"THE PROMOTIVE EFFECT OF NOSTOC AND AZOLLA AS BIOFERTILIZERS ON SORGHUM BICOLOR (L.) MOENCH"

DISSERTATION SUBMITTED TO GOA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR

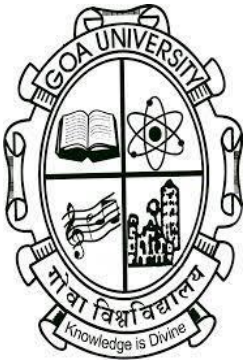
THE DEGREE OF MASTER OF SCIENCE IN BOTANY

BY

MR. ROYSTON D'SILVA

UNDER THE GUIDANCE OF

DR. RUPALI BHANDARI



DEPARTMENT OF BOTANY

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CERTIFICATE

This is to certify that this dissertation is a bonafide and an authentic record of this research entitled **"The promotive effect of *Nostoc* and *Azolla* as biofertilizers on *Sorghum* bicolor (L.) Moench"** carried out by **D'silva Royston,** student of Department of Botany, Goa University. This work is carried out under my supervision and guidance at the Department of Botany, Goa University, Taleigao Plateau, Goa, in partial fulfilment for the requirement for the award of 'MASTER OF SCIENCE IN BOTANY' degree of the University and that no part, therefore, has been presented before in any other degree or diploma of any University.

Dr. Rupali Bhandari

Dissertation Guide

Department of Botany,
Goa University

DECLARATION

I declare that the project entitled "**The promotive effect of *Nostoc* and *Azolla* as biofertilizers on Sorghum *bicolor* (L.) Moench**" submitted for the Master of Science in Botany to Goa University, is carried out by me under the supervision of Dr. Rupali Bhandari, Department of Botany, Goa University. The work is original and had not been submitted in any part or whole by me for any other degree or diploma to this or any other university.

Mr. Royston D'silva
Department of Botany
Goa University

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ABSTRACT

The present work was conducted to evaluate the response of *Sorghum bicolor*. (L.) Moench to biofertilizers, *Nostoc commune* and *Azolla pinnata* on morphological, physiological and biochemical parameters. The Sorghum plants were raised in vermiculite under a controlled environment and supplemented with a single or combination of biofertilizers and chemical fertilizer and Hoagland solution containing all nutrients and Hoagland solution with the absence of nitrates. It was observed that plants grown in Hoagland solution containing all the nutrients treated with chemical fertilizer showed an increase in relative water content and biomass compared to other treatments. While plants grown in Hoagland solution containing no nitrates treated with *Nostoc* showed greater RWC than other treatments, the shoot biomass of plants grown in Hoagland solution containing all nutrients increased in plants treated with *Azolla* while greater root biomass was observed in *Nostoc*. In Hoagland solution, chemical fertiliser treated plants (absence of nitrates) showed more shoot growth than other treated plants. However, plants treated with a combination of *Azolla*+*Nostoc* showed greater root biomass. The seed germination rate increased in seeds treated with biofertilizers and chemical fertilizer in Hoagland solution containing all nutrients. Hoagland solution with the absence of nitrates and biofertilizers showed reduced germination rate compared to control plants (lack of nitrates). The photosynthetic efficiency decreased in plants grown in Hoagland solution containing all nutrients with *Azolla*, *Nostoc*, its combination and chemical fertilizer compared to control plants while it increased in plants grown in Hoagland solution (absence of nitrates) with *Azolla* and *Azolla*+*Nostoc*. Plants in Hoagland solution containing all nutrients with *Azolla*, *Nostoc*, 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+Nostoc* 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 treated with a combination of *Azolla+Nostoc* showed an increase in total sugar content compared to control. At the same time, Plants in the absence of nitrates treated with *Azolla* and *Azolla+Nostoc* showed an upsurge in total sugar content. Plants treated with *Nostoc* showed higher protein content than all the treatments grown in Hoagland solution containing all nutrients. On the other hand, Plants grown in nitrate absence treated with *Azolla* showed more protein content than all the treatments. The glycolipid content in plants treated with *Azolla* was high compared to all the treatments grown in Hogland solution containing all nutrients. Whereas in plants in nitrate absence treated with *Azolla+Nostoc*, the glycolipid content drastically increased compared to all the treatments. Biofertilizers treatment with *Nostoc commune* and *Azolla pinnata* increased nitrogen uptake and enhanced the yield of sorghum plants with better physiological and biochemical attributes even in the absence of inorganic nitrogen fertilizer application. The results indicated that biofertilizer would be an excellent substitute for the 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 biofertilizers might be crucially important in small to medium input structures in cultivation. The outcomes can be practised to provide better instruction for root-level farmers on biofertilizers.

1. INTRODUCTION

Global population growth poses a threat to food security in an era of increased ecosystem degradation, climate change, soil erosion, and biodiversity loss. In modern agriculture, due to heavy usage of chemical fertilizers and harmful pesticides on the crops, the sustainability of the agriculture systems collapsed, the cost of cultivation soared at a high rate, farmers' income stagnated, and food security and safety became a challenge. The indiscriminate and imbalanced use of chemical fertilizers, especially urea, chemical pesticides, and the unavailability of organic manures have led to a considerable reduction in soil health. In modern agriculture, chemical fertilizers have degraded soil fertility, making it unsuitable for raising crop plants. In addition, the intensive use of these inputs has also led to severe health and environmental hazards such as soil erosion, water contamination, pesticide poisoning, falling groundwater table, waterlogging and depletion of biodiversity (Lipper *et al*., 2014).

1.1. Chemical fertilizers

1.1.1 Chemical fertilizers based on agriculture

Fertilizers increase efficiency and obtain a better quality of product recovery in agricultural activities. Non-organic fertilizers mainly contain phosphate, nitrate, ammonium and potassium salts, and these are required to enhance plants' natural ability to resist stress from drought and cold, pests and diseases (Tsai *et al.*, 2007). Current soil and agriculture management strategies mainly depend on the continuous use of inorganic chemical-based fertilizers, which are industrially manipulated substances, predominantly water-soluble and contain high available nutrient concentrations. However, chemical

fertilizer use has increased exponentially worldwide, causing severe environmental problems. Fertilization may lead to heavy metals in soil and affect the plant system by absorbing the fertilizers through the ground and entering the food chain. Thus, fertilization leads to water, soil and air pollution (Youssef *et al.,* 2014).

1.1.2 Effects of Chemical Fertilizers on Water Pollution

Nowadays, human beings are aware of nitrogenous fertilizers' harmful effects on the environment. Nitrogen in agricultural areas reaches the water environment in three ways: Drainage, leaching and flow. Nitrate leaching is mainly linked to agricultural practices such as fertilizing and cultivation. The majority of nitrogenous fertilizers are not absorbed products, and they interfere with both underground and surface water. In some of the arid and semiarid regions, the irrigated agricultural land increased nitrate accumulation in the soil and the evaporation of water. One of the most critical water pollution parameters is nitrates, the fundamental component of fertilizer. Agricultural activities increase both the nitrate concentration of groundwater and surface water. Nitrate is the most common form of dissolved nitrogen in groundwater. However, it can be found in the form of nitrite (NO_2^-), nitrogen (N_2), nitrogen oxide (N_2O) and organic nitrogen. Nitrates from drinking water of the body are absorbed in the intestinal tract 4-12 h and are excreted by the kidneys. The mechanism, as well as the salivary glands, can concentrate nitrate. The primary toxic effect of nitrate concentrations in drinking water of 50 mg NO_3^- /L exceeds the value of the bowel in adults, digestive and urinary systems, and inflammation is seen (Sonmez *et al*., 2002).

1.1.3 Effects of Chemical Fertilizers on Soil Pollution

Soils have strong buffering power due to their components; thus, soil fertility deterioration and degradation of soil reactions lead to the imbalance of the current soil element. In addition, toxic substances accumulate within the vegetables and cause adverse effects in humans and animals. The high sodium and potassium-containing fertilizers negatively impact soil pH, soil structure, and the increasing feature of acid irrigation or other agricultural operations. The continuous use of acid-forming nitrogen fertilizers causes a decrease in soil pH, which leads to the declining efficiency of field crops and a sudden drop in the yield and quality drops (Savci, 2012).

1.1.4 Effects of Chemical Fertilizers on Air Pollution

Chemical fertilizers are one of the most critical inputs of fertilizers in agricultural production. When it is applied inadequate, productivity and quality rates cause significant losses. When used in excess, it causes air pollution by nitrogen oxides (NO, N_2O, NO_2) emissions. Gases in the atmosphere such as water vapour, carbon dioxide, methane, and hydrogen sulfide (H_2S) with chloro-fluoro hydrocarbons, such as halon gases, contribute to the greenhouse effect (Atilgan *et al*., 2007). Calcareous and alkaline soils are mainly applied to the soil surface structure, and ammonium fertilizers with urea can evaporate NH_3. Many soil and environmental factors can be controlled and directly proportional to ammonia concentration in the soil solution. Ammonia emissions from fertilized lands result in deposition on ecosystems and vegetation damage. NH_3 may oxidize and turn into nitric acid and sulfuric acid from industrial sources, creating acid rain after the chemical

transformations. Acid rain can damage vegetation and organisms that live in both lakes and reservoirs (Shaviv, 2001).

In this context, harnessing naturally-occurring processes such as those provided by soil and plant-associated micro-organisms presents a promising strategy to reduce dependency on chemical fertilizers. Biofertilizers are living microbes that enhance plant nutrition by mobilizing or increasing soil nutrient availability. Various microbial taxa, including beneficial bacteria and fungi, are currently used as biofertilizers as they successfully colonize the rhizosphere, rhizoplane or root interior. Soil and plant-associated microbes play a crucial role in ecosystem functioning by carrying out numerous biogeochemical cycles and organic matter degradation (Paul, 2015). For this reason, biofertilizers (microbial-based fertilizers) are considered crucial components of sustainable agriculture, with long-lasting effects on soil fertility (Bargaz *et al.*, 2018; Singh *et al.,* 2019). The term biofertilizer can be defined as formulations comprised of living microbial cells, either a single strain or multiple strains (mixed or consortium), that promote plant growth by increasing nutrient availability and acquisition (Riaz *et al.*, 2020). Biofertilizers can also provide other direct and indirect benefits for plant growth, such as phytostimulation, abiotic stress tolerance and biocontrol (Ferreira *et al.*, 2019; Liu *et al.,* 2020; Shirmohammadi *et al.,* 2020). Biofertilizers naturally activate the micro-organisms found in the soil. Being cheaper, effective, and environment friendly, biofertilizers are gaining importance for crop production, restoring the soil's natural fertility and protecting it against drought, soil diseases, and stimulating plant growth.

Biofertilizers are most commonly referred to as the fertilizer containing living soil micro-organisms to increase plants' availability and uptake of mineral nutrients (Vessey, 2003). It is expected that their activities will influence the soil ecosystem and produce supplementary substances for the plants. Biofertilizers also include organic fertilizers (manure, etc.), which are rendered in an available form due to the interaction of micro-organisms or due to their association with plants (Sujanya and Chandra, 2011). When biofertilizers are applied as seed or soil inoculants, they multiply and participate in nutrient cycling and benefit crop productivity (Singh *et al.*,2011) (**Fig.1**).

Biofertilizers keep the soil environment rich in all kinds of micro-and macro-nutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization, the release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha *et al.,* 2014; Sivakumar *et al.,* 2013) providing better nutrient uptake and increased tolerance towards drought and moisture stress. Biofertilizers differ from chemical and organic fertilizers because they do not directly supply any nutrients to crops and are cultures of particular bacteria and fungi, are relatively simple and have low installation costs. Biofertilizers produce higher growth rates and rice yields than chemical fertilizers (Alam and Seth, 2012). Therefore, biofertilizers can solve the problem of feeding an increasing global population when agriculture is facing various environmental stresses and changes (**Fig.2**).

Biofertilizers, (microbial inoculants) are artificially multiplied cultures of certain soil organisms that can improve soil fertility and crop productivity. Although the benefits

of legumes in enhancing soil fertility have been known since ancient times, and their role in biological N-fixation was discovered more than100 years ago, commercial exploitation of such natural processes is of most interest and practice. Latent cells of efficient strains of nitrogen-fixing, phosphate solubilizing or cellulolytic micro-organisms are used for application to seed, soil or composting areas to increase the number of such micro-organisms and accelerate those microbial processes which augment the availability of nutrients that can be easily assimilated by plants (Mazid *et al.*, 2011b).

The commercial history of biofertilizers dates back to 1895 using "Nitragin" by Nobbe and Hiltner with laboratory culture of *Rhizobium* sp. (Singh *et al.*,2019). In the late 1950s, several studies with arbuscular mycorrhizal fungi inoculants reported positive plant growth promotion (PGP) effects through phosphorus (P) uptake (Koide and Mosse, 2004). However, despite their numerous advantages and low cost, the commercialization of biofertilizers is not widespread. The reasons limiting their use are mostly related to inconsistent responses over different soils, crops and environmental conditions, along with practical aspects related to mass production, shelf-life, appropriate recommendations and ease of use for farmers (Debnath *et al.*, 2019).

1.2. Role of nutrients

1.2.1. Nitrogen: N_2-Fixation

Nitrogen (N) is an essential element for life, and it is the fourth most abundant element in all living biomass after hydrogen, carbon, and oxygen (Howarth, 2009). For example, N is an essential component of chlorophyll, amino acids, nucleic acids, and the

energy transfer molecule adenosine triphosphate (ATP) (Werner and Newton, 2005). One important source of N in soils is organic N which requires microbial mineralization to be converted to plant-available inorganic N, a combination of ammonification and nitrification (Paul, 2015). However, the major N reservoir is in the atmosphere as N_2, which is not directly used by plants and only becomes available through biological nitrogen fixation. This is an energy-intensive process by which the enzyme nitrogenase converts atmospheric N_2 to ammonia (NH_3), which is readily available for assimilation by plants and microbes (Dakora *et al.,* 2008). Nitrogenases can be found in a small and diverse group of micro-organisms called diazotrophs (N_2-fixing), including symbiotic bacteria, free-living bacteria and archaea (Moreira-Coello *et al.,* 2019). In agriculture, the most studied symbiotic N_2-fixing organisms are bacteria known as rhizobia, comprised chiefly of the family Rhizobiaceae i.e., *Rhizobium*, *Bradyrhizobium, Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Sinorhizobium (Ensifer*) (Shamseldin *et al.,* 2017). Rhizobia can establish symbiotic relationships with legumes (family Fabaceae) by forming nodules on their roots or stems (Masson-Boivin and Sachs, 2018). These nodules provide an advantage for N_2-fixation in which nitrogenases are protected in bacteroids from atmospheric O_2. The oxygen concentration is essential in determining the fixed amount of N since oxygen is a negative regulator of *nif* gene expression and inhibits nitrogenase activity (Glick, 2015). Plants can acquire a significant proportion of their N requirement through associations with the diazotrophs (Dakora *et al.*,2008). For example, N_2-fixation could supply ~20–25% of the total N requirement in rice, ~30–50% in wheat and up to 70% in sugarcane (Hurek *et al.,* 2002; Gupta and Paterson, 2006; Santi *et al.,* 2013). Yet, the amount of N provided by BNF will vary depending on the plant species

and environmental factors, ultimately determining successful colonization (Parnell *et al.*, 2016).

1.2.2. Phosphorus: Solubilization and Mineralization

Phosphorus is one of the essential plant nutrients that directly or indirectly affects all biological processes. Phosphorus is critical in all significant plant metabolic processes such as photosynthesis, energy transfer, signal transduction, biosynthesis of molecules, and respiration. A considerable amount of P is present in soils, in both inorganic and organic forms, but its availability is one of the main factors limiting plant growth in many ecosystems worldwide (Raghothama, 2015). This is because most soil P is insoluble and unavailable for plants, which can uptake P from the soil solution as orthophosphate ions $H_2PO{-}4PO_4{-}$ and $HPO_4^{2}{-}HPO_4^{2}$- (Soumare *et al.,* 2020). Soil microbes can convert insoluble soil P into plant-available forms through various mechanisms of solubilization and mineralization (Alori *et al.,* 2017). Phosphate-solubilizing microbes (PSM) solubilize inorganic P (e.g., tricalcium phosphate, hydroxyapatite, and rock phosphate) via the production and release of different compounds. One mechanism consists of the excretion of organic acids, hydroxyl ions, and CO_2, which dissolves the insoluble phosphates directly by lowering the soil pH, leading to ion exchange of $PO_4^{2}{-}PO_4^{2}$- by acid ions (Wei *et al.,*2018). Microbes can also release chelating compounds that capture and mobilize cations from different insoluble phosphates such as Ca^{+2}, Al^{+3}, and Fe^{+3}, resulting in the release of associated soluble phosphates (Riaz *et al.,* 2020). The most studied P solubilizers belong to *Pseudomonas, Bacillus, Rhizobium, Enterobacter, Penicillium,* and *Aspergillus* (De Freitas *et al.,* 1997; Anand *et al.,* 2016). Another

essential process by which soil micro-organisms can increase P bioavailability is by mineralizing organic phosphate compounds (e.g., inositol hexaphosphate and phytate) (Alori *et al.,* 2017).

1.2.3. Potassium: Solubilization

Potassium (K) is a vital plant macronutrient and a major inorganic cation in the plant cytoplasm, essential for cell constitution and functioning, and implicated in photosynthesis, protein synthesis, and other primary metabolic functions. Potassium is also the second most abundant nutrient in the soil after N and one of the most abundant elements on Earth. However, ~98% of soil K is present in a non-exchangeable form, trapped within crystal structures of the minerals feldspar and mica (e.g., muscovite, biotite). Micro-organisms can increase K availability via solubilization, which plays a crucial role in the K cycle by making K available to plants (Sattar *et al.,* 2019; Macik *et al.,* 2020). Similar to P, the most well-known mechanism of microbial K solubilization involves the synthesis and discharge of organic acids (i.e., tartaric, citric, oxalic, gluconic, lactic, and malic acid) (Sattar *et al.,* 2019). These organic acids lead to the acidification of the surrounding environment and, therefore, the release (acidolysis) of K^+ from minerals (Sattar *et al.,* 2019). Other important K release mechanisms include chelation and exchange reactions involving organic acids (Sharma *et al.,* 2016). Several groups of soil bacteria (e.g., *Bacillus, Rhizobium, Acidithiobacillus, Paenibacillus*, *Pseudomonas*, and *Burkholderia*) and fungi (*Aspergillus, Cladosporium, Macrophomina*, *Sclerotinia, Trichoderma, Glomus*, and *Penicillium*) can solubilize K minerals (Kour *et al.,* 2020).

1.2.4. Sulfur: Oxidation

Sulfur (S) is an essential nutrient for plant growth, implicated in the conformation of biomolecules such as proteins, glutathione, chloroplast membrane lipids, coenzymes, and vitamins. Most S in soils (~95%) is in an organic form (C-bonded S or sulfate esters), while inorganic forms are less common (5–10%). The most common form of inorganic S is sulfate, which is readily available for plant uptake and either dissolved in the soil solution or adsorbed to soil particles (Scherer, 2009). The application of S-oxidizing microbes can help by optimizing S fertilization and minimizing environmental risks caused by S leaching. Sulfur-oxidizing bacteria can use S^0 as an energy source, releasing plant-available sulfate. Hence, their inoculation with S^0 fertilizers can speed up its conversion to sulfates, potentially leading to higher crop yields (Pujar *et al.*, 2014). Sulfur-oxidizing biofertilizers have been recommended for grain crops (e.g., oilseed species, oats) and horticultural crops (e.g., onion, cauliflower, ginger, garlic) (Santra *et al.*,2015). Sulfur oxidation in the soil is carried out by a variety of archaea and bacteria such as the genera *Xanthobacter*, *Alcaligenes, Bacillus, Pseudomonas*, *Streptomyces*, and *Thiobacillus*, as well as fungi including *Fusarium, Aspergillus*, and *Penicillium* (Grayston *et al.,* 1986; Germida and Janzen, 1993; Macik *et al.*, 2020).

1.2.5. Micronutrients: Chelation and Solubilization

Micronutrients such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), boron (B), molybdenum (Mo), chlorine (Cl), nickel (Ni), cobalt (Co), and silicon (Si), are essential for plants (Shukla *et al.*, 2018). These are essential for plant development as

they are involved in critical enzymatic reactions, including photosynthesis, respiration, water oxidation, and oxidative stress protection (Castro *et al.*, 2018). Several studies revealed that micronutrient deficiencies hamper crop production in many world areas, especially in alkaline soils with low organic matter content (Rashid and Ryan, 2004). One of the most studied mechanisms for increasing micronutrient availability is iron sequestration via siderophores (Rroço *et al.*, 2003).

1.3. Biofertilizers

Biofertilizers are live formulating of micro-organisms (beneficial bacteria and fungi) that are ready to be used and improve the quality and the health of the soil and the plant species by increasing the nutrient availability for the soil and plants (Abbasniayzare *et al.*, 2012). The specific micro-organisms used as microbial inoculants (biofertilizers) can be divided into two groups: symbiotic systems such as *Rhizobium spp.*, *Frankia spp*. and *Azolla spp*. and non-symbiotic systems such as *Azotobacter spp*., *Azospirillum spp*. and blue, green algae (Bashan and Holguin, 1997). Biofertilizers thus include the following, symbiotic nitrogen fixers *Rhizobium* spp., symbiotic free nitrogen fixers (*Azotobacter*, *Azospirillum*, etc.), algae biofertilizers (blue-green algae or BGA in association with Azolla), phosphate solubilizing bacteria, mycorrhizae, organic fertilizers (Goel *et al.*, 1999).

1.3.1. Blue-Green Algae (BGA) / Cyanobacteria

These phototropic prokaryotic bacteria are effective only in submerged paddy in the presence of bright sunlight by forming bluish-green algae on standing water and by

fixing N to the tune of 2-30 kg/ha, thereby raising the crop yield by 10-15% when applied at 10kg/ha/BGA biomass. These are phototrophic in nature and produce Auxin, Indole acetic acid (auxin) and Gibberellic acid; fix 20-30 kg N/ha in submerged rice fields as they are abundant in paddy (paddy organisms). Nitrogen is the key input required for low land rice production in large quantities. Soil nitrogen and biological nitrogen fixation by associated organisms are significant nitrogen sources for soft-land rice. The 50-60% nitrogen requirement is met by mineralizing soil organic nitrogen and nitrogen fixation by free-living and rice plant-associated bacteria.

Cyanobacteria are photosynthetic nitrogen fixers and are free living. They add growth-promoting substances, including vitamin B_{12}, thus improving the soil's aeration and water holding capacity and biomass when decomposed. Most nitrogen-fixing cyanobacteria are filamentous, consisting of a chain of vegetative cells, including specialized cells called heterocysts which function as micronodules for synthesis and nitrogen-fixing machinery. Cyanobacteria form symbiotic associations capable of fixing nitrogen with fungi, liverworts, ferns and flowering plants, but the most common symbiotic association has been found between a free-floating aquatic fern, the Azolla and *Anabaena azollae* (Cyanobacteria) (Rahman *et al.,* 2009).

1.3.2 Aquatic fern: *Azolla*

Azolla is an aquatic fern found in small, shallow water bodies and rice fields. The most common species occurring in India is *A. pinnata*. *Azolla* can be used as green manure or as a dual crop. *Azolla* is sown in the field or in a separate shallow pond for green manuring. It is a free-floating symbiotic fern found on the water surface in low land

fields and water bodies. Water is drained off the field, and Azolla is incorporated into the soil before transplanting the paddy. The critical factor in using *Azolla* as biofertilizers for rice crops is its quick decomposition in the soil and efficient availability of its nitrogen to rice plants. It has a symbiotic relationship with cyanobacteria and can help rice or other crops through dual cropping or green manuring of soil.

1.3.3. Cyanobacteria as biofertilizers:

Cyanobacteria have great potential as a source of fine chemicals, biofertilizers, and renewable fuel accumulators and degrade different kinds of environmental pollutants, including metal ions, salinity, and pesticides. Cyanobacteria can both photosynthesize and fix nitrogen, and at the same time, they can quickly adapt to different soil types. The critical role played by cyanobacteria is the maintenance and build-up of soil fertility, which further results in increasing rice growth and yield. The contributions of these organisms include (1) enhancement in soil porosity by a group of cyanobacteria having filamentous structure and production of adhesive substances; (2) excretion of growth-promoting substances such as hormones (auxin, gibberellin), vitamins, and amino acids; (3) increase in water holding capacity through their jelly structure; (4) increase in soil biomass following their death and decomposition ; (5) decrease in soil salinity; (6) prevention of weed growth and (7) increase in soil phosphate by excretion of organic acids. Beneficial effects of cyanobacterial inoculation were also reported on several other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli, and lettuce.

While working on the algae of Indian paddy fields, Gupta and Lata (1964) observed that cyanobacteria accelerated seed germination and promoted seedling growth. In addition, they also observed that both yield and quality of the grains were improved in protein content. The mechanisms used by microbes to stimulate plant growth include bio fertilization (increasing the supply of mineral nutrients to the plant), biological control (elimination of the plant enemies, including microbial pathogens, insects and weeds) and direct plant growth production by delivering plant growth hormones (Lugtenberg *et al.*, 1991). Bio fertilization techniques using cyanobacteria are recommended for increasing the rate of seed germination and growth parameters of many plants (Strick *et al.,* 1997).

Algae extract foliar application was recommended for increasing the growth parameters of potato (Awad *et al.,* 2006), tomato (Nour *et al.,* 2010), green gram (Pramanick *et al.,* 2013) and garlic plants (Shalaby and El-Ramady, 2014), Arafa *et al.,* 2011 on potato plants and Abo El-Yazied *et al.,* 2012 on snap beans, Zodape *et al.,* 2010 on green gram; Sarhan *et al.,* 2011 on cucumber. Most studies on the use of cyanobacteria as biofertilizers have concerned rice and a few crops like wheat, maize, and cotton, generally with an enhancement of the yield of rice (Mishra and Pabbi 2004; Karthikeyan *et al.,* 2007; Pereira *et al.,* 2009), and contents of N and other nutrients, sugar, amino acids, growth regulators, and protein in wheat (Wang *et al.,* 1991; Adam 1999; Nisha *et al.,* 2007). Inoculation of soil cultivated with maize with Tolypothrix tenuis and inoculation with only *Nostoc* increased maize yield (Maqubela *et al.,* 2009). Studies have also been carried out on cyanobacteria as a partial substitute for chemical fertilizers. De Cano *et al.,*. (1993) found that soil inoculation with *Tolypothrix tenuis* and

fertilization with urea increased stem length and rice growth. Zaccaro *et al.,*. (1999) found that soil fertilization with urea and soil inoculation with *Nostoc muscorum and T. tenuis* increased carbon content, dry weight, and shoot length of rice compared to control. Saswati-Nayak *et al.,*. (2004) reported that bio fertilization with blue-green algae and Azolla and fertilizer with urea significantly increased chlorophyll content of plant and rice yield chlorophyll content. Moreover, Pereira *et al.,*. (2009) reported that bio fertilization with a mixture of N fixing cyanobacteria (*Nostoc commune, Nostoc linckia, Nostoc sp., and Anabaena iyengarii var. tenuis*) decreased the use of nitrogen fertilizer by 50%, to get the exact grain yield and quality of rice compared with the total dose of chemical fertilizer.

1.4. Components of Biofertilizers:

1.4.1. The elements of biofertilizers include:

1. Bio Compost

It is one of the eco-friendly products composed of waste material released from sugar industries which are decomposed. It is magnified with human-friendly bacteria, fungi, and various plants.

2. Tricho-Card

It is an eco-friendly and nonpathogenic product used in a variety of crops as well as in horticultural and ornamental plants, such as paddy apple, sugar cane, brinjal, corn, cotton, vegetables, citrus, etc. It acts as a productive destroyer and antagonistic hyper parasitic against eggs of several bores, shoot, fruit, leaves, flower eaters and other pathogens in the field.

3. Azotobacter

It protects the roots from pathogens present in the soil and plays a crucial role in fixing atmospheric nitrogen. Nitrogen is an essential nutrient for the plant, and about 78% of the total atmosphere comprises nitrogen.

4. Phosphorus

Phosphorus is one of the essential nutrients for plants' growth and development. Phosphate solubilizing micro-organisms hydrolyze insoluble phosphorus compounds to the soluble form for plants uptake. Many fungi and bacteria are used for the purpose such as *Penicillium, Aspergillus, Bacillus, Pseudomonas, etc.*

5. Vermicompost

It is an Eco-friendly organic fertilizer that comprises vitamins, hormones, organic carbon, sulfur, and antibiotics that help increase the quantity and quality of yield. Vermicompost is one of the quick fixes to improve soil fertility.

1.5. Importance of Biofertilizers:

Biofertilizers are essential for the following reasons:

- Biofertilizers improve the soil texture and yield of plants.
- Inhibit pathogen growth.
- Eco-friendly and cost-effective.
- They destroy many harmful substances present in the soil that can cause plant diseases.

- Biofertilizers are proved to be effective even under semiarid conditions.
- The health of the people consuming the vegetables grown by the addition of chemical fertilizers is more at risk.

1.6. Sorghum (*Sorghum bicolor* (L.) Moench)

Grain sorghum (*Sorghum bicolor* (L.) Moench) is an annual cereal crop of great importance, especially in Africa, where it comes in the fifth order after rice, wheat, corn and barley. Among the forage crops, sorghum is very popular in semiarid zones, particularly in drought-prone regions of the world (Wenzel and Van Rooyen, 2001) due to its short duration, fast-growing nature, high productivity and wider adaptability to varied agro-climatic conditions. Sorghum (*Sorghum bicolor* (L.) Moench), locally known as 'juvar' or 'chari', has been under cultivation for grain feed and fodder in tropical countries since ancient times. It is most important, widely adaptable, and extensively grown as a fodder crop. It can withstand heat and drought and tolerate water logging better than other forage crops. The yield potential of sorghum is much higher than other forage crops, but the production is low (Singh *et al.,* 2016). Sorghum is a highly nutrient exhaustive crop; therefore, maintaining native soil fertility and health is necessary to achieve sustainable higher productivity. The balanced and conjugated use of inorganic fertilizer, bio compost, and biofertilizer is to maintain or adjust the soil fertility and plant nutrient supply to sustain desired crop productivity (Rakshit *et al.,* 2008).

1.7. Future Perspectives

Biofertilizers can prove a boon to sustain our agricultural production and meet the demand of the increasing population for agricultural-based products while conserving and maintaining the natural resources for the future generation. The importance of biofertilizers in enhancing the productivity and quality of agricultural products has already been proven through various research works carried out worldwide. Despite demonstrating their potential, biofertilizers remain underutilized on a large scale. Therefore, the need of the hour is to promote the application of biofertilizers among farmers to obtain higher agricultural sustainability, which can be achieved through awareness. Awareness should be created among farmers regarding the benefits of biofertilizers in providing good soil health, sustaining productivity of natural resources, and attaining high productivity and higher cost–benefit ratios. The main emphasis should be on the quality control during the production process of biofertilizers to keep their potency intact for a long time. The subsidies on biofertilizers should be provided to farmers to accelerate the use of biofertilizers among farmers.

The research on biofertilizers with multi-strain and multi-microorganism consortia should be carried out on a large scale to improve crop productivity. Compared to single-strain biofertilizers, multi-strain and multi-microorganism consortia can achieve higher productivity even under hostile growing situations. Biofertilizers should be made readily available for farmers, and large-scale production of biofertilizers should be initiated by providing training, and capacity building to industrial people, farmers, and other growers regarding production, quality control, and use of biofertilizers.

Despite their great potential and long-term effects, biofertilizer products still face significant challenges limiting their use in agricultural settings. These are often associated with limited shelf-life and the survival of inoculated strains in vastly different environments. At the same time, emerging culture-based methods (e.g., culturomics) can be used to discover novel isolates with biofertilizer applications. As an alternative, or in combination with, we suggest using 'plant prebiotics', that act as signaling molecules to attract beneficial microbes, thus enhancing biofertilizer efficiency.

However, the success of biofertilizers depends not only on selecting specific micro-organisms or functions but also on developing new formulations to ensure the survival of inoculated strains. Ideally, new technologies should target carriers and additives that are cost-effective and easy to use but, most importantly, support more viable cells during storage and application. Simultaneously, the biosafety of inoculated microbes should be assessed through a 'One Health' approach. This step includes proper screening tests (e.g., toxicity and pathogenicity testing) to ensure their safety before exposing personnel, consumers and natural resources.

One of the new challenges of the new millennium is obtaining more and more agricultural food production from shrinking per capita arable land. Biofertilizers have important and long-term environmental implications, negating the adverse effects of chemical fertilizers. Liquid bio-fertilizers are unique liquid formulations containing the desired micro-organisms and their nutrients and special cell protectants or chemicals that

promote the formation of resting spores or cysts for longer shelf-life and tolerance to adverse conditions. Despite the great potential of biofertilizers to improve soil fertility, it has yet to replace conventional chemical fertilizers in commercial agriculture. Moreover, continued studies on ecological interactions and how plants shape their microbiome in agricultural systems are still essential. This is particularly critical in climate change, where key biogeochemical processes carried out by soil micro-organisms may be affected. Finally, the public and private sectors need significant resource inputs to fill critical knowledge gaps. This effort must be accompanied by the encouragement of regulatory agencies and policymakers supporting sustainable practices and biofertilizers, i.e., by creating awareness about biofertilizers among the public and farmers.

OBJECTIVES

The present study aimed to assess the comparative effect of biofertilizers (*Nostoc commune* and *Azolla pinnata*) and chemical fertilizer on morphological, physiological and biochemical parameters in *Sorghum bicolor*. (L.) Moench ensuring healthy environment by avoiding chemical pollution. This work is important to layout the response of *Sorghum bicolor*. (L.) Moench to biofertilizers and chemical fertilizer, by analyzing below mentioned parameters:

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

2. MATERIALS & METHODS

2.1. Plant material and growth conditions

Sorghum (*Sorghum bicolor*. (L.) Moench) seeds were surface sterilized with 0.2% sodium hypochlorite for 5 mins and repeatedly washed with distilled water to remove all the traces of the sterilizing agent. The seeds were soaked for 2 h 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 ℃±2 ℃ with a light intensity of ≈ 200 μmol $m^{-2}s^{-1}$.

2.1.1. Azolla pinnata

Azolla pinnata is a free-floating fresh water fern belonging to the family Azollaceae and order Pteridophyta (Kumar *et al.,* 2018). It is distinguishable into stem, leaves and roots. The stem is often called the rhizome. It is profusely branched and its upper surface is covered with dense leaves. The leaves are alternate and are arranged in two rows. Each leaf has two lobes, the upper lobe being aerial and green in colour. The lower lobe is thin and colourless and is completely submerged in water. The dorsal lobe encloses large mucilage filled cavities. Inhabiting these mucilage cavities is found a Cyanophycean Alga-*Anabaena azollae*. The relationship between alga and *Azolla* is symbiotic. While the alga provides nitrogen to the plant the latter gives it shelter. The rhizome on its lower surface produces simple roots either singly or in clusters. These roots help in stabilizing the plants in water (Raja *et al.,* 2012).

2.1.2. Nostoc commune

Nostoc commune is a species of cyanobacterium in the family Nostocaceae. It is a colonial species of cyanobacterium. It initially forms a small, hollow gelatinous globule which grows and becomes leathery, flattened and convoluted, forming a gelatinous mass with other colonies growing nearby. It is a terrestrial or freshwater species and forms loose clumps on soil, gravel and paved surfaces, among mosses. *Nostoc commune* can fix nitrogen from the atmosphere and can therefore live in locations where no nitrogenous compounds are available from the substrate. *Nostoc commune* contains photosynthetic pigments and the energy storing photosystems in membrane structures called thylakoids located in cytoplasm of the cells. It also contains pigments that absorb long and medium wavelength ultraviolet radiation, which enables it to survive in places with high levels of radiation (Wright *et al.,* 2001).

2.2. Collection and preparation of biofertilizer

Nostoc commune and *Azolla pinnata* were used as biofertilizers for this study. *Nostoc* was collected from the rocks of the Goa University plateau, and *Azolla* was collected from rice fields in Taleigao. After collection, both the specimens were washed with running tap water to remove micro-organisms and other extraneous matter. The samples were dried at room temperature and placed in the oven at 60ºC for 5 h to complete the drying process. The dried *Nostoc/Azolla* were ground to fine powder by mortar and pestle (**Fig. 3, 4**).

The liquid fertilizers were prepared by mixing 1g biofertilizer powder or chemical fertilizer from *Jai Kisaan Samarth from Zuari Agro Chem Ltd* per 1 litre of Hoagland solution or Hoagland-Nitrate solution.

2.3. Treatments conditions

The biofertilizer treatment was given as follows: -

Hogland solution (HS)+Nitrates	**Hogland solution (HS)-Nitrates**
CONTROL	CONTROL-NO_3 (C-N)
Azolla (A)	*Azolla*-NO_3 (A-N)
Nostoc (N)	Nostoc-NO_3 (No-N)
Azolla+Nostoc (A+No)	*Azolla+Nostoc*-NO_3 (A+No-N)
Chemical fertilizer (CH)	Chemical fertilizer-NO_3 (CH-N)

The biofertilizer application was imposed right from germination, and the plants were allowed to grow for 12 days, and watering was done every fourth day. For analysis, plants were harvested on the 13^{th} day.

2.4. Physiological and Biochemical analysis

2.4.1. Relative water content

Relative water content (RWC) of sorghum 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℃ 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:

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

2.4.2. Total biomass

Biomass analysis was carried out according to Chen *et al.*, (2014) using Ten random plantlets were 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:

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

2.4.3. Thin Layer Chromatography (TLC) analysis of pigments

2.4.3.1. Extraction of photosynthetic pigments

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 pigments analysis for TLC.

2.4.3.2. Qualitative separation of photosynthetic pigments

Separation of pigments was carried out using silica TLC plates, according to Sankhalkar (2000). 50 µL of pigment sample was loaded as discrete spots on TLC plates about 2 cm away from the bottom of the plate using a micropipette. The leaves were developed using an n-hexane: ethyl acetate: triethanolamine (2:1:0.5) solvent system. The spots were identified by calculating colour and their R_f values.

2.4.4. 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 2mL. The extract was kept overnight for incubation at 4℃. After 24 h the homogenate was centrifuged at 7000-8000 rpm for 10 mins at 4℃. The supernatant was used to measure the absorbance at 663, 645 and 470 nm using a UV-visible spectrophotometer (UV-2450, Shimadzu).

Chlorophyll a (Chl a) (mg/g FW) = $12.27 \times A_{663} - 2.69 \times A_{645}$

Chlorophyll b (Chl b) (mg/g FW) = $22.9 \times A_{645} - 4.86 \times A_{663}$

Carotenoids (mg/g FW) = $4.7 \times A_{443} – 0.27 \times (20.2 \times A_{665} + 8.02 \times A_{663})$

2.4.5. Measurements of photosynthetic efficiency

According to Sharma *et al.*, (1997), Photosynthetic efficiency measurements were done using a chlorophyll fluorescence monitoring system. Sorghum leaves were adapted to dark for 5 mins 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 $m^{-2}s^{-1}$. Maximum fluorescence (Fm) was measured by exposing the sample to a saturation light pulse (≈ 4000 μmol $m^{-2}s^{-1}$ for 0.06 s). Variable fluorescence (Fv) was calculated as **Fv = Fm – Fo** and the maximum quantum yield (Fv/Fm) ratio. Actinic light of ≈ 600 μmol $m^{-2}s^{-1}$ was allowed to reach the steady fluorescence yield (Fs), followed by a far-red pulse for 5 s.

2.4.6. Determination of seed germination

According to Mazhar *et al.*, (2016), determining seed germination was carried out. 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 **2.3**. 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

2.4.7. Total sugars content

2.4.7.1. Extraction of total sugars

According to Dubois *et al.*, (1956), total sugars were estimated with slight modifications. 0.5g of leaf tissue was weighed, cut into small pieces and hydrolyzed in 5 mL of 2.5 N Hydrochloric acid by placing 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 mins. The supernatant was used to estimate total carbohydrates.

2.4.7.2. Estimation of total sugars

0.5 mL of sample was taken, making the final volume 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 mins 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).

2.4.8. Protein Content

2.4.8.1. Extraction of Proteins

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

2.4.8.2. 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 mins. 0.5 mL of Folin-Ciocalteau reagent was added with appropriate mixing. The reagent mix was further incubated for 30 mins 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.

2.4.9. Total lipid content

2.4.9.1. 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 (1:2 v/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℃. 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 mins 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 mins, and 2.5 mL of 0.88% potassium chloride was added. On vigorous shaking for 5 mins, the extract was kept for separation for 30 mins. 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.

2.4.9.2. 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 lipid 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 mins 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).

3. RESULTS

3.1. 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 biofertilizers on relative water content was measured in *Sorghum bicolor* (L.) Moench. Leaf (**Fig. 5**; **Table 1**). RWC was increased in plants grown in Hoagland solution containing all nutrients treated with *Azolla* (3%), *Nostoc* (3%), a combination of *Azolla+Nostoc* (2%), and chemical fertilizer by 4% as compared to untreated plants. Plants treated with chemical fertilizer showed higher RWC than plants treated with *Azolla*, *Nostoc*, and a combination of *Azolla+Nostoc*. Individually *Azolla* and *Nostoc* treated plants showed an increase in RWC compared to the combination of *Azolla + Nostoc* treatment.

Plants grown in Hoagland solution (absence of nitrates) treated with *Azolla*, *Nostoc*, and a combination of *Azolla+Nostoc* and chemical fertilizer showed an increase in RWC by 3%, 4%, 0.2%, and 1.6%, respectively, as compared to control plants (absence of nitrates). On the other hand, plants grown in *Nostoc* showed greater RWC than other biofertilizers and chemical treatments. Results obtained in this study depict that treatment with *Azolla* and *Nostoc* alone and chemical fertilizer increased the RWC as compared to other treatments.

3.2. Determination of Biomass

Shoot and root biomass were determined from plants treated with biofertilizers and chemical fertilizers grown in Hoagland and Hoagland solutions without nitrates (**Fig. 6, 7, 8 and Table 1**). Plants grown in Hoagland solution containing all nutrients treated with *Azolla* showed an increase in the shoot by 5% compared to control plants. Whereas plants treated with *Nostoc*, a combination of *Azolla+Nostoc,* and chemical fertilizer showed a decline in shoot biomass by 0.5%, 18%, and 11%, respectively, compared to control plants. The root biomass of plants treated with *Azolla*, *Nostoc*, and chemical fertilizer increased by 39%, 65%, and 4%, respectively, as compared control plants. Plants treated with a combination of *Azolla+Nostoc* showed a decline in shoot and root biomass by 18% and 23%.

Plants grown in Hogland solution (absence of nitrates) with *Nostoc*; a combination of *Azolla+Nostoc* and chemical fertilizer showed an increase in shoot biomass by 8%, 15%, and 38%, respectively, as compared to control plants (absence of nitrates). In comparison, plants treated with *Azolla* showed a decline in shoot biomass by 28% as compared to control plants (absence of nitrates). Chemical fertilizer treated plants in Hoagland solution (absence of nitrates) showed more shoot growth than *Azolla*, *Nostoc,* and a combination of *Azolla+Nostoc* treated plants. The root biomass of plants treated with *Nostoc*, a combination of *Azolla+Nostoc,* and chemical fertilizer showed an increase of 25.8%, 85.7%, and 44.1%, respectively, compared to control plants (absence of nitrates). In comparison, plants treated with *Azolla* showed a decline by 16% compared to control plants (absence of nitrates). Plants treated with a combination of Azolla +

Nostoc showed greater root biomass than Azolla, Nostoc, and chemical fertilizer-treated plants.

3.3. Determination of seed germination

The effect of fertilizers on seed germination rate was measured in control and treated plants (**Fig. 9, 10 and Table1**). Seeds treated with Hoagland solution containing all nutrients with *Azolla*, *Nostoc*, a combination of *Azolla+Nostoc*, and chemical fertilizer showed an increase in germination rate by 15%, 40%, 50%, and 20%, respectively, as compared to the control plants. Seeds treated with a combination of *Azolla+Nostoc* showed a higher rate in comparison to *Azolla* and *Nostoc* alone.

Seeds treated with Hoagland solution (absence of nitrates) with *Azolla*, *Nostoc*, a combination of *Azolla+Nostoc*, and chemical fertilizer showed reduced germination rates by 9%, 27%, 30%, and 8%, respectively, as compared control plants (absence of nitrates).

3.4. Determination of Photosynthetic efficiency (Fv/Fm ratio)

The Fv/Fm ratio, which indicates photosynthetic efficiency, was measured in control and treated plants (**Fig.11 and Table 2**). The Fv/Fm ratio decreased in plants grown in Hoagland solution containing all nutrients with *Azolla*, *Nostoc*, a combination of *Azolla+Nostoc* and chemical fertilizer by 5%, 17.5%, 12.5%, and 17.5% as compared to control plants. Plants treated with *Nostoc* showed the lowest Fv/Fm values as compared to control plants. In plants grown with Hoagland solution (absence of nitrates) with *Azolla* and a combination of *Azolla+Nostoc*, the photosynthetic efficiency increased

by 15% and 12%, respectively, as compared to control plants (absence of nitrates). However, *Nostoc* and chemical fertilizer treated plants showed a decrease in photosynthetic efficiency by 0.02% and 40% as compared to control plants (absence of nitrates).

3.5. Estimation of Photosynthetic pigments

Various photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoids were measured in control and treated plants (**Fig.12, 13 and Table 2**). It was observed that in control as well as treated plants, the amount of chlorophyll a was maximum, followed by chlorophyll b and carotenoids. Plants were grown in Hoagland solution containing all nutrients with *Azolla*, *Nostoc*, and chemical fertilizer showed an increase in Chl a concentration by 26%, 21%, and 6%, respectively, compared to control plants. However, plants treated with a combination of *Azolla+Nostoc* showed reduced concentration by 21.4% as compared to control. A similar trend was observed in the amount of Chl b. The concentration of chlorophyll b increased in *Azolla*, *Nostoc*, and chemical fertilizer by 2%, 26%, and 0.7%, respectively, as compared to control plants. In comparison, plants treated with a combination of *Azolla+Nostoc* showed a reduced concentration of chlorophyll b by 26% as compared to control. The carotenoid concentration was reduced in all treated plants as compared to control.

Chl a concentration reduced in plants grown in Hoagland solution (absence of nitrates) with *Azolla*, *Nostoc,* and chemical fertilizer by 1.5%, 5.9%, and 16% as compared to control plants (absence of nitrates). In comparison, plants treated with a

combination of *Azolla+Nostoc* showed an increase in Chl a by 18% as compared to control (absence of nitrates). The Chl b concentration was reduced in all treated plants by 9%, 13.6%, 4%, and 21%, respectively. A similar trend was observed in carotenoid concentration. The levels of carotenoids were lowered in all treated plants as compared to control (absence of nitrates).

3.6. Qualitative separation of photosynthetic pigments

Qualitative separation of photosynthetic pigments was done by Thin Layer Chromatography (TLC) (**Fig.14**). TLC profile showed the presence of chlorophyll a, Chlorophyll b, and β-carotene. There were qualitative changes observed in the photosynthetic pigment profile due to the different treatments.

3.7. Estimation of total sugar content

Total sugar content was determined in plants grown in Hoagland solution and Hoagland solution containing no nitrates along with biofertilizers and chemical fertilizer (**Fig.15 and Table 3**). Plants grown in Hoagland solution containing all nutrients with *Azolla* showed higher total sugar content by 10.7%, whereas plants treated with *Nostoc* and chemical fertilizer showed a decline by 2.5 % and 0.18%, respectively, as compared to control plants. On the other hand, plants treated with a combination of *Azolla+Nostoc* showed an increase as compared to *Azolla* and *Nostoc* alone.

Total sugar content was observed to be higher in plants grown in Hoagland solution containing no nitrates along with biofertilizers as compared to plants grown in

Hoagland solution containing all the nutrients along with biofertilizers. Plants grown in Hoagland solution (absence of nitrates) treated with *Azolla* and a combination of *Azolla+Nostoc* showed an upsurge in total sugar content by 2% and 1.71%, respectively, as compared to control plants grown in Hoagland solution (absence of nitrates). However, plants treated with chemical fertilizer (absence of nitrates) showed a decline of 4% as compared to plants grown in Hoagland solution (absence of nitrates).

3.8. Estimation of protein content

Protein content was measured in control and treated plants in Hoagland solution and Hoagland solution containing no nitrates along with biofertilizers and chemical fertilizer (**Fig.16 and Table 3**). The plants grown in Hoagland solution containing all nutrients treated with *Azolla*, *Nostoc*, a combination of *Azolla+Nostoc*, and chemical fertilizer showed an increase in protein content by 26.6%, 31.8%, 11.3%, and 17% as compared to control plants. Plants treated with *Nostoc* showed higher protein content as compared to *Azolla*, a combination of *Azolla+Nostoc* and chemical fertilizer.

In plants grown in Hoagland solution (absence of nitrates) treated with *Azolla* and *Nostoc* exhibited increased protein content by 11% and 3%, respectively, compared to control (absence of nitrates) plants. However, plants treated combination of *Azolla+Nostoc* and chemical fertilizer showed a decline by 1.5% and 18.6% as compared to control (absence of nitrates) plants. Plants treated with *Azolla* showed more protein content as compared to *Nostoc*, a combination of *Azolla+Nostoc*, and chemical fertilizer.

3.9. Estimation of glycolipid content

Glycolipid content was measured in control and treated plants in Hoagland solution and Hoagland solution containing no nitrates along with biofertilizers and chemical fertilizer **(Fig.17 and Table 3)**. Plants grown in Hoagland solution containing all nutrients treated with *Azolla* showed higher glycolipid content by 21%. In contrast, plants treated with *Nostoc*, a combination of *Azolla+Nostoc,* and chemical fertilizer showed lesser content by 49%, 71%, and 28%, respectively, as compared to control plants. The glycolipid content in plants treated with *Azolla* was high as compared to *Nostoc*, a combination of *Azolla+Nostoc* and chemical fertilizer.

In plants grown in Hoagland solution (absence of nitrates) treated with *Azolla*, a combination of *Azolla+Nostoc* and chemical fertilizer showed an increase in glycolipid by 25%, 400%, and 136%, respectively, as compared to control plants (absence of nitrates). In comparison, plants treated with *Nostoc* showed a decrease of 76% compared to control plants (absence of nitrates). The glycolipid content in plants treated with a combination of *Azolla+Nostoc* drastically increased as compared to *Azolla*, *Nostoc*, and chemical fertilizer.

4. DISCUSSION

Our results showed that different biofertilizer treatment increased biomass and RWC as compared to control and plants treated with chemical fertilizers **(Fig. 5, 6, 7, 8 and Table 1)**. This increase in biomass may be due to the increased nitrogen uptake being responsible for higher yield of crops (Hirel *et al.,* 2011) and increase in nitrogen content in plants was due to sustained availability of nitrogen because of nitrogen fixing ability of the biofertilizers (Razie and Anas, 2008). We observed individual biofertilizer treatment has beneficial effect on Sorghum growth compared to treatment with combination of biofertilizers. Garcha and Maan, (2017) reported increased crop yields in cereals (e.g., wheat, rice, and corn) (Khan, 2018) and a variety of other crops such as sunflower, carrot, oak, sugar beet, sugarcane, tomato, eggplant, pepper, and cotton due to *Azospirillum* species, which can carry out several PGP functions but are also the most well-known free-living diazotrophs, shown to enhance nitrogen availability and acquisition in more than 113 plant species (Pereg *et al.*, 2016; Zeffa *et al.,* 2019). Sghir *et al.,* (2014) reported that the application of different biofertilizers (alone or in combination) benefited plant growth mainly leaf number, shoot height, root length, leaf area, and total dry biomass production. The beneficial effect of these biofertilizers could be explained by the greater uptake of nutrients with low mobility such as P and N contained in the substrate. Biofertilizers also have been reported to integrate nutrition and fertilizer uptake by crop plants (Yedidia *et al.,* 2001), enhance plant development and improve leaf greenness (Harman, 2006).

Our results showed that the application of *Nostoc* and *Azolla* biofertilizers promoted the seed germination in comparison to the control and chemical fertilizer treatment **(Fig. 9, 10 and Table 1)**. This could be due to secretion of certain phytohormones such as auxins and gibberellins, etc., which are known to enhance seed germination and early development. Also, during metabolism, there is excretion of organic acids (citric acid, malic acid etc.), thus helping nutrient uptake at a later stage of growth. Early seed germination and better seedling establishment of mustard seeds with application of *Trichoderma* sp. treated wastewater have been reported by Molla and Khan (2018). Biofertilizer inoculation is proven to help nitrogen uptake by plants and support different physiological aspects of plant performances (Sharma *et al.*, 2010; Malusa *et al.*, 2016; Simarmata *et al.*, 2016). There have been positive effects of inoculating wheat seed with various biofertilizer sources on the crop yields (Bahrani *et al.*, 2010). Ahmed *et al.*, (2011) indicated that all the growth characters were significantly affected by inoculation of wheat grain with bio-organic fertilizers. The applications of biofertilizers in agriculture are suggested as a sustainable way of increasing crop yields and economize their production as well (Wali Asal, 2010). Bio-fertilization is very safe for humans, animals and environment to get lower pollution and saving fertilization cost. In addition, their application in soil improves soil biota and minimizes the sole use of chemical fertilizers (Sabashini *et al.*, 2007).

We reported increase in the photosynthetic efficiency and photosynthetic pigments in plants treated with different biofertilizers **(Fig. 11, 12, 13, 14 and Table 2)**. This positive effect of biofertilizers on the photosynthetic pigments may be due to the improvement of chlorophyll formation, and photochemical efficiency of leaf. 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 Al-Aghabary *et al.,* (2004). The high chlorophyll content indicates the better and healthy root system that functions properly leading to empower the plants to conquer better performance in water and nutrient up-take (Thakur *et al.,* 2010). Khan *et al.,* (2010) reported the positive effects of biofertilizers in the counteraction of the adverse effects of salt and water stress which may be due to the stabilization and protection of the photosynthetic pigments and the photosynthetic apparatus from oxidization. The different biofertilizers can mitigate the adverse effects of drought through increasing the content of IAA and GA3 and decreasing ABA level, which may be involved in protecting the photosynthetic apparatus and consequently increasing the photosynthetic pigments (Saeidi-Sar *et al.,* 2013). A positive correlation between leaf nitrogen fertilization and rate of the chlorophyll content is well documented for a number of plant species and has been investigated for rapid nitrogen determination for most major crops including corn, rice, 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 that reported by Ramakrishnan and Selvakumar (2012) who found that *Azotobacter* treated plants had the highest chlorophyll and protein contents. Similarly, biofertilizer 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 favourable 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 significant role in leaf chlorophyll content and photosynthetic improvement have also been reported (Guler *et al.,* 2016; Harman, 2011).

We also reported an increase in sugar content, protein content and glycolipid content in sorghum plants due to the treatment with different biofertilizers **(Fig. 15, 16, 17 and Table 3)**. The effect of biofertilizers on carbohydrate biosynthesis, especially soluble sugars, is considered to be the principle organic osmotica in a number of glycophytes subjected to saline conditions (Hassanein, 2004). Biofertilizer treatments results of the present study are in agreement with that reported by Ramakrishnan and Selvakumar (2012) who found that *Azotobacter* treated plants had the highest carbohydrate contents. Similarly, biofertilizer significantly improved sugar concentration in chilli plants (Selvakumar and Thamizhiniyan, 2011) and in black gram plants (Selvakumar *et al.,* 2012).

Plants treated with biofertilizer compared with other treatments, produced higher protein content, therefore their protein yield was highest. 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 increases the amount of protein. In addition, Shehata and Khawas (2003) showed that application of biological fertilizer on sunflower increased seed protein. The increase in the total proteins 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 biofertilizer treatments caused marked increase in glycolipid content of the sorghum plants. Zarei *et al.*, (2012) also observed that biofertilizer treatment caused the highest increase in total unsaturated fatty acid of three flax cultivars. Darzi *et al.*, (2009) stated that using organic and biofertilizers lead to a change in the composition of essential oil in the different plant species.

The current result showed that the biofertilizers 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 sorghum plants with better photosynthetic rate, stomatal conductance, specific relative chlorophyll contents and crop yield (Doni *et al.*, 2017). Biofertilizer 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.

Biofertilizers treatment with *Nostoc commune* and *Azolla pinnata* provided significant increase in nitrogen uptake and enhanced the yield of sorghum plant with better physiological and biochemical attributes even in the absence of inorganic nitrogen fertilizer application. The results indicated that use of biofertilizer 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 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.

5. CONCLUSION

Our study suggests that all the applied biofertilizers and chemical fertilizers caused changes in the Sorghum .plant's morphological, physiological and biochemical parameters. Compared to all the treatments, relative water content was increased in plants grown in Hoagland solution containing all nutrients treated with chemical fertilizer. However, Nostoc plants grown in nitrate absence showed greater RWC than other biofertilizers and chemical treatments. The shoot biomass increased in plants grown in Hoagland solution containing all nutrients treated with *Azolla*. Also, The root biomass of plants treated with *Azolla*, *Nostoc*, and chemical fertilizer increased compared to control plants. In the absence of nitrates, chemical fertiliser treated plants showed more shoot growth than *Azolla*, *Nostoc*, and *Azolla+Nostoc* treatment. However, plants treated with a combination of *Azolla+Nostoc* showed greater root biomass. The seed germination rate increased in seeds treated with biofertilizer and chemical fertilizer in Hoagland solution containing all nutrients. However, seeds treated with Hoagland solution (absence of nitrates) with biofertilizer and chemical fertilizer showed a reduced germination rate as compared control plants (absence of nitrates). The photosynthetic efficiency decreased in plants grown in Hoagland solution containing all nutrients with *Azolla*, *Nostoc*, a combination of *Azolla+Nostoc* and chemical fertilizer compared to control plants. Plants treated with *Nostoc* showed the lowest Fv/Fm values compared to control plants. In plants grown in the absence of nitrates with *Azolla* and a combination of *Azolla+Nostoc*, the photosynthetic efficiency increased compared to its control plants. Plants grown in Hoagland solution containing all nutrients with *Azolla*, *Nostoc*, and chemical fertilizer

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+Nostoc* showed an increase in Chlorophyll a compared to control in nitrate absence; however, The Chlorophyll b and carotenoids concentration was reduced. Plants grown in Hoagland solution containing all nutrients and treated with a combination of *Azolla+Nostoc* showed an increase in total sugar content. Plants grown in Hoagland solution (absence of nitrates) treated with *Azolla* and a combination of *Azolla+Nostoc* showed an upsurge in total sugar content compared to its control. Plants treated with *Nostoc* showed higher protein content than to all the treatments containing complete nutrients. Plants treated with *Azolla* showed more protein content than all the treatments grown in Hoagland solution containing no nitrates. The glycolipid content in plants treated with *Azolla* was high compared to all the treatments grown in Hogland solution containing all nutrients. Whereas in plants treated with a combination of *Azolla+Nostoc* the glycolipid content drastically increased compared to all the treatments grown in Hoagland solution (absence of nitrates). Biofertilizers treatment with *Nostoc* sp. and *Azolla* sp. provided a significant increase in nitrogen uptake and enhanced the yield of sorghum plants with better physiological and biochemical attributes even in the absence of inorganic nitrogen fertilizer application. The results indicated that the use of 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 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.

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Chapter – 1

INTRODUCTION

Chapter – 2

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DISCUSSION

Chapter – 5

CONCLUSION

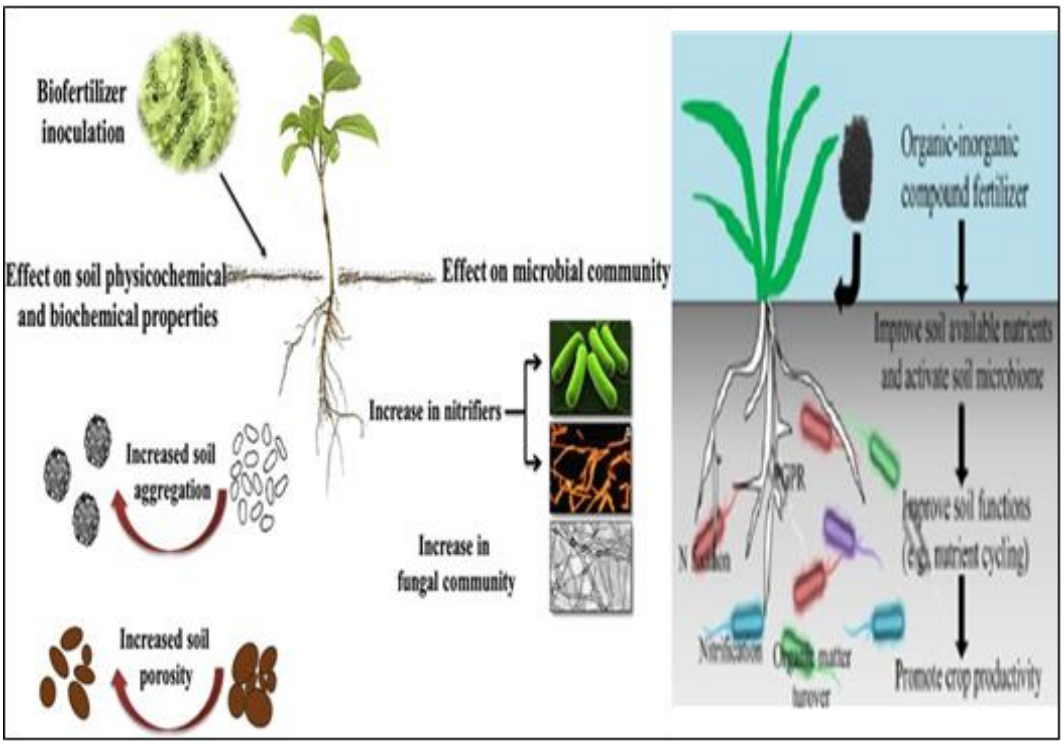


Fig.1. Biofertilizers: mode of action

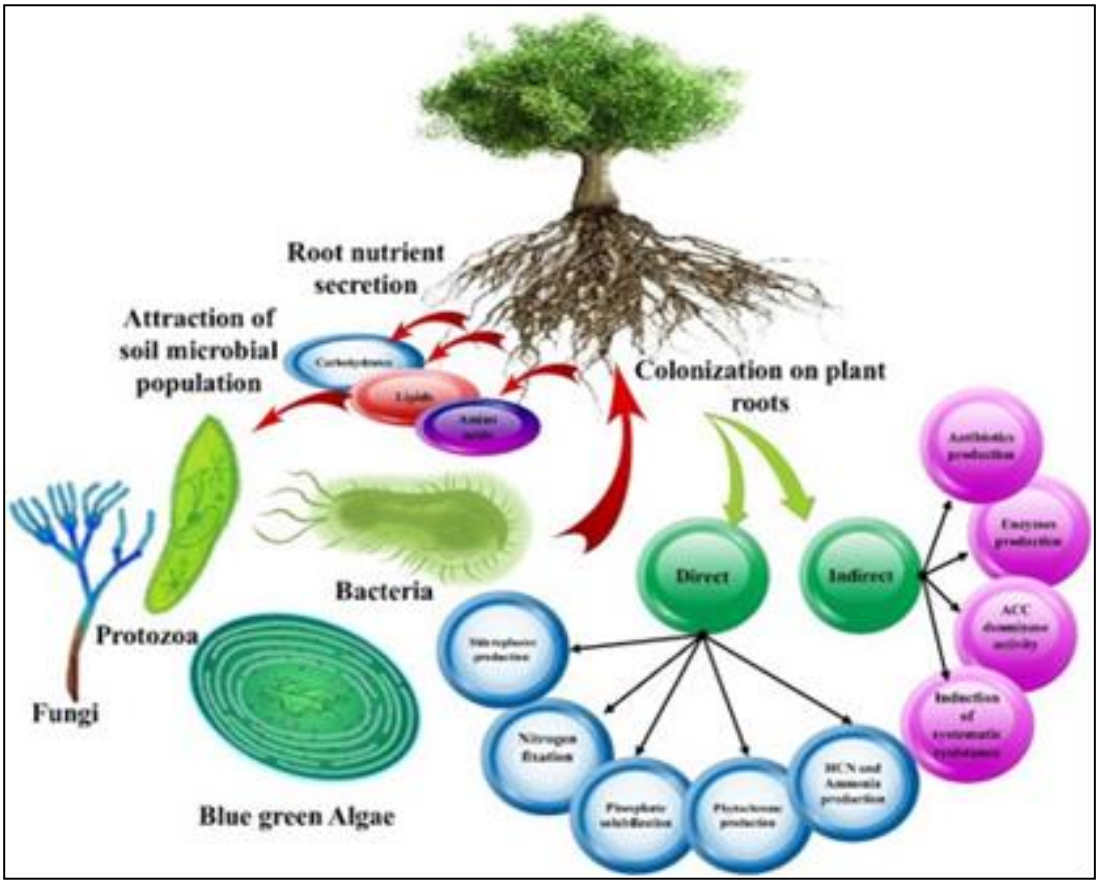


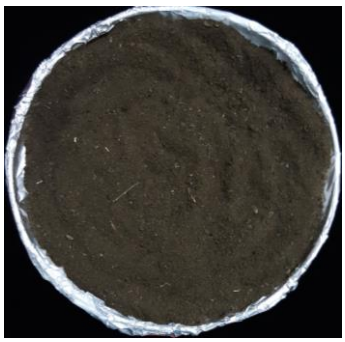
Fig.2. Role of Biofertilizers in a sustainable environment

Azolla pinnata collected from rice fields in Taleigao

Sample washed with running tap water

Microscopic image of *Anabaena azollae*

The sample dried at room temperature, placed in the oven at 60ºC for 5 h

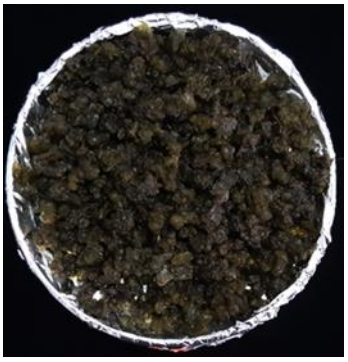


The dried *Azolla* ground to fine powder by mortar and pestle

Liquid biofertilizer prepared by mixing 1g *Azolla* powder per 1 litre of Hogland solution or Hogland – Nitrate solution

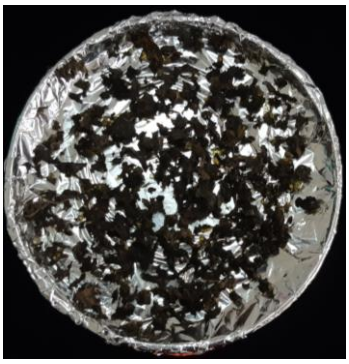
Fig. 3. Collection and preparation of *Azolla pinnata* biofertilizer

Nostoc commune was collected from the rocks of the Goa University plateau



Sample washed with running tap water

Microscopic image of *Nostoc commune*



The sample dried at room temperature, placed in the oven at 60ºC for 5 h

The dried *Nostoc* ground to fine powder by mortar and pestle.

Liquid biofertilizer prepared by mixing 1g *Nostoc* powder per 1 litre of Hogland solution or Hogland – Nitrate solution.

Fig. 4. Collection and preparation of *Nostoc commune* biofertilizer

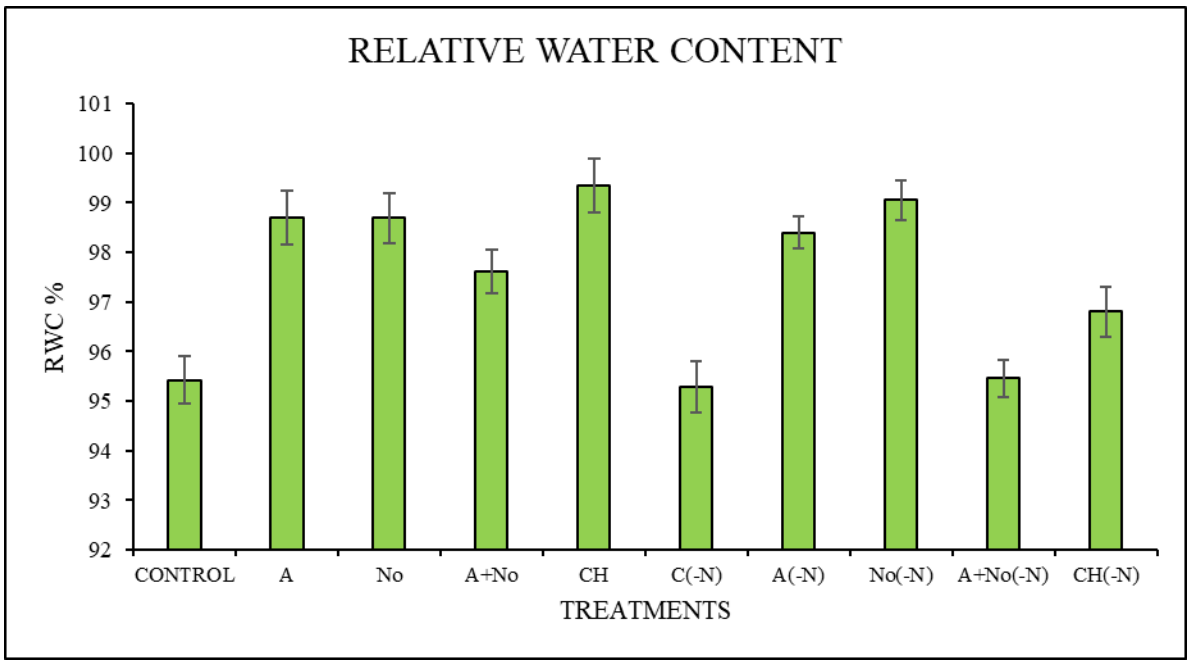


Fig. 5. Effect of fertilizers on RWC in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+Nostoc, CH: Chemical fertilizer, C(-N): Control-NO_3, A(-N): *Azolla*-(NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

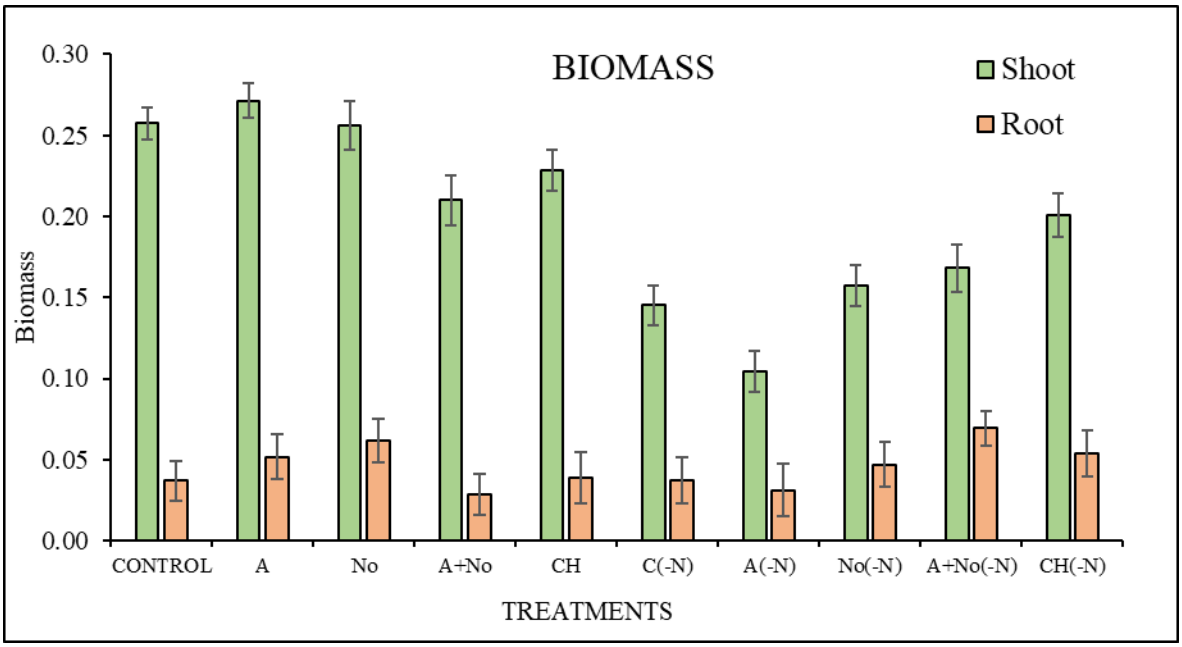


Fig. 6. Effect of fertilizers on Biomass (Shoot and Root) in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+*Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

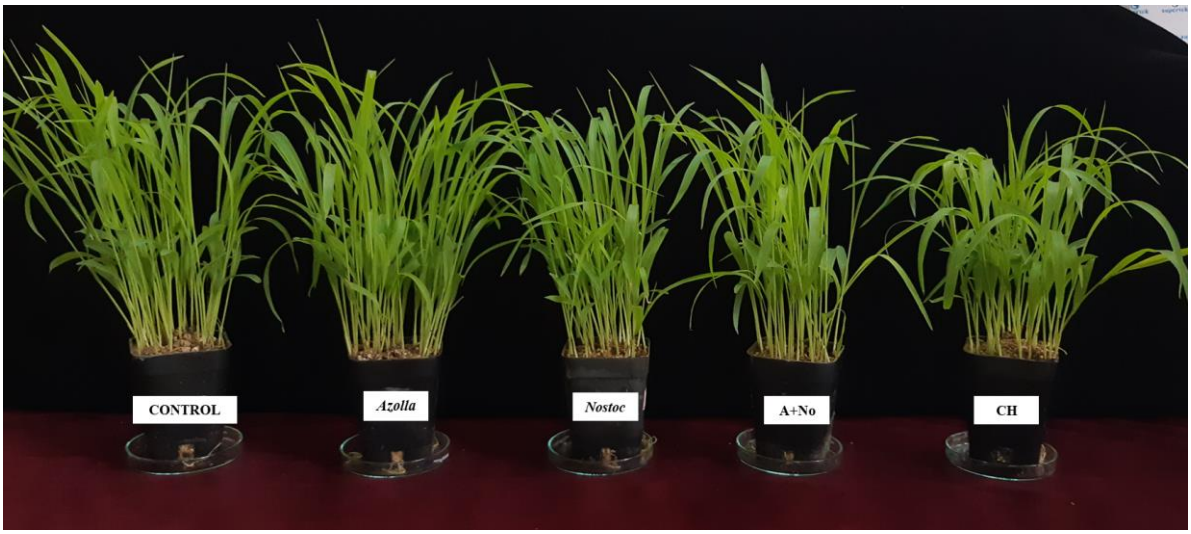


Fig. 7. Effect of fertilizers on the growth of *Sorghum bicolor*. (L.) Moench A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+ *Nostoc*, CH: Chemical fertilizer

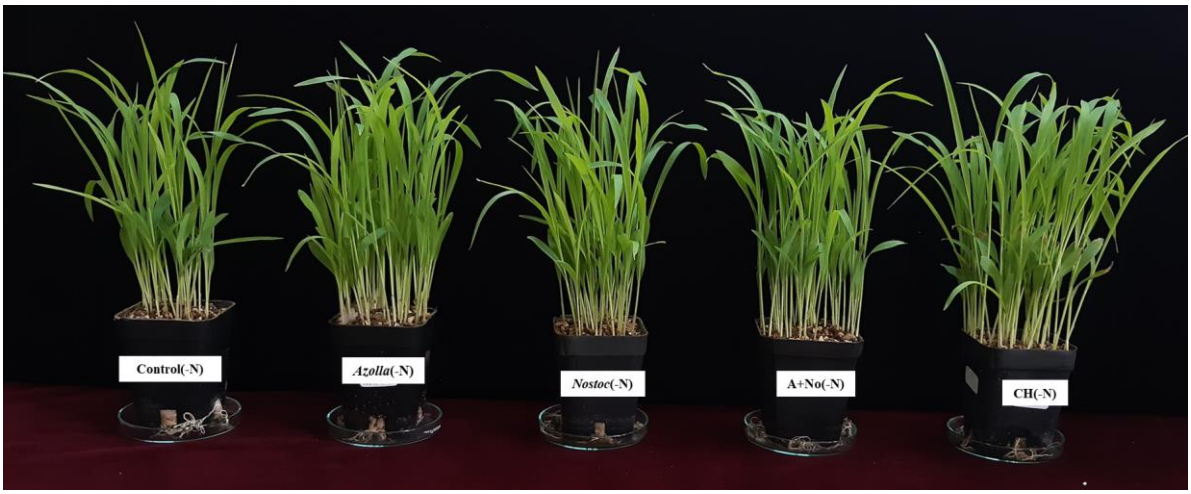


Fig. 8. Effect of fertilizers on the growth of *Sorghum bicolor*. (L.) Moench A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

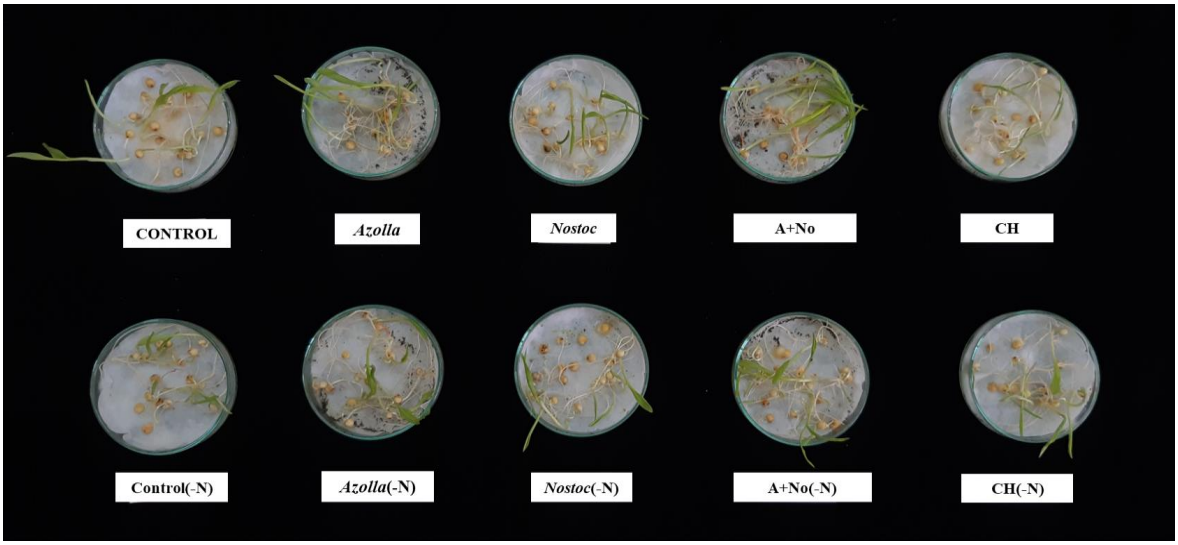


Fig. 9. Effect of fertilizers on Percent Germination in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+ *Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

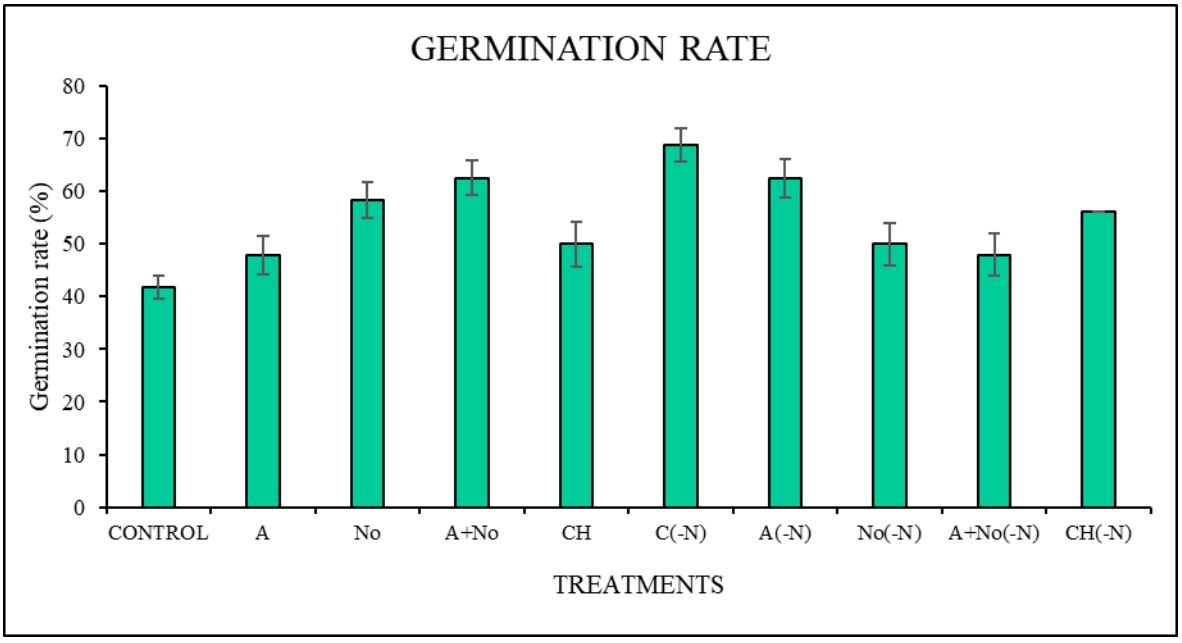


Fig. 10. Effect of fertilizers on Percent Germination in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+ *Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

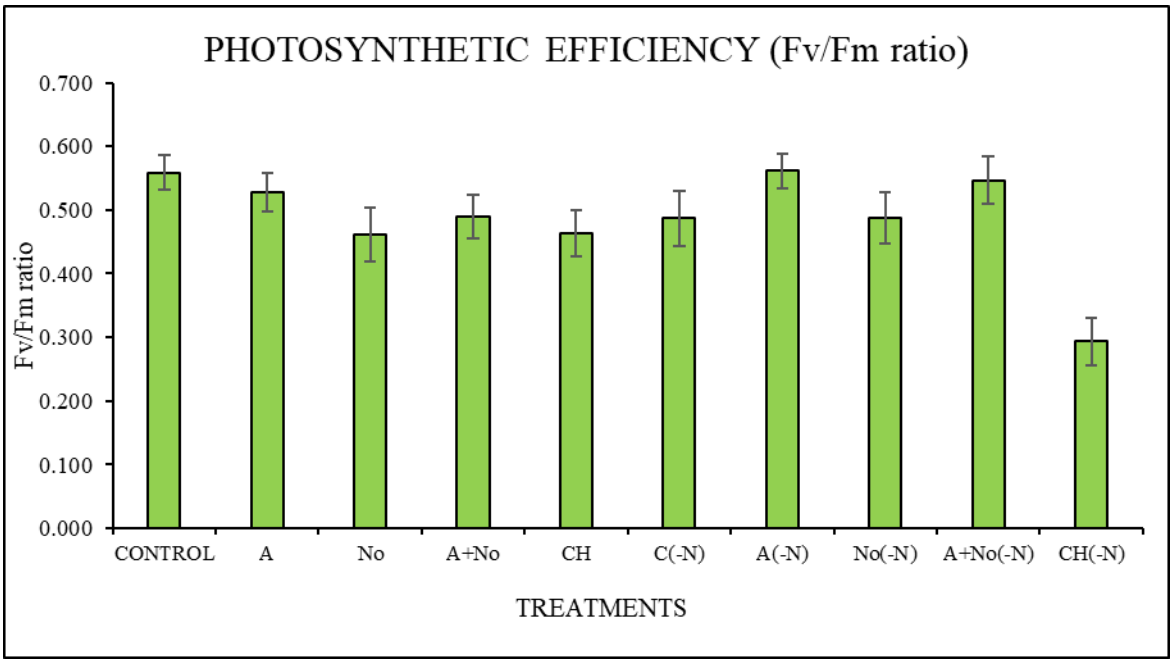


Fig. 11. Effect of fertilizers on Photosynthetic Efficiency in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+ *Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

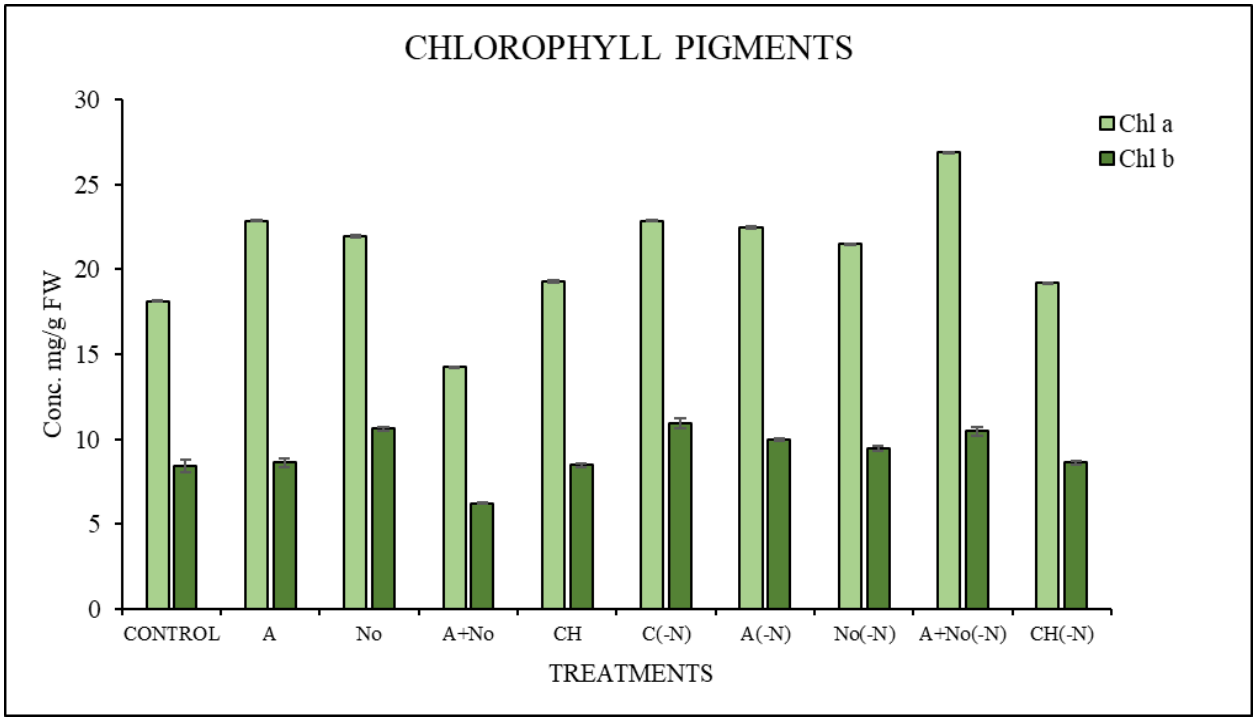


Fig. 12. Effect of fertilizers on Chlorophyll Pigments in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+ *Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

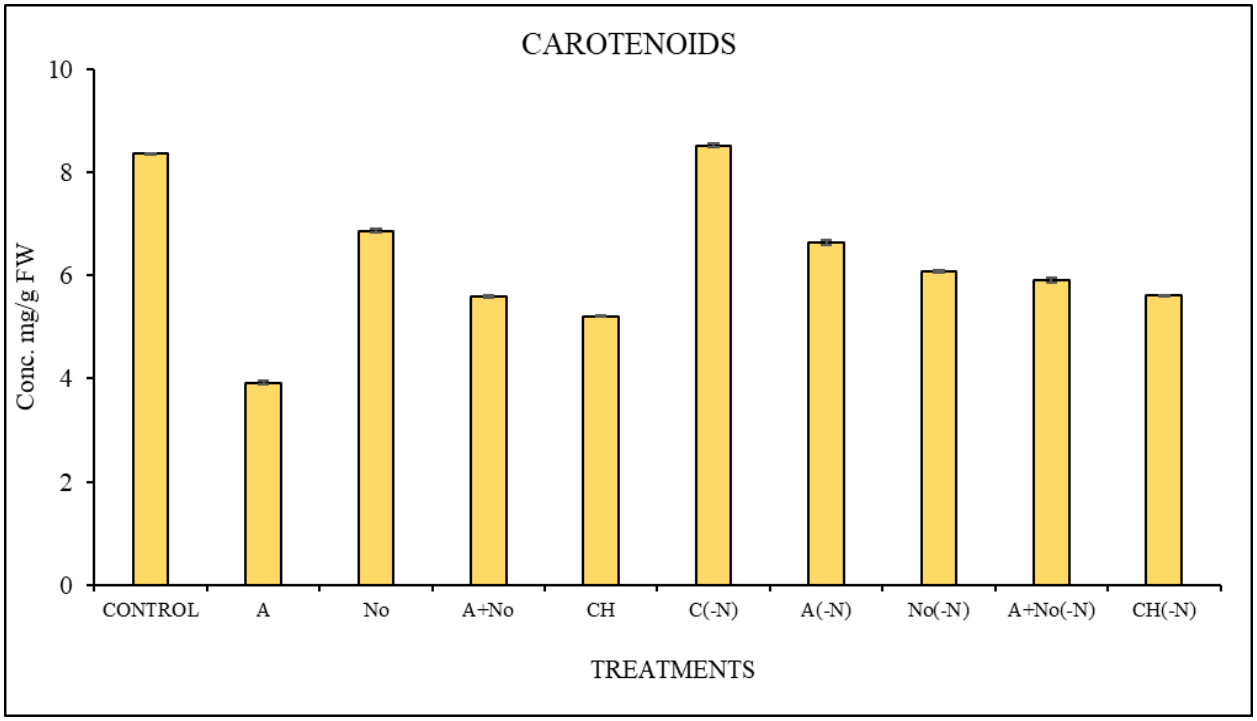


Fig. 13. Effect of fertilizers on Carotenoids in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+*Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

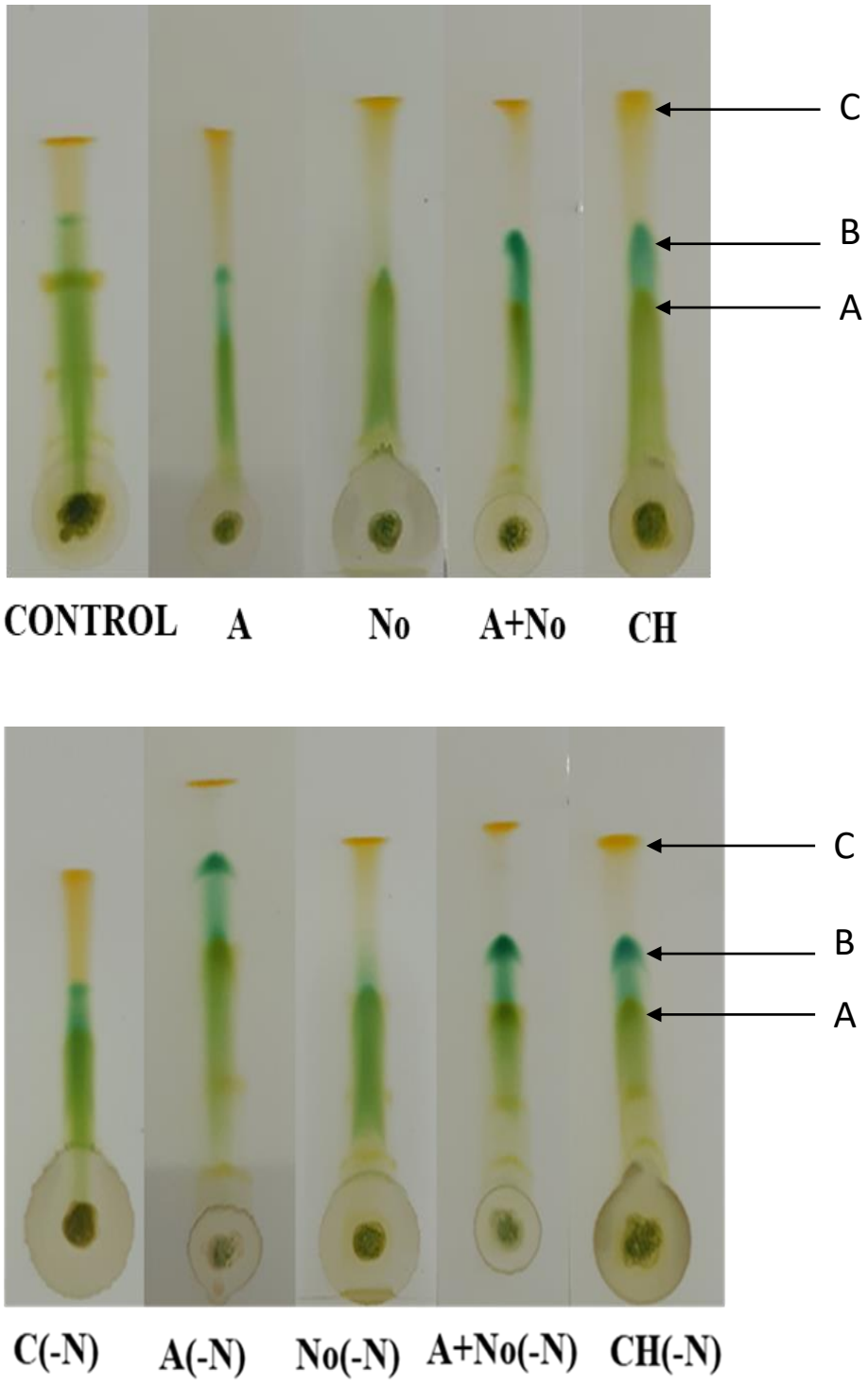


Fig. 14. Thin layer chromatogram of photosynthetic pigments in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+*Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla* +*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3); A-Chl b, B- Chl a, C- β-carotene.

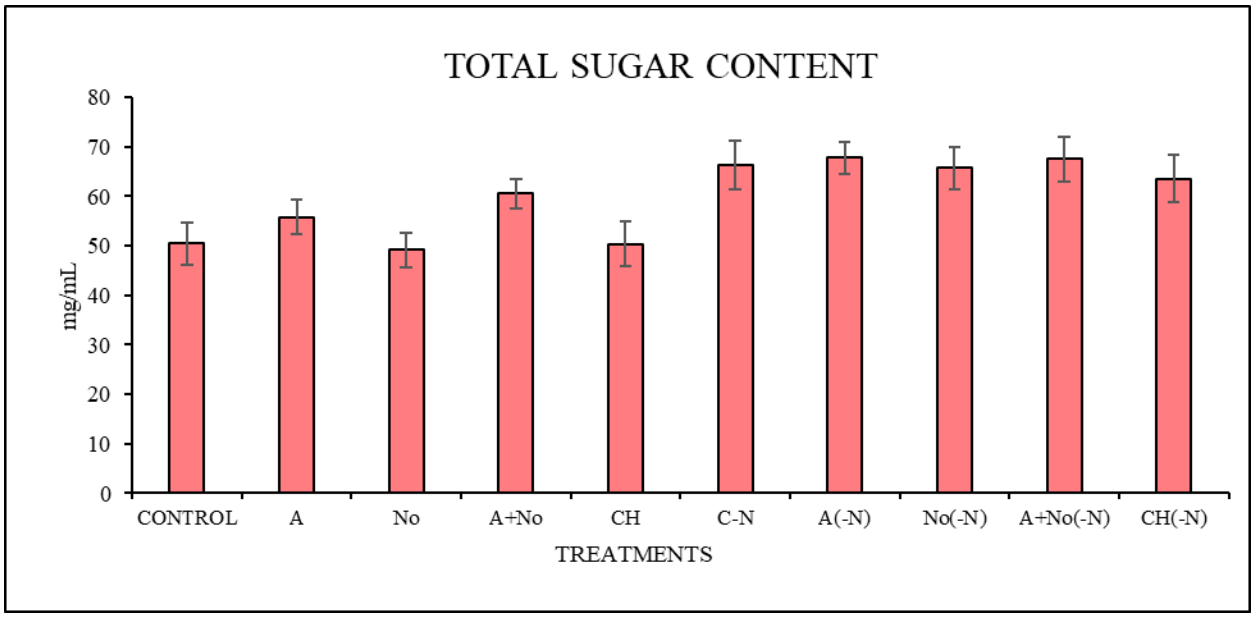


Fig. 15. Effect of fertilizers on total sugar content in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+ *Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla* +*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

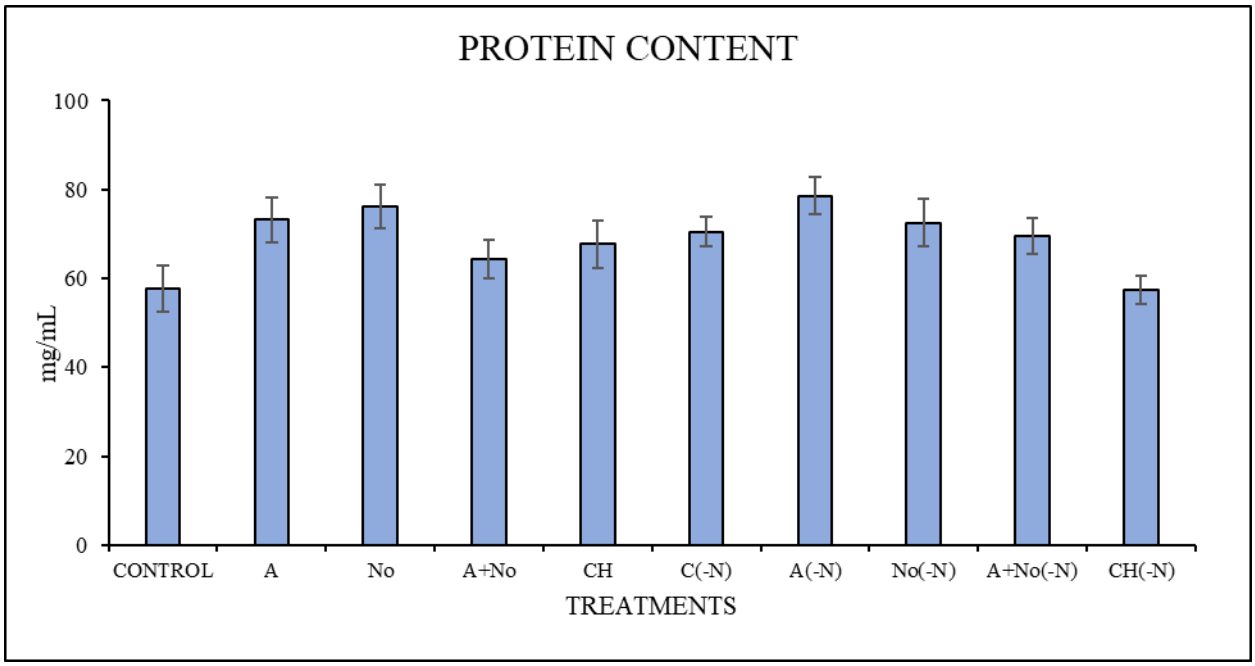


Fig. 16. Effect of fertilizers on protein content in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+*Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla*+*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

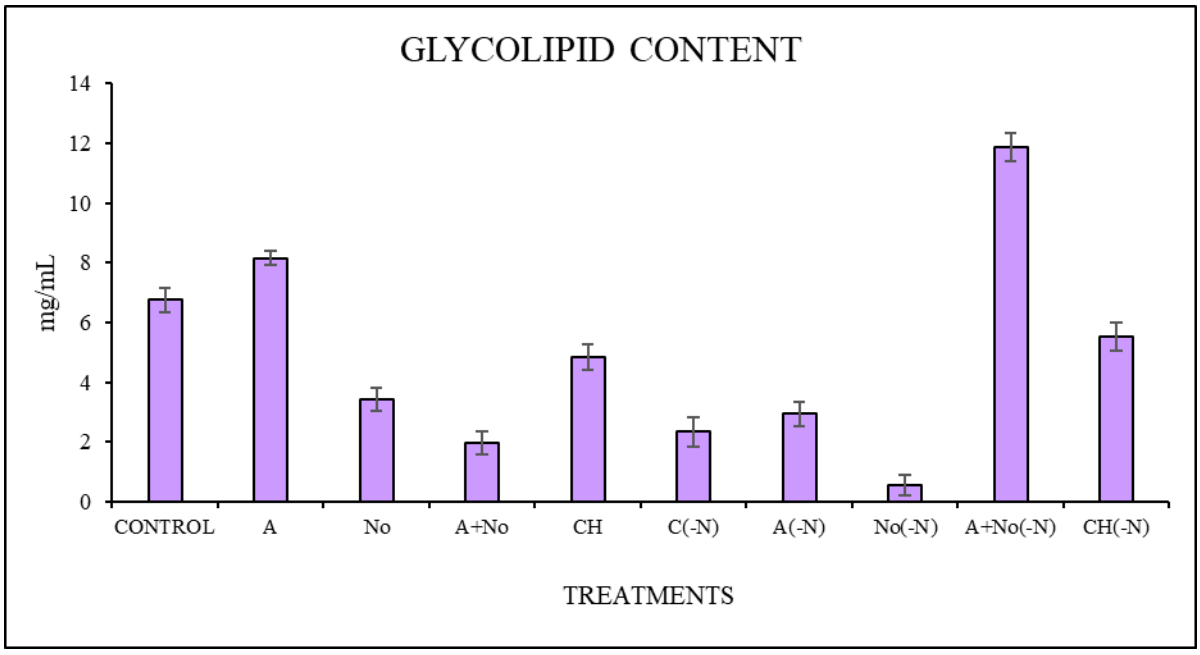


Fig. 17. Effect of fertilizers on glycolipid content in *Sorghum bicolor*. (L.) Moench. A: *Azolla*, No: *Nostoc*, A+No: *Azolla*+*Nostoc*, CH: Chemical fertilizer, C(-N): Control(-NO_3), A(-N): *Azolla*(-NO_3), No(-N): *Nostoc*(-NO_3), A+No(-N): *Azolla* +*Nostoc*(-NO_3), CH(-N): Chemical fertilizer(-NO_3)

Table 1. Effect of biofertilizer treatment on Relative water content, Percent germination, and Biomass (root and shoot) of *Sorghum bicolor*. (L.) Moench. (-N): absence of NO_3; where ± indicates standard deviation, n=3

Treatments	Relative water content (RWC) (%)	Germination (%)	Biomass	
			Shoot	Root
Control	95.42 ± 0.67	41.67 ± 2.18	0.257 ± 0.010	0.037 ± 0.012
Azolla	98.70 ± 0.73	47.92 ± 3.61	0.271 ± 0.011	0.052 ± 0.014
Nostoc	98.69 ± 0.53	58.33 ± 3.43	0.256 ± 0.015	0.062 ± 0.013
Azolla + *Nostoc*	97.61 ± 0.63	62.50 ± 3.25	0.210 ± 0.015	0.028 ± 0.013
Chemical	99.35 ± 0.63	50.00 ± 4.25	0.228 ± 0.013	0.039 ± 0.016
Control(-NO_3)	95.29 ± 0.52	68.75 ± 3.25	0.145 ± 0.012	0.037 ± 0.014
Azolla(-NO_3)	98.39 ± 0.32	62.50 ± 3.65	0.105 ± 0.012	0.031 ± 0.016
Nostoc(-NO_3)	99.05 ± 0.50	50.00 ± 4.83	0.157 ± 0.012	0.047 ± 0.014
Azolla + *Nostoc*(-NO_3)	95.45 ± 0.54	47.92 ± 4.02	0.168 ± 0.015	0.069 ± 0.011
Chemical(-NO_3)	96.80 ± 0.60	56.25 ± 0.00	0.201 ± 0.014	0.054 ± 0.014

Table 2. Effect of biofertilizer treatment on Photosynthetic efficiency and Photosynthetic pigments of *Sorghum bicolor*. (L.) Moench; (-N): absence of NO_3; where ± indicates standard deviation, n=3

Treatments	Fv/Fm ratio	Photosynthetic pigments (mg/g FW)		
		Chlorophyll a	Chlorophyll b	Carotenoids
Control	0.559 ± 0.027	18.14 ± 0.036	8.429 ± 0.352	8.352 ± 0.021
Azolla	0.528 ± 0.037	22.858 ± 0.037	8.618 ± 0.256	3.918 ± 0.032
Nostoc	0.461 ±0.041	21.957 ± 0.048	10.625 ± 0.117	6.863 ± 0.030
Azolla + *Nostoc*	0.489 ± 0.033	14.254 ± 0.046	6.242 ± 0.067	5.590 ± 0.016
Chemical	0.463 ± 0.035	19.292 ± 0.049	8.496 ± 0.110	5.210 ± 0.031
Control(-NO_3)	0.487 ± 0.043	22.853 ± 0.040	10.929 ± 0.272	8.515 ± 0.031
Azolla(-NO_3)	0.561 ± 0.027	22.503 ± 0.073	9.944 ± 0.070	6.634 ± 0.039
Nostoc (-NO_3)	0.487 ± 0.040	21.49 ± 0.032	9.439 ± 0.144	6.074 ± 0.026
Azolla + *Nostoc*(-NO_3)	0.547 ± 0.037	26.92 ± 0.013	10.467 ± 0.272	5.909 ± 0.044
Chemical(-$N0_3$)	0.293 ± 0.036	19.188 ± 0.024	8.631 ± 0.109	5.609 ± 0.010

Table 3. Effect of biofertilizer treatment on Total sugars (mg/mL), Protein content (mg/mL), Glycolipid content (mg/mL) of *Sorghum bicolor*. (L.) Moench; (-N): absence of NO_3; where ± indicates standard deviation, n=3

Treatments	Total sugar content (mg/mL)	Protein content (mg/mL)	Glycolipid content (mg/mL)
Control	50.441 ± 4.250	57.768 ± 5.152	6.749 ± 0.389
Azolla	55.819 ± 3.603	73.160 ± 4.991	8.159 ± 0.245
Nostoc	49.174 ± 3.491	76.180 ± 5.037	3.421 ± 0.385
Azolla + Nostoc	60.499 ± 2.990	64.315 ± 4.335	1.967 ± 0.367
Chemical	50.349 ± 4.601	67.752 ± 5.338	4.832 ± 0.435
Control(-N)	66.355 ± 4.922	70.537 ± 3.215	2.343 ± 0.503
Azolla(-N)	67.731 ± 3.243	78.531 ± 4.170	2.939 ± 0.406
Nostoc (-N)	65.712 ± 4.270	72.491 ± 5.328	0.569 ± 0.341
Azolla + Nostoc(-N)	67.493 ± 4.599	69.452 ± 4.043	11.870 ± 0.479
Chemical(-N)	63.565 ± 4.801	57.370 ± 3.220	5.532 ± 0.465