (shoot and root) in Jaya rice variety. Az-N: *Azolla* (-NO_3), mult-N:Multani (-NO_3), Az+mult-N: *Azolla*+multani (-NO_3), curry+N: Curry leaves (-NO_3), Az+curry-N: *Azolla*+curry leaves (-NO_3), char+N: charcoal (-NO_3), Az+char-N: *Azolla*+charcoal (-NO_3), chemical-N: Chemical (-NO_3), control-N: Control (-NO_3). (-N): absence of NO_3; where ± indicates standard deviation, n=3.

Table 5. Effect of carrier based biofertilizer treatment on Photosynthetic efficiency of Jaya rice variety. (+N): presence of NO_3; where ± indicates standard deviation, n=3.

TREATMENT	Fv/Fm ratio	% Change
CONTROL + N	0.666±0.0011	0
***AZOLLA* + N**	0.687±0.0012	3.2
MULTANI + N	0.767±0.0030	15.2
***AZOLLA*+ MULTANI +N**	0.796±0.0020	19.5
CURRY LEAVES + N	0.709±0.00047	6.4
***AZOLLA*+CURRY LEAVES+N**	0.777±0.0012	16.7
CHARCOAL + N	0.707±0.0021	6.3
***AZOLLA*+ CHARCOAL + N**	0.732±0.0014	9.9
CHEMICAL + N	0.668±0.00071	0.3

Table 6. Effect of carrier based biofertilizer treatment on Photosynthetic efficiency of Jaya rice variety. (-N): absence of NO_3; where ± indicates standard deviation, n=3.

TREATMENT	Fv/Fm ratio	% Change
CONTROL - N	0.668±0.00071	0
***AZOLLA* -N**	0.689±0.00071	3.1
MULTANI - N	0.770±0.00283	15.4
***AZOLLA*+ MULTANI -N**	0.798±0.00071	19.5
CURRY LEAVES - N	0.708±0.00141	6.1
***AZOLLA*+CURRY LEAVES-N**	0.778±0.00141	16.6
CHARCOAL - N	0.709±0.00212	6.1
***AZOLLA*+ CHARCOAL - N**	0.734±0.00354	9.9
CHEMICAL - N	0.671±0.00071	0.4

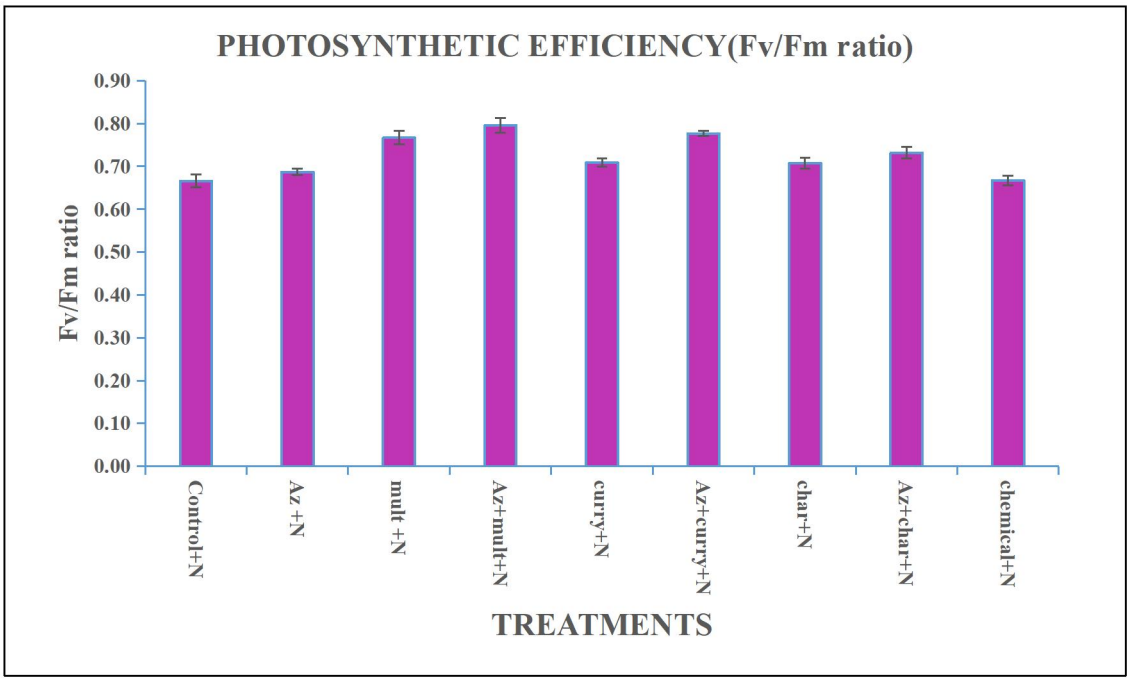

Fig.21. Effect of carrier based biofertilizer on Photosynthetic Efficiency in Jaya rice variety. Az+N:*Azolla* (+NO_3), mult+N:Multani (+NO_3), Az+mult+N: *Azolla*+multani(+NO_3), curry+N: Curry leaves(+NO_3), Az+curry+N: *Azolla*+curry leaves(+NO_3), char+N: charcoal(+NO_3), Az+char+N: *Azolla*+charcoal(+NO_3), chemical+N: Chemical(+NO_3), control+N: Control(+NO_3). (+N): presence of NO_3; where ± indicates standard deviation, n=3.

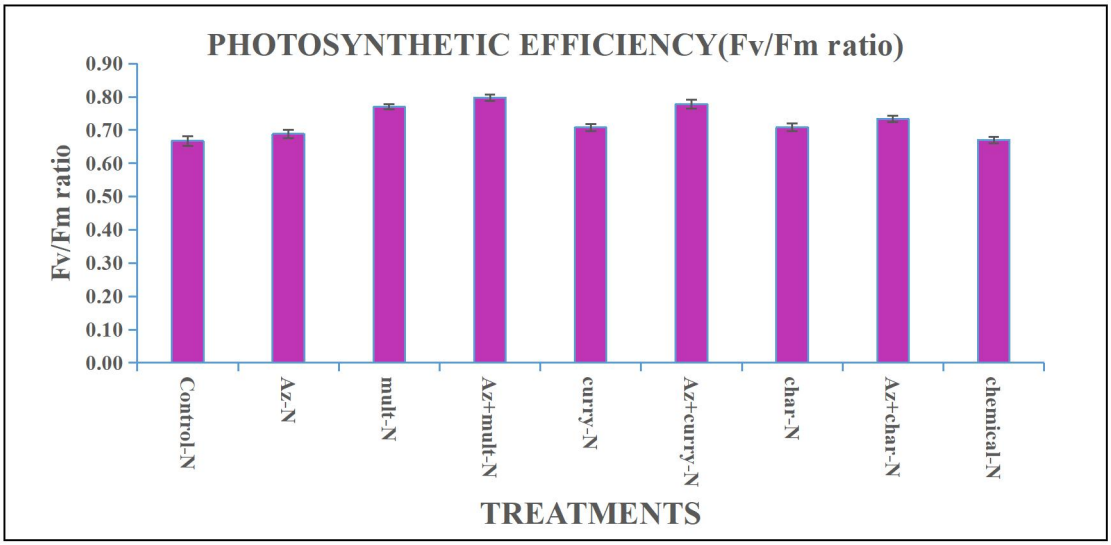

Fig.22. Effect of carrier based biofertilizer on Photosynthetic Efficiency in Jaya rice variety. Az-N: *Azolla*(-NO_3), mult-N:Multani(-NO_3), Az+mult-N: *Azolla*+multani(-NO_3), curry+N: Curry leaves(-NO_3), Az+curry-N: *Azolla*+curry leaves(-NO_3), char+N: charcoal(-NO_3), Az+char-N: *Azolla*+charcoal(-NO_3), chemical-N: Chemical(-NO_3), control-N: Control(-NO_3). (-N): absence of NO_3; where ± indicates standard deviation, n=3.

Table 7. Effect of carrier based biofertilizer treatment on Photosynthetic pigments of Jaya rice variety. (+N): presence of NO_3 ; where ± indicates standard deviation, n=3

TREATMENT	Chl a	% Change	Chl b	% Change	Carotenoid	% Change
CONTROL+N	20.923± 0.044	0	8.529± 0.042	0	0.852±0.061	0
***AZOLLA* + N**	22.877± 0.05	9.3	8.866± 0.051	3.9	1.266±0.057	-36.7
MULTANI + N	26.502± 0.055	26.6	9.685± 0.043	13.5	1.452±0.067	70.4
***AZOLLA*+ MULTANI +N**	31.406± 0.054	50.1	13.881± 0.053	62.7	1.638±0.082	92.2
CURRY LEAVES+N	25.292± 0.047	20.8	10.132± 0.056	18.7	1.644±0.065	92.9
***AZOLLA*+ CURRY LEAVES+N**	28.314± 0.048	35.3	10.914± 0.059	27.9	1.764±0.084	107
CHARCOAL +N	24.678± 0.062	17.9	9.845± 0.065	15.4	4.083±0.089	379.2
***AZOLLA*+ CHARCOAL +N**	21.474± 0.043	2.6	9.994± 0.041	17.1	5.730±0.060	572.5
CHEMICAL +N	16.941± 0.048	-19	8.160± 0.016	-4.3	0.855±0.007	0.35

Table 8. Effect of carrier based biofertilizer treatment on Photosynthetic pigments of Jaya rice variety. (-N): absence of NO_3 ; where ± indicates standard deviation, n=3

TREATMENT	Chl a	% Change	Chl b	% Change	Carotenoid	% Change
CONTROL-N	4.283±0.543	0	1.989±0.294	0	0.852±0.314	0
***AZOLLA* -N**	5.273±0.643	23.1	2.258±0.411	13.5	1.170±0.436	-97.9
MULTANI - N	7.185±0.741	67.7	2.255±0.473	13.3	1.452±0.583	70.4
***AZOLLA*+ MULTANI -N**	8.459±0.835	97.5	3.221±0.457	61.9	1.638±0.568	92.2
CURRY LEAVES-N	5.310±0.896	23.9	3.012±0.472	51.4	1.644±0.533	92.9
***AZOLLA*+ CURRY LEAVES-N**	5.385±0.890	25.7	3.021±0.518	51.8	1.764±0.527	107
CHARCOAL -N	5.003±0.914	16.8	3.115±0.723	56.6	2.002±0.517	134.9
***AZOLLA*+ CHARCOAL -N**	5.124±0.733	19.6	3.220±0.561	61.8	2.150±0.433	152.3
CHEMICAL -N	4.007±0.553	-6.4	1.997±0.220	0.4	0.855±0.392	0.3

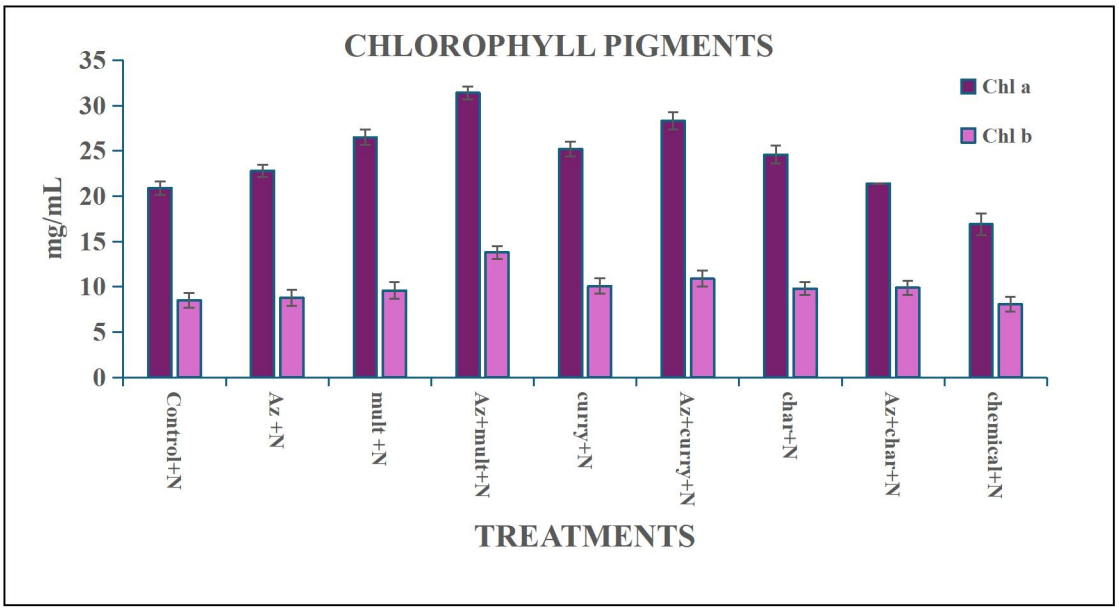

Fig.23. Effect of carrier based biofertilizer on Chlorophyll Pigments in Jaya rice variety. Az+N: *Azolla*(+NO_3), mult+N:Multani(+NO_3), Az+mult+N: *Azolla*+multani(+NO_3), curry+N: Curry leaves(+NO_3), Az+curry+N: *Azolla*+curry leaves(+NO_3), char+N: charcoal(+NO_3), Az+char+N: *Azolla*+charcoal(+NO_3), chemical+N: Chemical(+NO_3), control+N: Control(+NO_3). (+N): presence of NO_3; where ± indicates standard deviation, n=3.

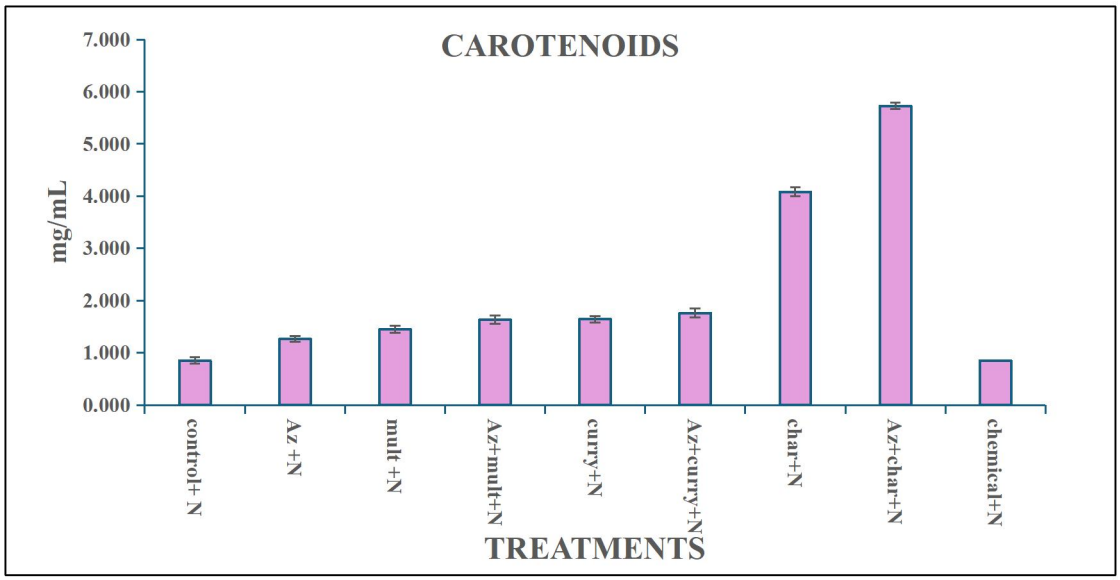

Fig.24. Effect of carrier based biofertilizer on Carotenoids in Jaya rice variety. Az+N: *Azolla*(+NO_3), mult+N:Multani(+NO_3), Az+mult+N: *Azolla*+multani(+NO_3), curry+N: Curry leaves(+NO_3), Az+curry+N: *Azolla*+curry leaves(+NO_3), char+N: charcoal(+NO_3), Az+char+N: *Azolla*+charcoal(+NO_3), chemical+N: Chemical(+NO_3), control+N: Control(+NO_3). (+N): presence of NO_3; where ± indicates standard deviation, n=3.

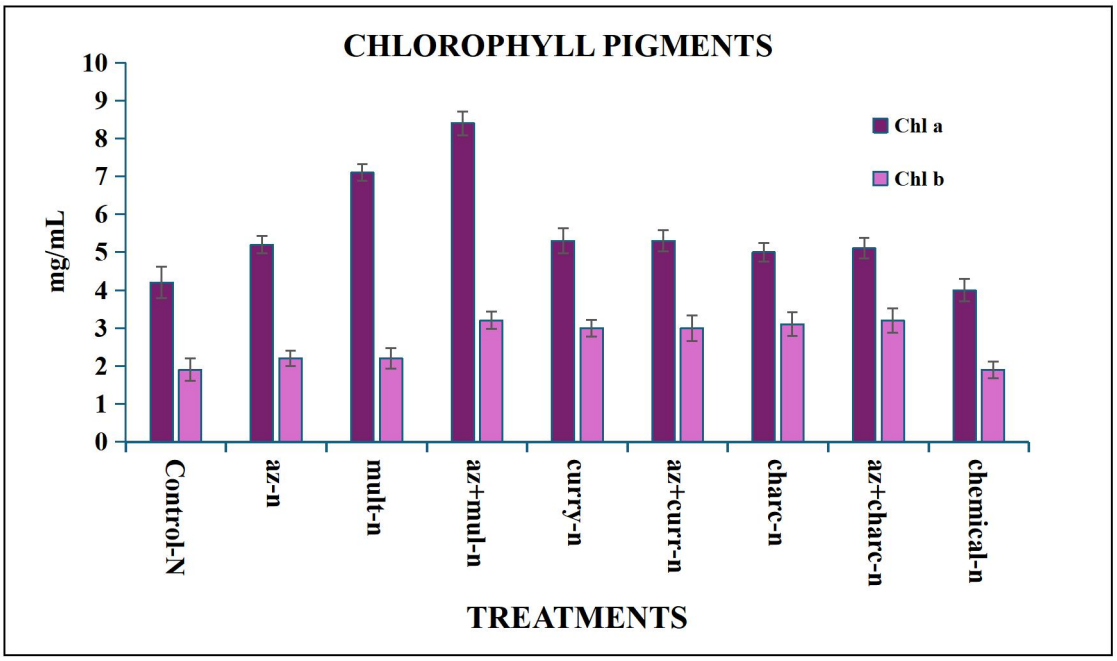

Fig.25. Effect of carrier based biofertilizer on Chlorophyll Pigments in Jaya rice variety. Az-N: *Azolla*(-NO_3), mult-N:Multani(-NO_3), Az+mult-N: *Azolla*+multani(-NO_3), curry+N: Curry leaves(-NO_3), Az+curry-N: *Azolla*+curry leaves(-NO_3), char+N: charcoal(-NO_3), Az+char-N: *Azolla*+charcoal(-NO_3), chemical-N: Chemical(-NO_3), control-N: Control(-NO_3). (-N): absence of NO_3 ; where ± indicates standard deviation, n=3.

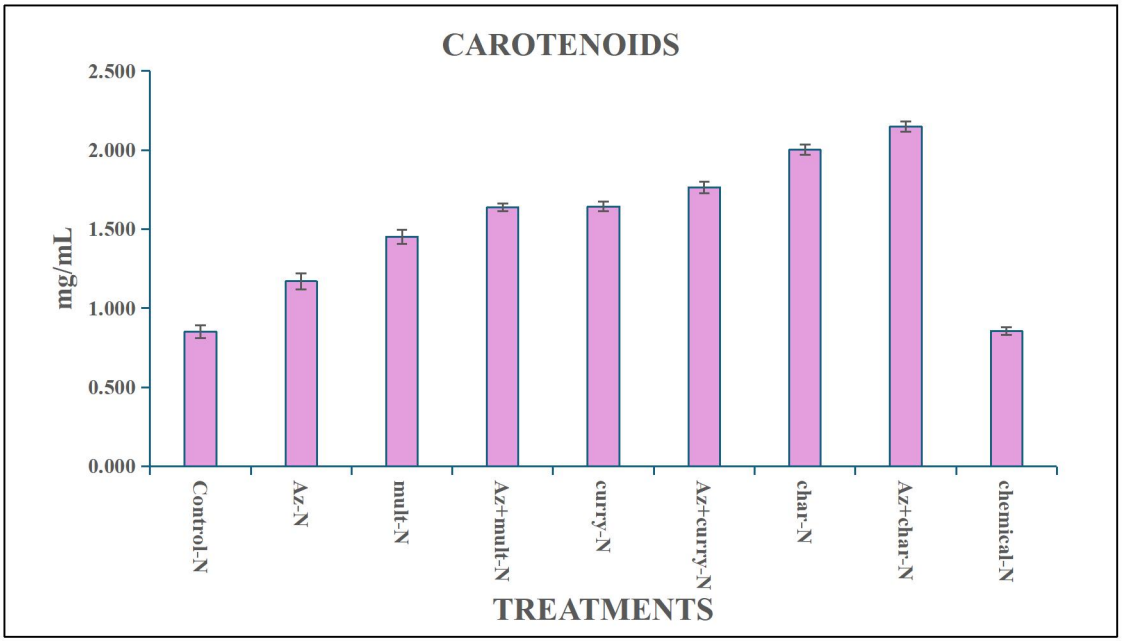

Fig.26. Effect of carrier based biofertilizer on Carotenoids in Jaya rice variety. Az-N: *Azolla*(-NO_3), mult-N:Multani(-NO_3), Az+mult-N: *Azolla*+multani(-NO_3), curry+N: Curry leaves(-NO_3), Az+curry-N: *Azolla*+curry leaves(-NO_3), char+N: charcoal(-NO_3), Az+char-N: *Azolla*+charcoal(-NO_3), chemical-N: Chemical(-NO_3), control-N: Control(-NO_3). (-N): absence of NO_3 ; where ± indicates standard deviation, n=3.

Table 9. Effect of carrier based biofertilizer treatment on Total sugar (mg/mL) and Protein content (mg/mL) of Jaya rice variety. (+N): presence of NO_3 ; where ± indicates standard deviation, n=3.

TREATMENT	TOTAL SUGAR	% Change	PROTEIN	% Change
CONTROL + N	32.211 ± 0.027	0	43.507 ± 0.029	0
AZOLLA + N	42.365 ± 0.019	31.5	52.544 ± 0.035	20.7
MULTANI + N	46.426 ± 0.02	44.1	53.633 ± 0.028	23.2
AZOLLA+ MULTANI +N	50.860 ± 0.018	57.8	59.990 ± 0.023	37.8
CURRY LEAVES + N	52.595 ± 0.028	63.2	61.543 ± 0.021	41.4
AZOLLA+ CURRY LEAVES +N	59.942 ± 0.024	86	70.777 ± 0.033	62.6
CHARCOAL + N	60.542 ± 0.022	87.9	60.561 ± 0.036	39.1
AZOLLA+ CHARCOAL + N	69.881 ± 0.021	116.9	62.234 ± 0.037	43
CHEMICAL + N	37.389 ± 0.010	16	50.237 ± 0.020	15.4

Table.10. Effect of carrier based biofertilizer treatment on Total sugar (mg/mL) and Protein content (mg/mL) of Jaya rice variety. (-N): absence of NO_3; where ± indicates standard deviation, n=3.

TREATMENT	TOTAL SUGAR	% Change	PROTEIN	% Change
CONTROL - N	32.848 ± 0.021	0	39.182 ± 0.029	0
AZOLLA - N	37.995 ± 0.019	15.6	48.213 ± 0.033	23
MULTANI - N	39.231 ± 0.01	19.4	54.312 ± 0.025	38.6
AZOLLA+ MULTANI - N	52.163 ± 0.014	58.8	58.432 ± 0.026	49.1
CURRY LEAVES - N	59.865 ± 0.024	82.2	62.865 ± 0.020	60.4
AZOLLA+CURRY LEAVES- N	67.119 ± 0.018	104.3	71.633 ± 0.028	82.8
CHARCOAL - N	62.681 ± 0.024	90.8	60.579 ± 0.032	54.6
AZOLLA+ CHARCOAL - N	70.202 ± 0.029	113.7	64.276 ± 0.039	64
CHEMICAL - N	42.505 ± 0.017	29.3	50.612 ± 0.024	29.1

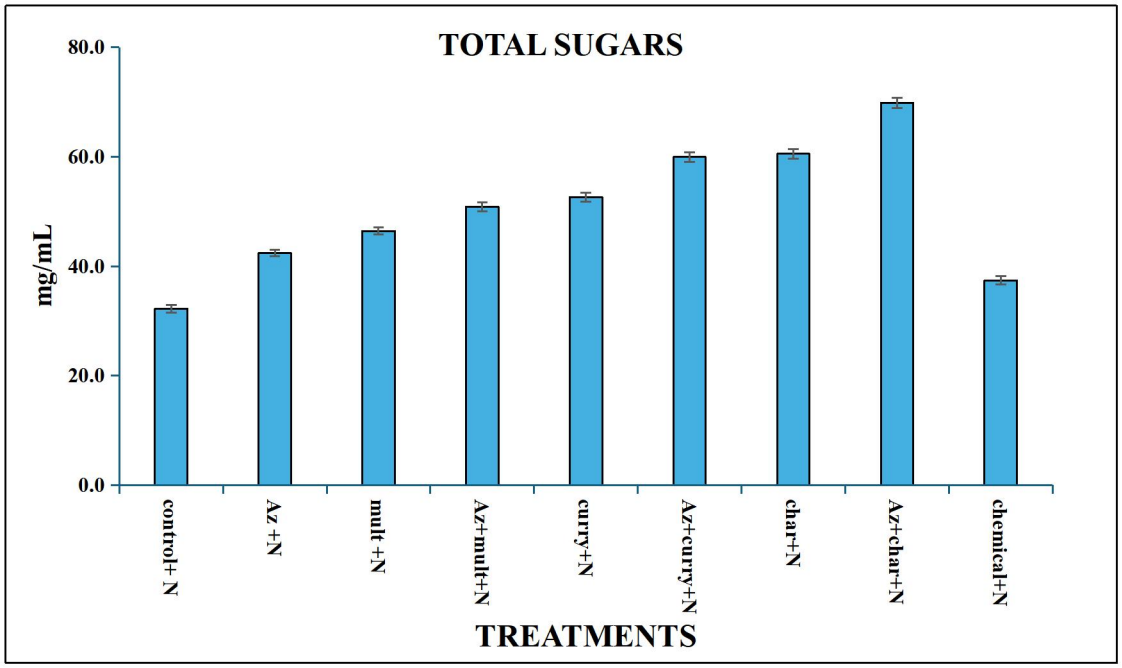

Fig.27. Effect of carrier based biofertilizer on total sugar content in Jaya rice variety. Az+N: *Azolla*(+NO_3), mult+N:Multani(+NO_3), Az+mult+N: *Azolla*+multani(+NO_3), curry+N: Curry leaves(+NO_3), Az+curry+N: *Azolla*+curry leaves(+NO_3), char+N: charcoal(+NO_3), Az+char+N: *Azolla*+charcoal(+NO_3), chemical+N: Chemical(+NO_3), control+N: Control(+NO_3). (+N): presence of NO_3 ; where ± indicates standard deviation, n=3.

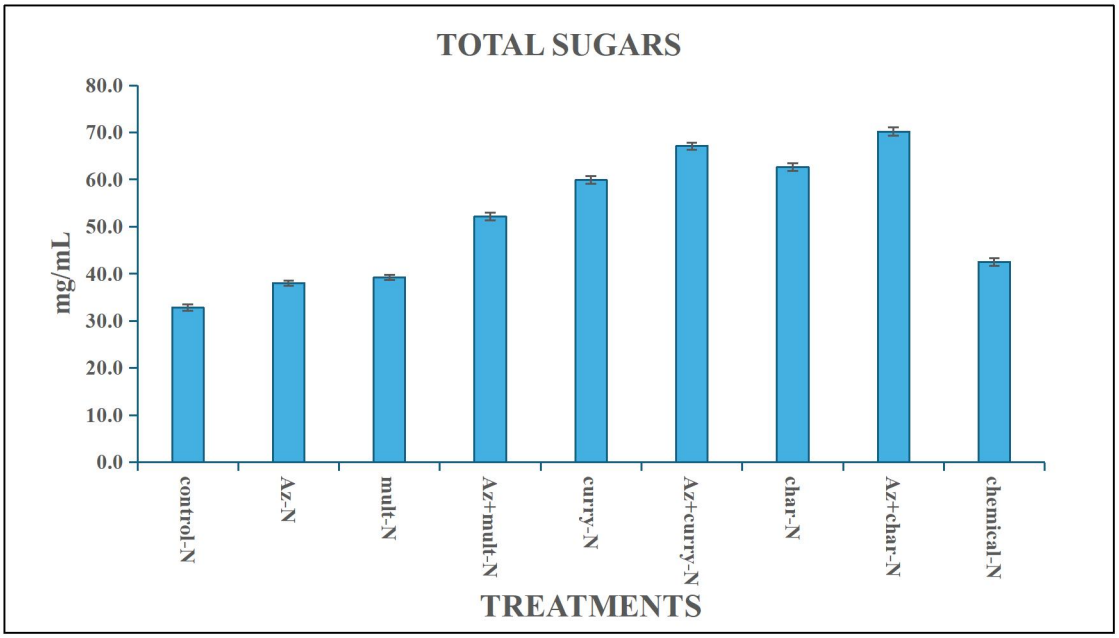

Fig.28. Effect of carrier based biofertilizer on total sugar content in Jaya rice variety. Az-N: *Azolla*(-NO_3), mult-N:Multani(-NO_3), Az+mult-N: *Azolla*+multani(-NO_3), curry+N:Curry leaves(-NO_3),Az+curry-N:*Azolla*+curry leaves(-NO_3),char+N:charcoal(-NO_3),Az+char-N: *Azolla*+charcoal(-NO_3), chemical-N: Chemical(-NO_3), control-N: Control(-NO_3). (-N): absence of NO_3; where ± indicates standard deviation, n=3.

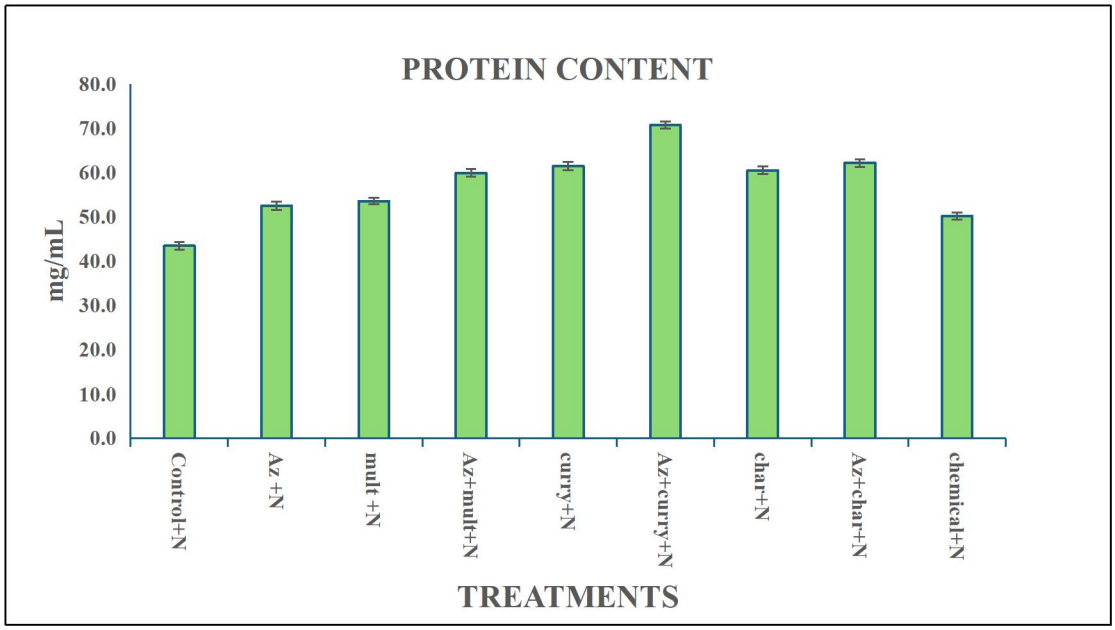

Fig.29. Effect of carrier based biofertilizer on protein content in Jaya rice variety. Az+N: *Azolla*(+NO_3), mult+N:Multani(+NO_3), Az+mult+N: *Azolla*+multani(+NO_3), curry+N: Curry leaves(+NO_3), Az+curry+N: *Azolla*+curry leaves(+NO_3), char+N: charcoal(+NO_3), Az+char+N: *Azolla*+charcoal(+NO_3), chemical+N: Chemical(+NO_3), control+N: Control(+NO_3). (+N): presence of NO_3 ; where ± indicates standard deviation, n=3.

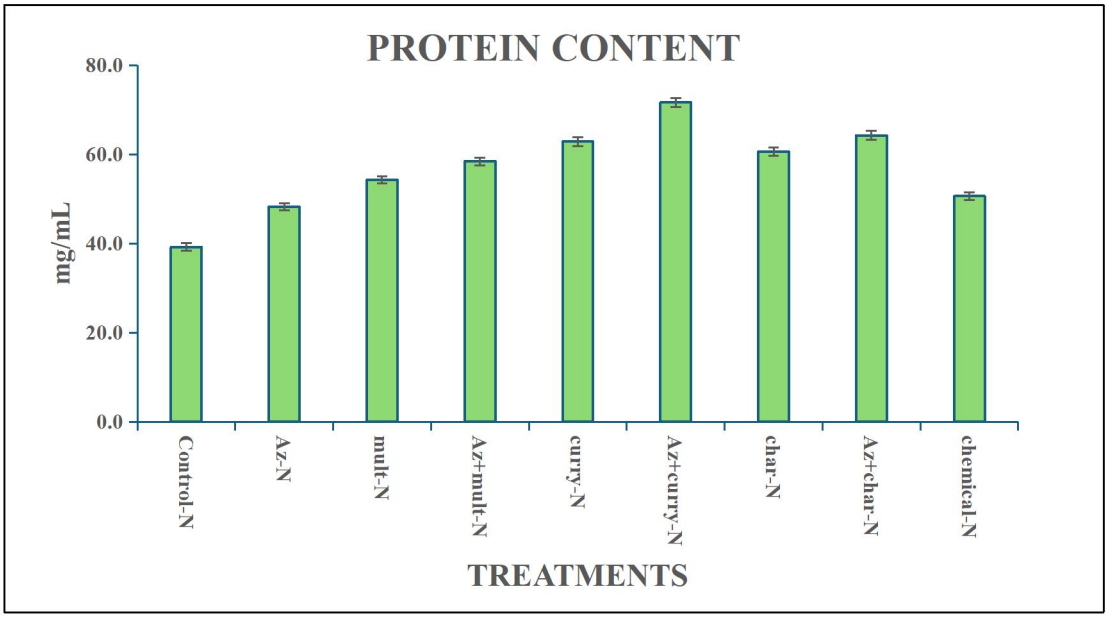

Fig.30. Effect of carrier based biofertilizer on protein content in Jaya rice variety. Az-N: *Azolla*(-NO_3), mult-N:Multani(-NO_3), Az+mult-N: *Azolla*+multani(-NO_3), curry+N: Curry leaves(-NO_3), Az+curry-N: *Azolla*+curry leaves(-NO_3), char+N: charcoal(-NO_3), Az+char-N: *Azolla*+charcoal(-NO_3), chemical-N: Chemical(-NO_3), control-N: Control(-NO_3). (-N): absence of NO_3; where ± indicates standard deviation, n=3.

Table 11. Effect of carrier based biofertilizer treatment on Glycolipid (mg/mL) of Jaya rice variety. (+N): presence of NO_3; where ± indicates standard deviation, n=3.

TREATMENT	Glycolipid	% Change
CONTROL + N	125.235±0.0172	0
***AZOLLA* + N**	245.053±0.0803	95.675
MULTANI + N	263.112± 0.0155	110.095
***AZOLLA*+ MULTANI +N**	292.064±0.0681	133.213
CURRY LEAVES + N	304.132 ±0.04054	142.849
***AZOLLA*+ CURRY LEAVES +N**	339.064±0.0205	170.742
CHARCOAL + N	314.417±0.0139	151.062
***AZOLLA*+ CHARCOAL + N**	372.101±0.0372	197.122
CHEMICAL + N	103.246±0.0245	-17.559

Table 12. Effect of carrier based biofertilizer treatment on Glycolipid content (mg/mL) of Jaya rice variety.(-N):absence of NO_3 ; where ± indicates standard deviation, n=3.

TREATMENT	Glycolipid	% Change
CONTROL - N	120.507±0.00259	0
***AZOLLA* -N**	214.246±0.01390	77.787
MULTANI - N	225.103±0.06858	86.797
***AZOLLA*+ MULTANI -N**	230.553±0.11101	91.319
CURRY LEAVES - N	258.009±0.07966	114.103
***AZOLLA*+ CURRY LEAVES -N**	265.116±0.08885	120.000
CHARCOAL - N	327.176±0.05374	171.500
***AZOLLA*+ CHARCOAL - N**	351.447±0.09215	191.640
CHEMICAL - N	118.587±0.00966	-1.593

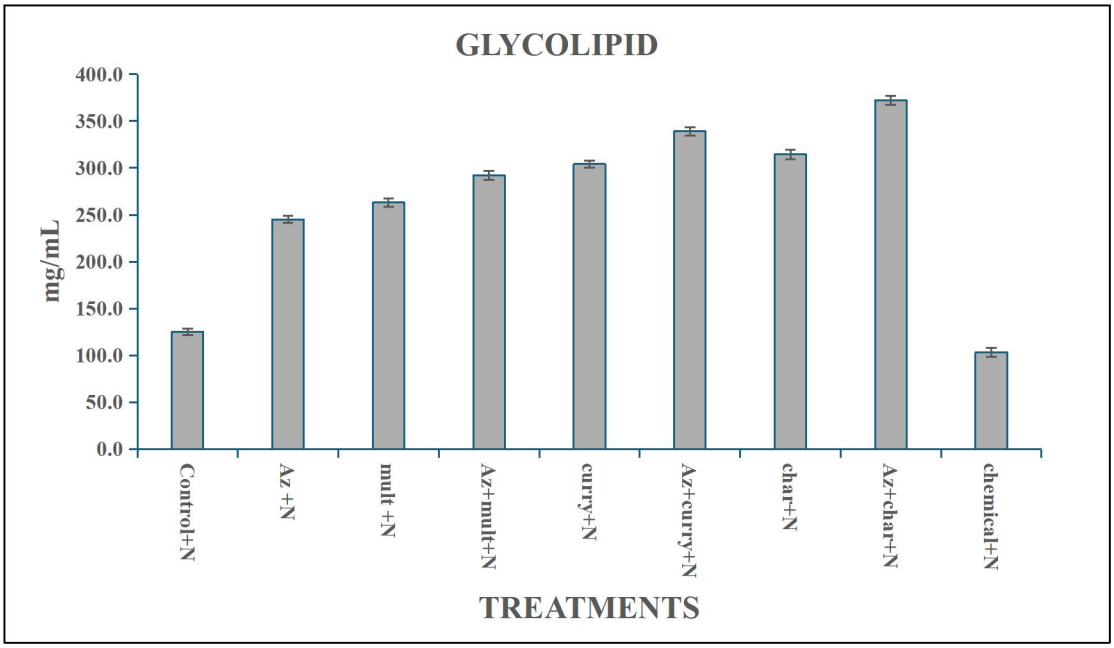

Fig.31. Effect of carrier based biofertilizer on glycolipid content in Jaya rice variety. Az+N: *Azolla*(+NO_3), mult+N:Multani(+NO_3), Az+mult+N: *Azolla*+multani(+NO_3), curry+N: Curry leaves(+NO_3), Az+curry+N: *Azolla*+curry leaves(+NO_3), char+N: charcoal(+NO_3), Az+char+N: *Azolla*+charcoal(+NO_3), chemical+N: Chemical(+NO_3), control+N: Control (+NO_3). (+N): presence of NO_3 ; where ± indicates standard deviation, n=3.

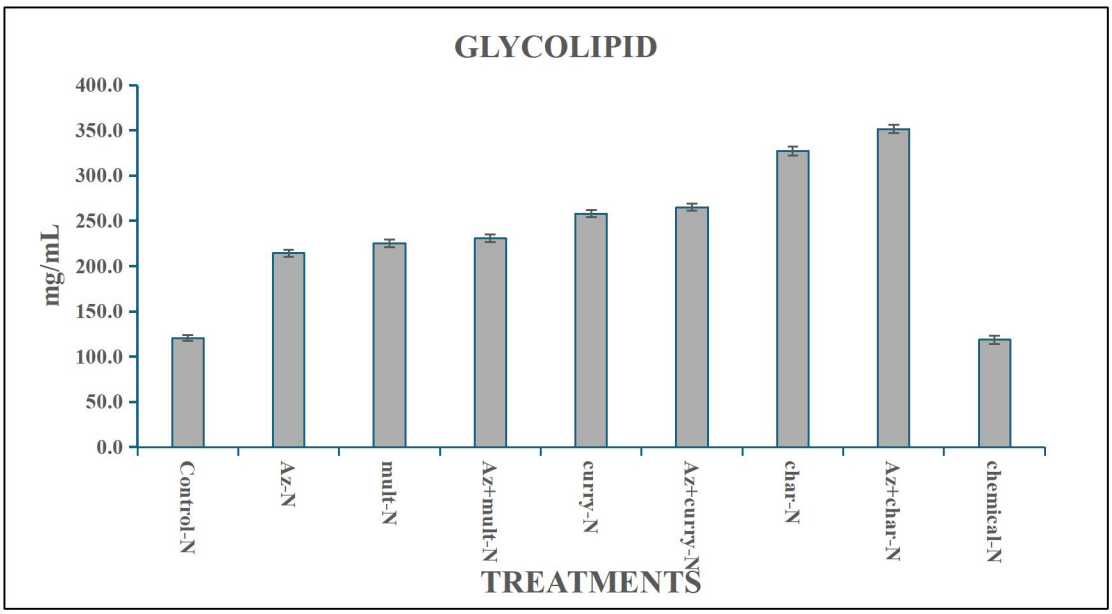

Fig.32. Effect of carrier based biofertilizer on glycolipid content in Jaya rice variety.Az-N:*Azolla*(-NO_3),mult-N:Multani(-NO_3),Az+mult-N:*Azolla*+multani(-NO_3),curry+N:Curryleaves(-NO_3),Az+curry-N:*Azolla*+curryleaves(-NO_3),char+N: charcoal (-NO_3), Az+char-N: *Azolla*+charcoal (-NO_3), chemical-N: Chemical (-NO_3), control-N: Control (-NO_3).

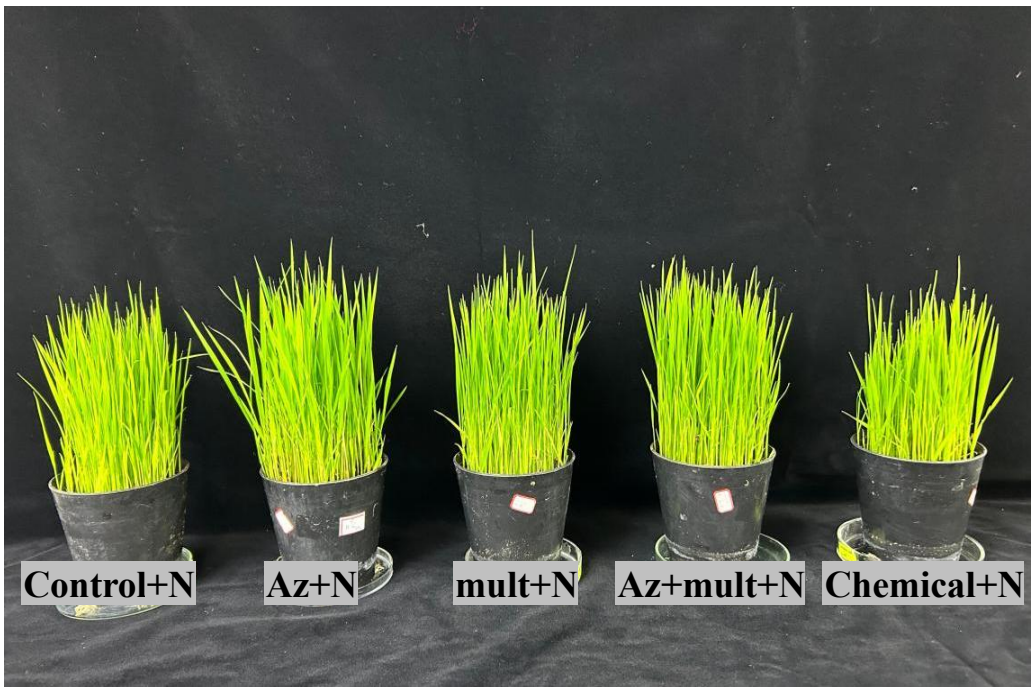

Fig.7. Effect of carrier based biofertilizer on the growth of Jaya rice variety. control+N: Control (+NO_3), Az+N: Azolla (+NO_3), mult+N: Multani (+NO_3), Az+mult+N: Azolla+multani(+NO_3), Chemical+N: Chemical(+NO_3).

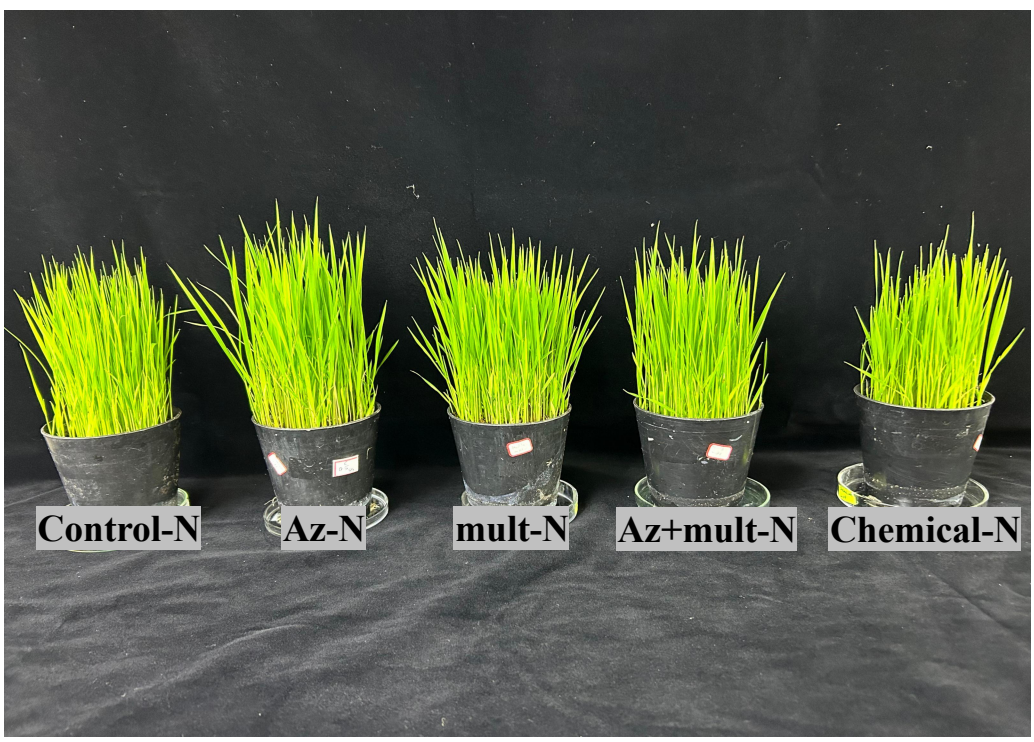

Fig.8. Effect of carrier based biofertilizer on the growth of Jaya rice variety. Az-N: Azolla (-NO3), mult-N:Multani (-NO3), Az+mult-N: Azolla+multani (-NO3), chemical-N: Chemical(-NO3), control-N: Control(-NO3).

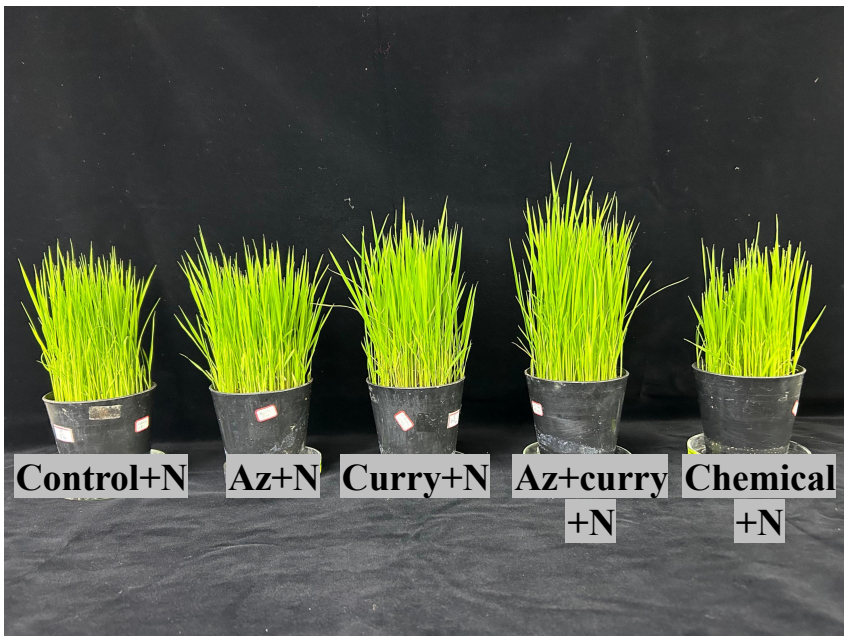

Fig.9. Effect of carrier based biofertilizer on the growth of Jaya rice variety. Az+N: Azolla(+NO3), curry+N: Curry leaves(+NO3), Az+curry+N: Azolla+curry leaves(+NO3), chemical+N: Chemical(+NO3), control+N: Control(+NO3).

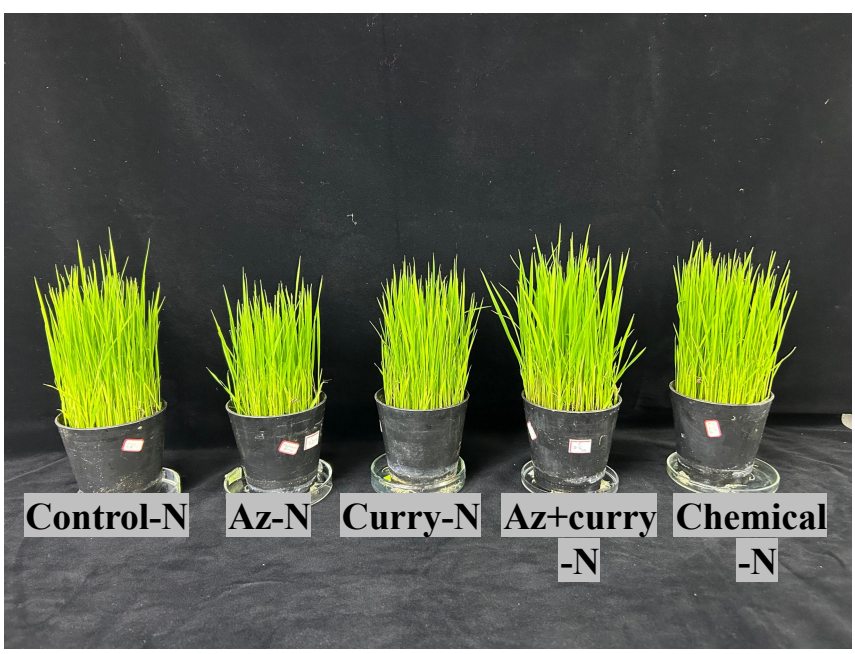

Fig.10. Effect of carrier based biofertilizer on the growth of Jaya rice variety. Az-N: Azolla(-NO3), curry-N: Curry leaves(-NO3), Az+curry-N: Azolla+curry leaves(-NO3), chemical-N: Chemical(-NO3), control-N: Control(-NO3).

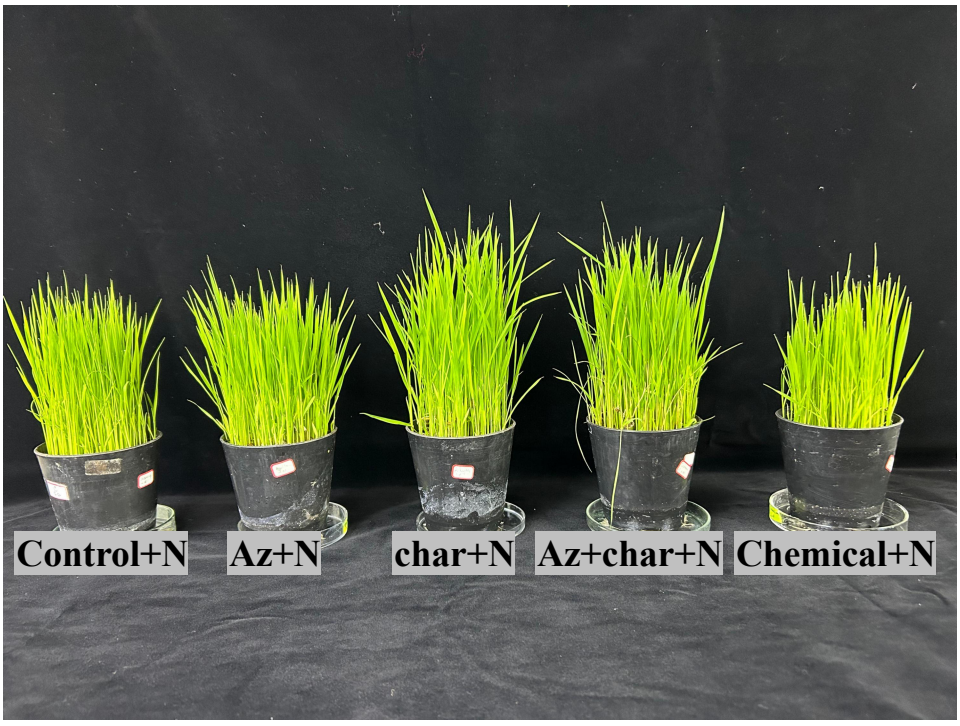

Fig.11. Effect of carrier based biofertilizer on the growth of Jaya rice variety. Az+N: Azolla(+NO3), char+N: charcoal(+NO3), Az+char+N: Azolla+charcoal(+NO3), chemical+N: Chemical(+NO3), control+N: Control(+NO3).

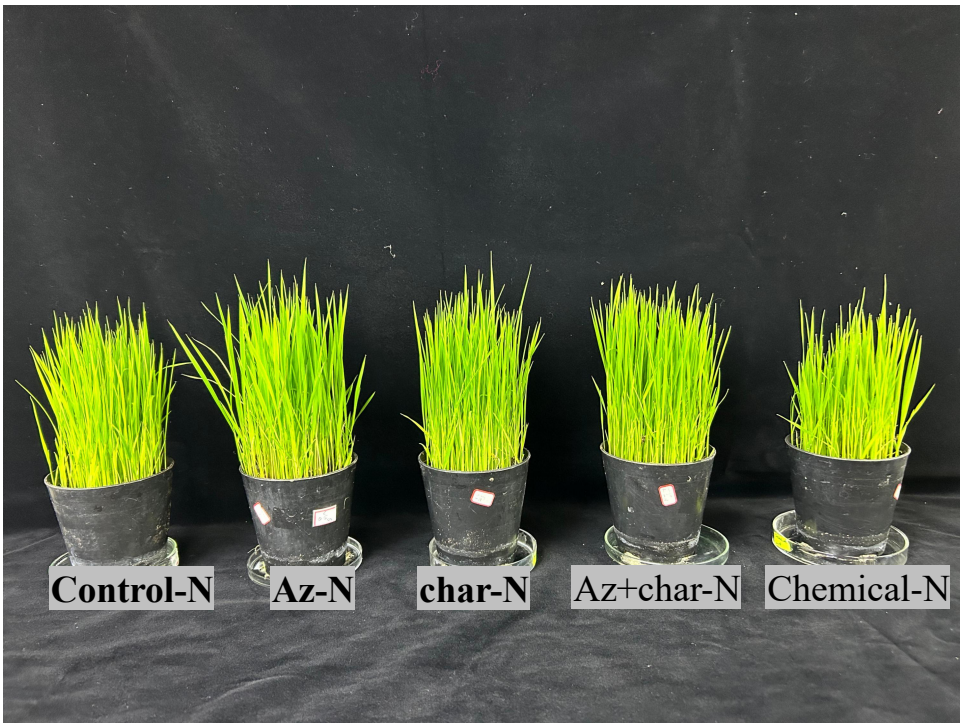

Fig.12. Effect of carrier based biofertilizer on the growth of Jaya rice variety. Az-N: Azolla(-NO3), char-N: charcoal(-NO3), Az+char-N: Azolla+charcoal(-NO3), chemical-N: Chemical(-NO3), control-N: Control(-NO3).

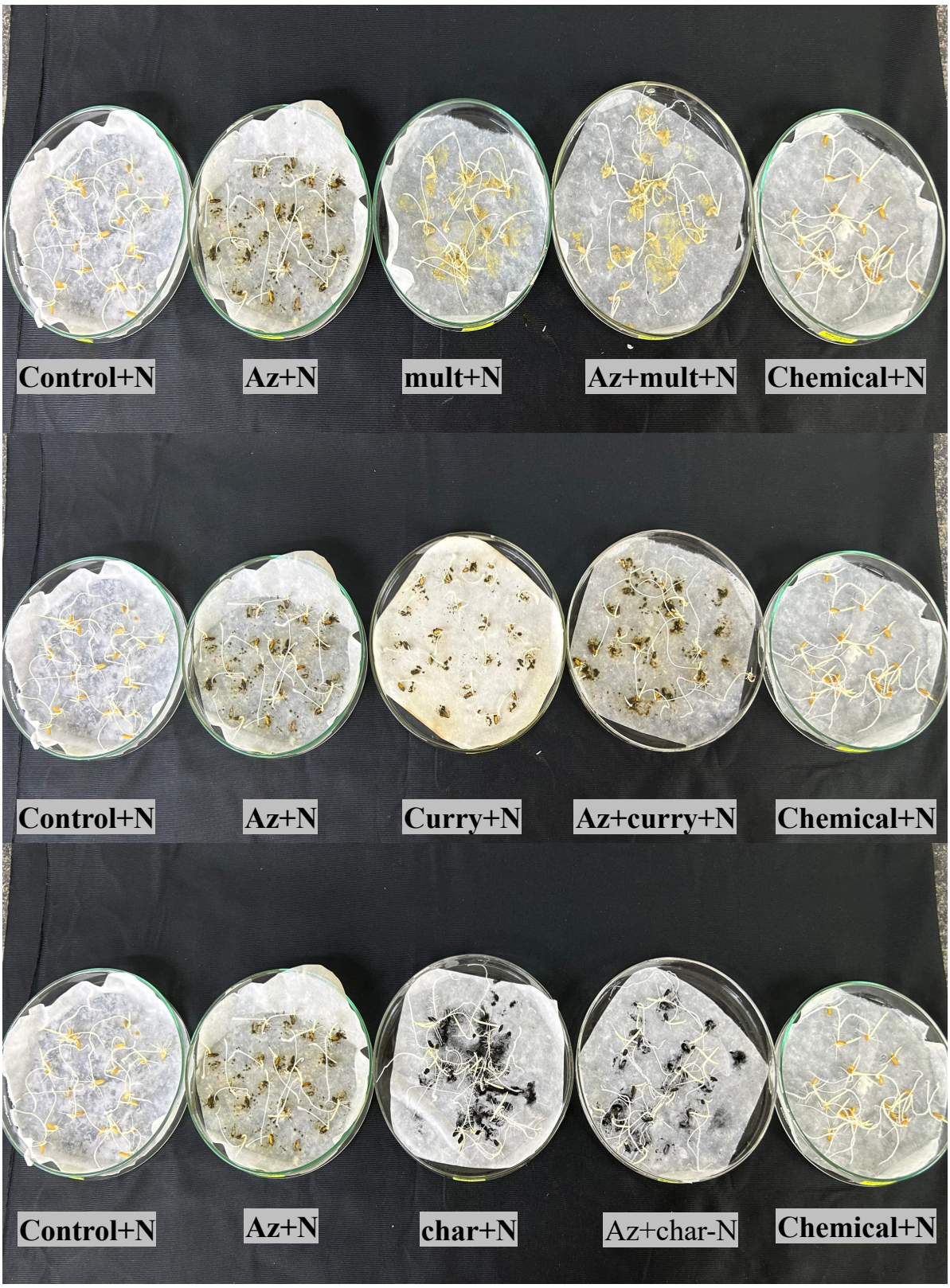

Fig.13. Effect of carrier based biofertilizers on Percent Germination in Jaya rice variety. Az+N: Azolla(+NO3), mult+N:Multani(+NO3), Az+mult+N: Azolla+multani(+NO3), curry+N: Curry leaves(+NO3), Az+curry+N: Azolla+curry leaves(+NO3), char+N: charcoal(+NO3), Az+char+N: Azolla+charcoal(+NO3), chemical+N: Chemical(+NO3), Control+N: Control(+NO3).

Fig.14. Effect of carrier based biofertilizers on Percent Germination in Jaya rice variety. Az-N: Azolla(-NO3), mult-N:Multani(-NO3), Az+mult-N: Azolla+multani(-NO3), curry-N: Curry leaves(-NO3), Az+curry-N: Azolla+curry leaves(-NO3), char+N: charcoal(-NO3), Az+char-N: Azolla+charcoal(-NO3), chemical-N: Chemical(-NO3), Control-N: Control(-NO3).

Fig.5. Azolla pinnata collected from Pilar rice field.

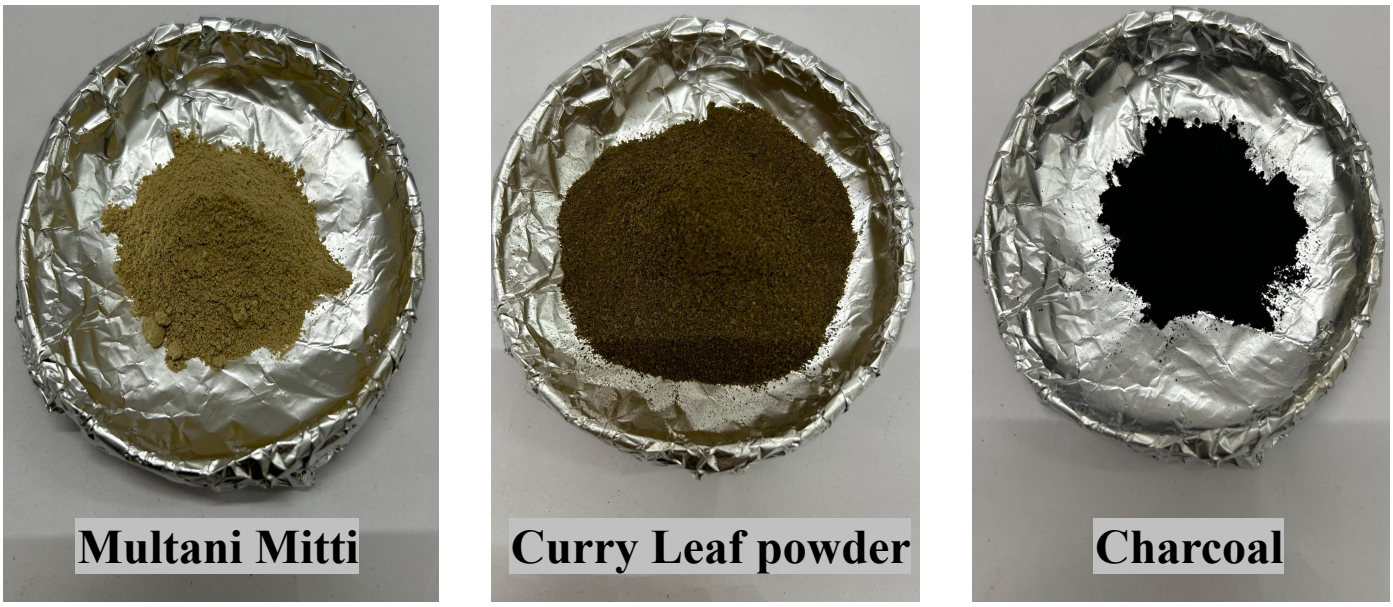

Fig.6. Carrier materials in powdered form.

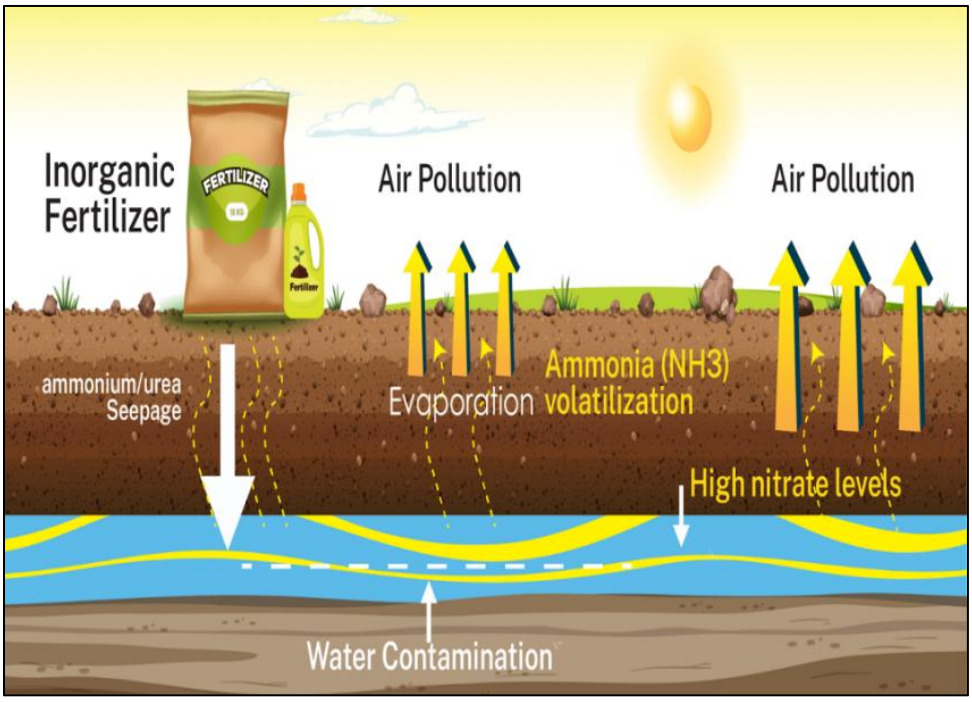

Fig.1. Effect of inorganic fertilizer on the environment.

Fig.2. Types of inorganic fertilizers.

Fig.3. Types of biofertilizers.

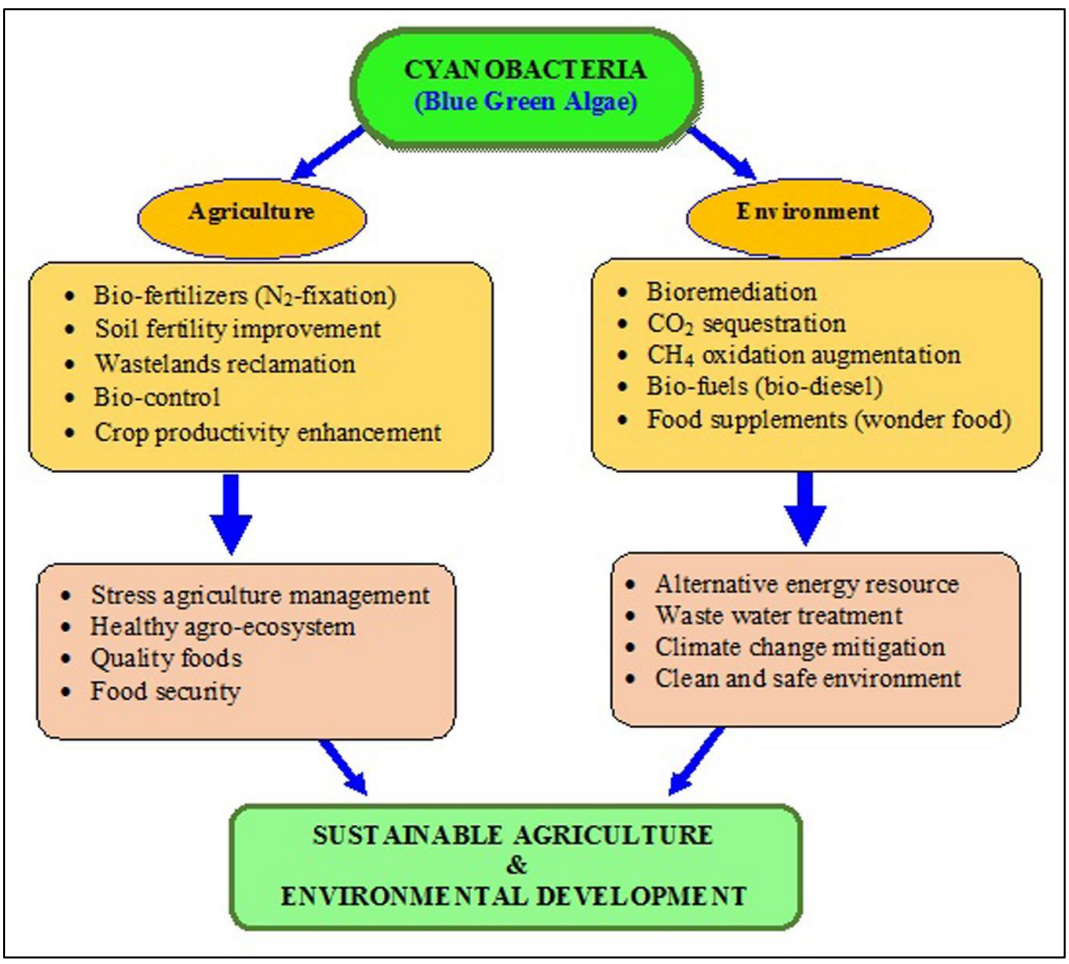

Fig.4. A hypothetical model exhibiting the potential roles of cyanobacteria in sustainable agriculture and environmental management.