"Promotive effect of different carrier based *Nostoc commune* biofertilizers on *Triticum aestivum*"

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Promotive effect of different carrier based *Nostoc commune* biofertilizers on *Triticum aestivum*" is based on the results of investigations carried out by me in Botany Discipline at 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 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 Different Carrier Based *Nostoc commune* Biofertilizers On *Triticum aestivum*" is a bonafide work carried out by Ms Shrutika Subhash Naik under my supervision in partial fulfilment of the requirements for the award of the degree of Master of Science in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University.

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ABSTRACT

Wheat (*Triticum aestivum L.*) is a staple crop worldwide, and enhancing its productivity through sustainable agricultural practices is imperative for global food security. Biofertilizers, particularly carrier-based formulations containing beneficial microorganisms, have gained attention as eco-friendly alternatives to chemical fertilizers. This paper investigates the promotive effect of carrier-based *Nostoc* biofertilizers on wheat plants, focusing on their influence on morphological, physiological and biochemical parameters of wheat plants parameters. 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 different carrier such as ash, straw and neem powder were used in combination with *Nostoc*. Key findings highlight the role of carrier materials in providing a conducive environment for microbial activity, enhancing nutrient availability, and increasing the plant growth. Moreover, carrier based *Nostoc* biofertilizer application promotes root development, improves nutrient uptake efficiency, and induces increasing levels of protein, sugar and lipid content in wheat plants. The paper concludes by discussing the implications of these findings for sustainable wheat production and suggesting avenues for future research to optimize biofertilizer formulations and application strategies. Overall, this research contributes to the advancement of eco-friendly agricultural practices aimed at enhancing wheat productivity and sustainability.

Keywords: Wheat, biofertilizer, carrier-based, growth promotion, nutrient uptake, yield improvement, sustainable agriculture.

1. INTRODUCTION

The world's population has been increasing, and this, along with the effects of global warming and climate change, has negatively impacted agricultural productivity. According to the FAO, an estimated 815 million people in the world are undernourished as of 2017. Therefore, it is crucial to take necessary steps to improve agricultural productivity. These steps may include enhancing seed quality, optimizing germination conditions, implementing effective farming practices, and improving soil quality. Soil quality can be improved by using either chemical or biological fertilizers. Chemical fertilizers have been in popular use since the 20th century, particularly since their contribution to the Green Revolution. However, excessive and extensive use of chemical fertilizers has resulted in a large number of environmental problems (Savci, 2012); which include water, soil, and air pollution. Nitrate content from chemical fertilizers can get into water bodies by drainage, leaching, and flow. This causes eutrophication, leading to algal bloom and suffocation of aquatic life. Also, chemical fertilizers contain heavy metals, such as cadmium and chromium. Hence long-term use may result in the accumulation of inorganic compounds in the soil, degrading the quality of soil. Continuous use of chemical fertilizers causes soil degradation and deterioration of soil fertility, as it affects soil pH, and usually causes negative effects on soil organisms, such as worms, and soil mites. Chemical fertilizers contribute to air pollution during ammonia evaporation; oxidize to nitric acid and cause acid rain. Nitrogen oxide emissions cause global warming.

Biofertilizers have emerged as a solution to mitigate the harmful effects of chemical fertilizers, while also providing additional benefits. Biofertilizers are fertilizers that contain

living microorganisms, which can impact the soil ecosystem positively and produce supplementary substances for the plants (Parr et al., 2002). They contain live and efficient formulates of bacteria, algae, and fungi either separately or in combination that are capable of fixing atmospheric nitrogen, solubilizing phosphorus, decomposing organic materials, or oxidizing sulphur and; on the application will enhance the availability of nutrients for the benefits of the plants (Hanapi et. al., 2012). They also accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants.

1.1 BIOFERTILIZER

Biofertilizers are preparations containing living or latent cells of efficient strains of microorganisms that help crop plants uptake nutrients by their intentions in the rhizosphere when applied through seed or soil. Abdullahi et. al., (2012) provide a simple definition, describing biofertilizers as preparations of living cells or efficient microorganisms that help in the uptake of nutrients for the growth of plants. Biofertilizer is a substance that contains living organisms Vessey (2003). When applied to seeds, plant surface, or soil, they colonize the rhizosphere or interior of the plant. This promotes growth by increasing the supply and availability of nutrients to the host plant.

Biofertilizers are substances that enhance and stimulate specific microbial processes in the soil, **(Fig.2)** which help to increase the availability of essential nutrients in a form that plants can easily absorb. Using biofertilizers is an essential part of the integrated nutrient management system as they are not only cost-effective but also renewable and highly effective in enhancing soil fertility. Bio-fertilizers contain bacterial, fungal, or algal strains

and enhance the productivity of the soil by fixing atmospheric nitrogen (Peter et. al., 2015) by solubilizing soil phosphate or by stimulating plant growth for the synthesis of growth-promoting substances by increasing the availability of primary nutrients. They play the main role in the selective adsorption of immobile P, Zn, Cu, and mobile C, S, Ca, K, Mn, Cl, Br, and N elements to plants (Sivakumar et. al., 2013).

Biofertilizers can be applied to seeds, soil, plant surfaces, or composting areas to accelerate microbial processes which augment the availability of nutrients that can be easily absorbed by plants, harvesting the naturally available biological system of nutrient mobilization (Patil et. al., 2013). Biofertilizers can add 20-200 kg N ha–1 by fixation and increase crop yield by 10-50% (Asad et. al., 2004). They include Symbiotic Nitrogen Fixers (*Rhizobium spp*.), Symbiotic free Nitrogen Fixers, (*Azotobacter, Azospirillum* , etc.), algal biofertilizers (blue green algae or BGA in association with Azolla), phosphate solubilizing bacteria, mycorrhizae, organic fertilizers and NPK (nitrogen, phosphorous and potassium) from organic sources, such as FYM (farm yard manure) can be used as a sole source or as a substitute for inorganic fertilizers (Sujanya et. al., 2011).

Biofertilizers are a great alternative to chemical fertilizers. They are made up of biological components and are produced from microorganisms like bacteria, fungi or blue-green algae. These microorganisms in biofertilizers add nutrients to the soil through natural processes such as nitrogen fixation, solubilizing, and mobilizing phosphorus. Additionally, they synthesize growth-promoting substances, which are beneficial for plant growth.

1.2 NITROGEN-FIXING BIOFERTILIZER

Nitrogen is the most important nutrient for plant growth because it is a major component of chlorophyll and amino acids. (Day & Ludake, 1993). Although atmospheric nitrogen is relatively abundant (about 78%), fixed nitrogen is a major limiting nutrient for plant growth (Ohyama, 2010; Bhat et al., 2014). Atmospheric nitrogen can only become available to plants as ammonia (NH3), through a biological process known as nitrogen fixation. Nitrogen fixation is a process in which nitrogen in the atmosphere is converted into ammonia (NH3) (Postgate, 1998).

Nitrogen fixation is a process that occurs naturally in the air through the production of nitrogen oxide (NOX) during lightning (Hill et al., 1979). The NOX can react with water to form nitrous acid or nitric acid, which then seeps into the soil and becomes nitrate. Apart from this, nitrogen fixation can also be carried out biologically by nitrogen-fixing bacteria, which accounts for approximately 90% of nitrogen fixation (Encyclopaedia Britannica, 2018). These bacteria can be free-living, symbiotic, or associative symbiotic.

Free-living nitrogen-fixing bacteria**:** These include cyanobacteria (blue-green algae), *Azotobacter, Clostridium*. The reduction of atmospheric nitrogen to ammonia (nitrogen fixation) is catalysed by the enzyme, nitrogenase (Burk, 1934; Burk et. al., 1934) which requires a lot of energy. Free-living bacteria obtain the necessary nutrients for supplying this energy. Even as they exist in relatively small concentrations, they are especially important in fixing nitrogen for crops that do not favour symbiotic bacteria.

Symbiotic nitrogen-fixing bacteria**:** These bacteria form a beneficial relationship with the plant's roots. The plant provides the bacteria with nutrients in the form of exudates, which are sugars that act both as carbon source and energy source. The bacteria, in turn, invade the root hair, where they multiply and stimulate the formation of root nodules; within which they convert free nitrogen to ammonia, which the host plant utilizes for its development. This relationship is most common in leguminous species e.g. beans, and peas, ensuring their optimum growth.

Associative symbiotic nitrogen-fixing bacteria**:** This group of bacteria does not form symbiotic structures in the host plant. They, however, invade the cortical and vascular tissues of the host and enhance the growth of more lateral root hairs. This results in an increase in mineral uptake, which is due to phytochrome production.

1.3. CYANOBACTERIA (BLUE-GREEN ALGAE) AS BIOFERTILIZERS

Nostoc, Anabaena, and Oscillatoria are types of prokaryotic organisms that exhibit phototropism. **(Fig 1**) They are essential for enriching the soil in paddy fields by fixing atmospheric nitrogen and supplying vitamin B complex, as well as growth-promoting substances that help the plants grow vigorously. When applied, cyanobacteria can increase crop yield by 10-15%. According to a study by Youssef & Ali (1998), three species of blue-green algae - *Anabaena oryzae*, *Nostoc calcicola*, and *Spirulina* sp. were found to reduce the number of galls and egg masses caused by the root-knot nematode Meloidogyne incognita, which infects cowpea. This resulted in an overall improvement in plant growth.

Cyanobacteria like *Nostoc linkia, Anabaena variabilis, Aulosira fertilisima, Calothrix sp., Tolypothrix sp*., and *Scytonema* sp. are efficient nitrogen-fixing microorganisms emerging for sustainable agriculture. They are commonly found in rice crop cultivation areas (Prasad et al., 2001). *Anabaena* and *Nostoc* are types of cyanobacteria that can survive on the surface of soil and rocks. According to Malliga et al. (1996), cyanobacteria are capable of fixing up to 20-25 kg/ha of atmospheric nitrogen and also enriching soil with organic matter. Unlike other microbes, these bacteria do not require a host for their growth, development, and production of valuable organic products. As per Song et al. (2005), cyanobacteria play a crucial role in maintaining and building up soil fertility, contributing to higher yields as a natural biofertilizer.

The major actions of blue-green algae include;

(a) Make porous soil and produce adhesive substances.

(b) Excretion of phytohormones (auxin, gibberellins, etc.), vitamins, amino acids.

(c) Improve the water holding capacity of soil through their characteristic jelly structure.

(d) Increase in biomass of soil after their death and decomposition.

(e) Decrease in soil salinity.

(f) Controls weeds growth.

(g) Availability of soil phosphate by excretion of organic acids.

(h) Efficient absorption of heavy metals on the microbial surface (bioremediation).

1.4. *NOSTOC* AS BIOFERTILIZER

Nostoc is a genus within the family Nostocaceae, and its name has been in use in Europe for around 500 years (Potts, 1997). *Nostoc commune*, a terrestrial cyanobacterium also known as "Dimuer" in China, has been used for over 2000 years as a dietary supplement and herbal medicine (Hu, 2006; Li et al., 2003). Earlier studies have described the morphological structure of the filament, trichome, vegetative cell, heterocyst, spherical colony, and discoid colony of *Nostoc sp.* (Hu, 2006; Briones et al., 2007; Yan et al., 2010). The Thalli of N. commune is filled with winding algae filament, which is a colony of trichomes embedded in a sheath and packed with a capsule outside. The vegetative cells of N. commune are about 4.5-6 μm in length and 5 μm in width. Heterocysts occur in the middle or at the end of the filaments which are about 7 μm in diameter and are larger than the vegetative cells. Recent studies have shown that *N. Commune* contains rich proteins, amino acids, fatty acids, polysaccharides, flavonoids, vitamins, and many kinds of minerals. These compounds possess antitumor, anti-viral, anti-bacterial, and anti-inflammatory effects. *N. commune* has edible and medicinal value, which contributes to its popularity in China. Besides, it also promotes the growth of crops. The study by Chittapun et.al., (2018) found that introducing *Nostoc* cyanobacteria improved rice seedling growth and yield compared to the control group and showed a significant increase in root length.

In a recent study conducted by Ördög et al., (2021), it was discovered that the use of cyanobacterium *N. piscinale* as a biostimulant on maize (*Zea may*s L.) has positive effects on crop production. The study found that the use of cyanobacterium-based biostimulants resulted in a sustainable increase in maize yield, and is also environmentally safe. Another interesting investigation by Prasanna et al., (2013) proved that strains of *N. piscinale* in combination

with strains of other cyanobacteria such as *Nostoc carneum, Anabaena torulosa*, *Anabaena doliolum* using vermicompost-based carrier revealed through microscopic examination of soil enrichment cultures that, inoculation of these combined cyanobacteria significantly enhanced soil condition, including microbial biomass carbon, humus content and nitrogen, phosphorous and potassium (NPK) content, which is statistically at par with the fertilizer treatment. Nitrogen is one of the key factors that limit the development of rice. However, nitrogen fixation can increase the amount of nitrogen in the plant during its growth, which promotes the growth and development of rice while maintaining the original composition of soil attributes such as pH (Bisht and Chauhan, 2020).

1.5 SOLID-CARRIER BIOFERTILIZER

A carrier material is used as a vehicle for microorganisms that are utilized as biofertilizers (Brar et al., 2012) for seed or soil inoculation. These materials play a crucial role in maintaining the viability of microorganisms before their release into the field and provide a suitable microenvironment for rapid growth upon release, thereby enhancing their effectiveness. A carrier could be a material, such as peat, vermiculite, lignite powder, clay, talc, rice bran, seed, rock phosphate pellet, charcoal, soil, paddy straw compost, wheat bran or a mixture of such materials.

In order to increase the shelf-life of biofertilizer formulation, it is common practice to select a carrier material or a mixture of carrier materials based on the viability of the microorganisms mixed with them. For the preparation of seed inoculant, the carrier material used is a fine powder with a particle size ranging from 10 to 40μm (Ma & Kalaiyarasi, 2015).

According to the “Handbook for Rhizobia” (Somasegaran & Hoben, 1994), the properties of a good carrier material for seed inoculation are:

(i) Non–toxic to inoculant bacteria strain.

(ii) Good moisture absorption capacity.

(iii) Easy to process and free of lump-forming material

(iv) Easy to sterilize by autoclaving or gamma-irradiation.

(v) Available in adequate amount.

(vi) Inexpensive.

(vii) Good adhesion to seed.

(viii) Good pH buffering capacity.

(ix) Non–toxic to plant.

1.6 *Triticum. aestivum* L

Botanical Name: *Triticum aestivum L.*

Common Name: Ghau, Wheat

Plant Family: Poaceae (Gramineae)

According to the Rules of ICBN the names of the families should end in -aceae. Thus, the new name for the family Gramineae became Poaceae. However, the name Gramineae is also exempted and conserved under 'Nomina Conservanda' because of their constant use for a long time.

Plant Form: Grasses

Habit ;A small, tufted annual.

Leaves: Long, linear, narrow, pointed, sheathing at base, ligule small, loose, membranous.

Inflorescence and Flowers: Flowers in terminal spikes with or without awns, spikelets distichous, glumes 5 or more, shorter than spikelet, the lower glume broader on the outer side with a protection tooth on the upper angle, the keel awned, the upper sometimes with a palea, the other 2 glumes have palea and bisexual, the upper staminate or neutral, stamens 3, anthers versatile. Ovary superior, truncate, hairy at the apex, stigmas short.

Fruits; Caryopsis free or remaining in hull.

Flowering and Fruiting Time: February

Significance Extensively cultivated everywhere as a staple food plant.

Triticum is a genus of the family *Graminae* (Poaceae) commonly known as the grass family. Of the cultivated wheats, common wheat, *T. aestivum*, is economically by far the most important. *T. aestivum* L. as described by Lersten (1987), is a mid-tall annual or winter annual grass with flat leaf blades and a terminal floral spike consisting of perfect flowers. The vegetative state of the plant is characterized by tillers bearing axillary leafy culms. Culms comprise five to seven nodes with three to four foliage leaves. The uppermost, or flag leaf, subtends the inflorescence. Each culm produces an inflorescence or composite spike, the basic unit of which is termed the spikelet. Spikelets are born on a main axis, or rachis, and are separated by short internodes. Each spikelet is a condensed reproductive shoot consisting of two subtending sterile bracts or glumes. The glumes enclose two to five florets which are born on a short axis, or rachilla. Wheat florets contain three stamens with large anthers and the pistil which comprises a single ovary, with a single ovule, two styles, and two branching plumose stigmas at the end of each style.

2. REVIEW OF LITRETURE

Research on *Nostoc* as biofertilizers has been conducted over several decades, contributing to our understanding of its potential applications in agriculture. Early research focused on characterizing the nitrogen-fixing capabilities of *Nostoc* and its potential as a biofertilizer. Studies by Stewart et al., (1974) and Fay & Steward (1981) investigated the symbiotic relationship between *Nostoc* and various host plants, demonstrating its ability to enhance nitrogen availability in soil and promote plant growth. Studies conducted by Rai et al., (1985) and Rai & Singh (1993) investigated the effectiveness of *Nostoc*-based biofertilizers in crops such as rice, wheat, and legumes. The studies demonstrated improvements in soil fertility, nitrogen fixation, and crop productivity. Further research focused on understanding the nutrient dynamics of *Nostoc* and its impact on soil health. Studies by Prasanna et al., (1999) and Kumar et al., (2007) investigated *Nostoc's* ability to enhance soil microbial activity, improve nutrient cycling, and suppress soil-borne pathogens, thereby promoting sustainable agricultural practices. Recent research has explored biotechnological applications of *Nostoc* in agriculture and its environmental benefits. Studies by Kaushik et al., (2012) and Singh et al., (2019) investigated the use of *Nostoc* in biofertilizer formulations, microbial consortia, and bioremediation strategies, highlighting its potential to enhance soil fertility, reduce chemical fertilizer usage, and mitigate environmental pollution.

These earlier research efforts have provided valuable insights into the agronomic, ecological, and environmental benefits of using *Nostoc* as a biofertilizer. While further studies are needed to optimize its utilization and address specific challenges, such as compatibility with different crop species and environmental conditions, the foundational

knowledge generated by earlier research serves as a valuable resource for advancing sustainable agriculture practices.

Jacob & Kumar (2020) reported that the application of cyanobacteria biomass production and its use as a liquid fertilizer for hydroponic cultivation is a feasible option, thus, empowering plant growth and more yields. The cyanobacteria liquid fertilizer allows and stimulates the microbiota in a liquid fertilizer medium, enhancing nitrogen fixation. Also, cyanobacteria and other groups of algae produce various bioactive compounds, such as growth hormones, enzymes, polysaccharides, and antibiotic agents, in liquid fertilizer and soil, thus sustainable microalgae and cyanobacteria-based plant biofertilizers and bio-stimulants, can be used in the hydroponic vegetable cultivation.

Thamida et al., (2011) conducted a study on the impact of cyanobacteria and urea-N inocula on the growth and yield of two HYV of rice (BR-28, BR-29) in the field. The use of fertilizer resulted in a significant increase in the number of tillers, panicles, length of panicle, weight of grains, and yields of grain and straw compared to the control group. Hasan (2020) in his study on cyanobacteria from rice fields and comparative study of their performances as biofertilizer on rice plants, cyanobacteria (Blue-Green Algae) were isolated, identified, multiplied, and used as inoculums in pot rice experiment. Uniyal & Singh (2023) studied the impact of different carrier materials on the viability of *Nostoc sp*. of five different low-cost carrier (neem leaves powder, curry leaves powder, fuller's earth, soil and sand) on the viability of cyanobacteria. The carriers were integrated with the cyanobacteria and the formulation was stored in room temperature for four months. The viability of cyanobacterial cells was studied by measuring the chlorophyll content of the formulation every month. The

highest increase in chlorophyll content was recorded in neem leaves powder (320%) followed by curry leaves powder (271.53%) and sand (5.12%). Thus, the present investigation highlights the possibility that neem leaves powder can be a suitable carrier for cyanobacterial bioformulation that can be used to enhance agriculture production. Aloo et al., (2022) studied the effects of carrier materials and storage temperatures on the viability and stability of three biofertilizer inoculants obtained from potato (*Solanum tuberosum* L.) rhizosphere. Esch (2014) stated that the addition of *Nostoc* can increase plant height and leaf number of plants thus demonstrating *Nostoc's* potential as a sustainable biofertilizer. Gupta et. al., (2022) studied to evaluate how different carrier-based formulations of salt-tolerant PGPR performed in seedlings of pea and maize plants. The chosen PGPR was mass replicated in the lab and put into seedlings via a variety of carriers, including charcoal powder, dry pea peel powder, tea leaf powder, hay+2%peptone, and cowdung powder and results showed that seedling germination was better with the bioformulation made with charcoal and tea leaf powder.

Abd-Alla et. al., (1993) indicated that live inoculant and live inoculant plus K, P and S significantly increased dry weight, total nitrogen, and pigment contents of wheat plants over control and other treatments. The increase in growth parameters was due to the substantial increases of N2-fixation due to the nitrogenase activity of the cyanobacteria. Burjus et. al., (2020) studied the effects of the application of cyanobacteria (*Anabaena circinalis* and *Nostoc commune*) alone or in combination with reducing the dose of chemical fertilizers (CFs), which consisted of diammonium phosphate (DAP) and urea (46% nitrogen), on growth, yield and yield components of wheat cv. IPA99. The results indicated that the use of wheat grains coated with compost amended with cyanobacteria, grains coated with compost, and foliar spray with cyanobacteria did not change yield, yield components and most of growth parameters tested in both stations. This study suggests that this approach can be

applied to reduce the input of chemical fertilizers into the field thereby reducing the cost and pollution of agroecosystems. Kaur & Goyal (2019) studied the effect of BGA biofertilizers on rice crops using different carrier materials, i.e. fly ash (100%), soil (100%), montmorillonite (100%), fly ash + soil (1:1) and fly ash + montmorillonite (1:1) and results showed that fly ash with combination of soil (1:1) was observed as a good carrier material in place of soil or MMT alone for showing highest nitrogen, carbon and phosphorus content promoting cheap and adaptable method by farmers for organic farming.

Ashour et. al., (2023) studied the response of wheat to cyanobacteria and compost tea applications as a tool to achieve bio-organic farming concept and showed that the combined treatment significantly enhanced wheat growth, nutrients uptake, photosynthetic pigments, yield, and its components as well as the nutritional value of wheat grains and straw. The results suggested that combining cyanobacteria and compost tea to improve wheat plant growth, productivity, and yield quality attributes might be a simple and cost-effective strategy Dhar et. al., (2008) studied the comparative performance of three carrier based Blue Green Algal Biofertilizers for sustainable rice cultivation and highest grain yields were obtained with the application of multani mitti based biofertilizer.

Research on biofertilizers, including organisms like *Azolla* and *Nostoc*, has made significant strides in recent decades, but several research gaps persist. Identifying and addressing these gaps are crucial for advancing the field and maximizing the potential benefits of biofertilizers in sustainable agriculture.

There is a need to investigate the effectiveness of biofertilizers across diverse agroecosystems, including various soil types, climates, and cropping practices.

Understanding how biofertilizers perform in different agricultural settings is essential for widespread adoption and scalability. Biofertilizer formulations and application methods can significantly impact their efficacy and practicality. Research is needed to optimize biofertilizer formulations, considering factors such as carrier materials, microbial consortia, and application techniques (e.g., seed coating, foliar spray, soil inoculation). Standardized protocols for biofertilizer production and application would facilitate comparison and adoption by farmers.

Research should evaluate the long-term effects of repeated biofertilizer application on soil fertility, microbial diversity, carbon sequestration, and crop yields to ensure the sustainability of agricultural systems. Research is also needed to assess the resilience of biofertilizers to climate variability and extremes, as well as their potential role in climate change mitigation and adaptation strategies, such as carbon sequestration, drought tolerance, and nutrient efficiency. Addressing these research gaps requires interdisciplinary collaboration among agronomists, microbiologists, ecologists, economists, and social scientists.

OBJECTIVES

The present study aimed to comparative effect of carrier based biofertilizers and chemical fertilizer on morphological, physiological and biochemical parameters in *Triticum aestivum*. This work is important to layout the response of *Triticum aestivum* to biofertilizers and chemical fertilizer, by analysing below mentioned parameters:

A. Morphological parameters

- Percent germination (% germination).

B. Physiological parameters

- Leaf turgor
- Biomass
- Photosynthetic efficiency (Fv/Fm ratio).
- Photosynthetic pigments (Chlorophyll, Carotenoids etc.)

C. Biochemical parameters

- Total sugar content.
- Protein content.
- Lipids content.

3. MATERIALS & METHODS

3.1. Plant material and growth conditions

Triticum aestivum 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 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}.

3.2. *Nostoc*

Nostoc is a species of cyanobacterium in the family *Nostocaceae*. It is a colonial species of cyanobacterium. It initially forms a small, hollow gelatinous globule that 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* can fix nitrogen from the atmosphere and can therefore live in locations where no nitrogenous compounds are available from the substrate. *Nostoc* contains photosynthetic pigments and the energy-storing photosystems in membrane structures called thylakoids located in the 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)

3.3. Collection and preparation of biofertilizer

Nostoc was used as biofertilizers for this study. Nostoc was collected from the rocks of the Goa University plateau **(Fig.3)**. After collection, the specimens were washed with running tap water to remove microorganisms and other extraneous matter. The samples were dried at room temperature and ground to fine paste by mortar and pestle. Biofertilizers were prepared by mixing 1 g of *Nostoc* paste with 1 g of carrier.

3.4. Carrier material

(Fig.4) The carriers used in the present study were neem leaves powder, paddy straw and wood ash.

3.5. Treatments conditions

The biofertilizer application was imposed right from germination, and the plants were allowed to grow for 10 days, and watering was done every fourth day. For analysis, plants were harvested on the 12th day.

The biofertilizer treatment was given as follows: -

Nostoc+ carrier + H.S+N	*Nostoc* + carrier + H.S -N
Control	Control - N
Neem leaves	Neem leaves -N
Wood ash	Wood ash -N
Paddy straw	Paddy straw - N
Chemical fertilizer	Chemical fertilizer -N

3.6. Physiological and Biochemical analysis

3.6.1. Relative water content

The 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:

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

3.6.2. Total biomass

Biomass analysis was carried out according to Chen et al., (2014) using ten random plantlets that 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:

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

3.6.3. Determination of seed germination

Seed germination was determined 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 2.3. 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 X 100

3.6.4. Analysis of pigments

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.

3.6.5. 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, 645 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)

3.6.6. Measurements of photosynthetic efficiency

Photosynthetic efficiency measurements were done using a chlorophyll fluorescence monitoring system according to Sharma et al., (1997), Sorghum 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. 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.

3.6.7. Total sugars content

3.6.7.a. Extraction of total sugars

The total sugars were estimated with slight modifications according to Dubois et al., (1956). 0.5g 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. The final volume was made to 15 mL and centrifuged at 5000 rpm for 10 min. The supernatant was used to estimate total carbohydrates.

3.6.7.b. Estimation of total sugars

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

3.6.8. Protein Content

3.6.8.a. Extraction of Proteins

Proteins were determined according to 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 min at 4°C. The supernatant was used to estimate protein content.

3.6.8.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 mix 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.

3.6.9. Total lipid content

3.6.9.a Extraction of total lipids

Total lipids were extracted according to Turnham & Northcote (1984). 2 g of leaf tissue was cut into small pieces and boiled in a sufficient amount of isopropanol for 10 min 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 et. 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.

3.6.9.b. Quantitative Estimation of glycolipids

Glycolipids were determined using phenol-sulphuric acid, according to Kushawa & 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 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)

4. RESULTS

4.1. Determination of Relative Water Content (RWC)

Relative water content indicates the plant's water use efficiency, it reflects on the water uptake and transpiration (Lugojan & Ciulca, 2011). In this study, the effect of carrier-based biofertilizers on relative water content was measured in plants (**Fig.13 and Table 1a**). RWC was increased in plants grown in Hoagland solution containing all nutrients treated with *Nostoc* (1.7%), Ash+ *Nostoc* (2.6%), Straw (0.8%), Straw + *Nostoc* (4.6%) and decreased in plants treated with ash (-0.7%), Neem (-6.3%), Neem + *Nostoc* (-4.4%), Chemical (-1.3%) as compared to untreated plants. Plants treated with straw as carriers with *Nostoc* showed higher RWC than plants treated with other combinations of carriers.

Plants grown in Hoagland solution (absence of nitrates) **(Fig 14 and Table 1b)** treated with *Nostoc*, Ash, Ash + *Nostoc*, Straw, Straw+ *Nostoc* and chemical fertilizer showed an increase in RWC by 2.5%, 0.5%, 1.5%, 3.4%, 4.9% and 0.9 % respectively, as compared to control plants and decreased RWC in plants treated with Neem (-5.1%), Neem + *Nostoc* (-3.1%). Results obtained in this study show that treatment with straw + *Nostoc* biofertilizer increased the RWC as compared to other treatments.

4.2. Determination of seed germination

The effect of biofertilizers on seed germination rate was measured in control and treated plants. **(Fig. 15 and Table 1a)**. Seeds treated with Hoagland solution containing all nutrients with *Nostoc*, Ash, Ash+*Nostoc*, Straw, Straw+ *Nostoc*, Neem+*Nostoc* and chemical fertilizer showed an increase in germination rate by 10%, 5%, 15%, 7.5%, 20%, 2.5% and 5%, respectively, as compared to the control plants and decline in germination of (-5%) was seen in seed treated with neem alone. Seeds treated with a combination of Ash+*Nostoc* and Straw+*Nostoc* showed a higher rate in germination comparison to other treatments.

Seeds treated with Hoagland solution (absence of nitrates) **(Fig. 16 and Table 1b)** with *Nostoc,* Ash, Ash+*Nostoc,* Straw, Straw+*Nostoc*, and chemical fertilizer showed an increase in germination rate by 12.5%, 10%, 15%, 9%, 20% and 7.5% respectively. The decline in germination was seen in seeds treated with Neem and Neem+*Nostoc* which was -7.5% and -2.5% respectively.

4.3. Determination of Biomass

Shoot and root biomass were determined from plants treated with carrier based biofertilizers and chemical fertilizer grown in Hoagland with nitrate and Hoagland solutions without nitrate **(Fig.17 and Table 2a)**. Plants grown in Hoagland solution containing all nutrients treated with *Nostoc*, Ash, Ash+*Nostoc,* Straw, Straw+ *Nostoc*, Neem+*Nostoc* and chemical fertilizer showed an increase in the shoot by 1%, 0.6%, 2.8%, 1.8%, 4.8%, 0.6% respectively and 3.3% in chemical fertilizer compared to control plants. Whereas plants treated with Neem without *Nostoc* showed a decline in shoot biomass by (–0.1%) respectively, compared to control plants. The root biomass of plants treated with *Nostoc*, Ash + *Nostoc*, Straw+*Nostoc*, Neem, Neem+*Nostoc* and chemical fertilizer increased by 0.7%, 2.1%, 3.2%, 0.1%, 0.9%, 1.6% respectively, as compared to control plants. Plants treated with ash and straw without Nostoc showed a decline in shoot and root biomass by 0.1% and 0.6%.

Plants grown in Hoagland solution (absence of nitrates) **(Fig 18 and Table 2b**) with *Nostoc*, Ash + *Nostoc,* Straw, Straw+ *Nostoc* and chemical fertilizer showed an increase in shoot biomass by 1.4%, 3.3% ,1.6%, 5% and 3%, respectively, as compared to control plants. In comparison, plants treated with Ash, Neem, Neem+*Nostoc* showed a decline in shoot biomass by (-0.4%) and (-0.5%) as compared to control plants. The root biomass of plants treated *Nostoc,* Ash, Ash+*Nostoc*, Straw, Straw+ *Nostoc* and chemical fertilizer increased by 0.9%, 0.1%, 2.3%, 1%, 2.9%, and 1.4% respectively, compared to control plants. In comparison, plants treated with Neem and Neem+*Nostoc* showed a decline by (-0.8%) and (–0.1%) compared to control plants. Plants treated with a combination of ash +*Nostoc* and Straw +*Nostoc* showed greater root and shoot biomass.

4.4. Estimation of Photosynthetic pigments

Various photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoids were measured in control and treated plants **(Fig.19, 21and Table 3a)**. It was observed that treated plants, the amount of chlorophyll a was maximum, followed by chlorophyll b and carotenoids. Plants were grown in Hoagland solution containing all nutrients *Nostoc*, Ash, Ash+*Nostoc*, Straw, Straw+ *Nostoc* and chemical fertilizer respectively showed increase in chlorophyll a and chlorophyll b compared to control plants that is 3.17%, 4.69%, 1.18%, 5.49% and 4.13% respectively. However, plants treated with Neem+*Nostoc* and Neem showed reduced concentration as compared to control.

A similar trend was observed in the amount of Chlorophyll b. In comparison, plants treated with a combination of carriers+*Nostoc* showed an increased concentration of chlorophyll a as compared to control. The carotenoid concentration was reduced in all treated plants as compared to control **(Fig 20, 22 and Table 3b).** Chlorophyll a concentration reduced in plants grown in Hoagland solution (absence of nitrates) with *Nostoc*, Ash, Straw and chemical fertilizer compared to control plants (absence of nitrates). In comparison, plants treated with a combination of *Nostoc*+Ash and *Nostoc*+Straw showed an increase in Chlorophyll a by 4.99%and 6.18% as compared to control. The Chlorophyll b concentration was decreased in all treated plants. A similar trend was observed in carotenoid concentration. The levels of carotenoids was decreased in all treated plants as compared to control.

4.5. 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. 23 and Table 4a).** The Fv/Fm ratio decreased in plants grown in Hoagland solution containing all nutrients with Neem and Neem+*Nostoc* compared to control plants.

In plants grown with Hoagland solution in the absence of nitrates, biofertilizer treatment with Ash, Ash+*Nostoc*, Straw, Straw+*Nostoc*, the photosynthetic efficiency increased by 0.14%, 0.15%, 0.13% and 0.16% respectively, as compared to control plants **(Fig 24 and Table 4b).**

4.6. Estimation of total sugar content

Total sugar content was determined in plants grown in Hoagland solution with nitrate and Hoagland solution containing no nitrate along with biofertilizers and chemical fertilizer **(Fig.25 and Table 5a).** Plants grown in Hoagland solution containing all nutrients with combination of Straw + *Nostoc* showed increase in total sugar content by 12.96%, followed by plants treated with *Nostoc* +Ash with 11.23% respectively, as compared to control plants. On the other hand, plants treated with a only carriers and chemical fertilizers showed reduced total sugar content as compared to other treatments.

Total sugar content was observed to be higher in plants grown in Hoagland solution containing no nitrates **(Fig 26 and Table 5b)** along with biofertilizers as compared to plants grown in Hoagland solution containing all the nutrients along with biofertilizers. Plants grown in Hoagland solution containing all nutrients with combination of straw + *Nostoc* showed increased total sugar content by 11.2%, followed by plants treated with *Nostoc* +Ash with 6.08% respectively, as compared to control plants. On the other hand, plants treated with only carriers and chemical fertilizers showed reduced total sugar content as compared to other treatments.

4.7. Estimation of protein content

The plants grown in Hoagland solution containing all nutrients **(Fig 27 and table 5a)** treated with *Nostoc*, Ash, Straw, a combination of Straw+*Nostoc*, Ash+*Nostoc* and chemical fertilizer showed an increase in protein content by15.4%, 11.6%, 21.3 .5%, 27.9% and 16.7% as compared to control plants.

Plants treated with *Nostoc*+carriers showed higher protein content as compared to other treatments. The decline in protein content was observed in plant treated with Neem and *Nostoc*+Neem combination. In plants grown in Hoagland solution (absence of nitrates) there was 20% and 30% increase in protein content in plants treated with *Nostoc* and combination of straw+*Nostoc* compared to other treatments **(Fig 28 and Table 5b**).

4.8. Estimation of glycolipid content

Glycolipid content was measured in control and treated plants in Hoagland solution with nitrates and Hoagland solution containing no nitrates along with biofertilizers and chemical fertilizer **(Fig 29 and Table 5a)**. Plants grown in Hoagland solution containing all nutrients treated *Nostoc*, Ash, Straw, a combination of Straw+*Nostoc,* Ash+*Nostoc* and chemical fertilizer showed an increase in glycolipid content by 40%, 33%, 70.7%, 41%, 63% and 43% respectively. Decline was seen in Neem (0.5%) and Neem+*Nostoc* (16.5%) treated plants. The study indicates that higher amount of glycolipid presence in plants treated with combination of *Nostoc* and carrier based biofertilizers.

In plants grown in Hoagland solution in absence of nitrates treated with *Nostoc,* combination of carriers+*Nostoc* and chemical fertilizer showed an increase in glycolipid by 26%, 38%, 31%, and 20% respectively, as compared to control plants **(Fig. 30 and Table 5b).** In comparison, plants treated with Neem, Neem+ *Nostoc* show decline in glycolipid content. The glycolipid content in plants treated with a combination of carriers+*Nostoc* drastically increased as compared to other treatments.

5. DISCUSSION

"Promotive Effect of Carrier-Based Biofertilizer on ***Triticum aestivum***" encapsulates a multifaceted exploration into the influence of carrier-based biofertilizers on the growth and development of wheat. This discussion highlights the key findings of the experiment that is carrier based biofertilizers shows positive changes in the morphological, physiological and biochemical parameters of wheat plants. Our results showed that different carrier based biofertilizer treatment **(Fig 13, 14 ,17 and 18 Table 1a, 1b, 2a and 2b)** increased biomass and RWC as compared to control and plants treated with chemical fertilizers . This increase in biomass may be due to revealed that, higher concentrations of cyanobacterial hormones, auxin and cytokinin have a positive response to the plant growth, including the weight of the seeds and shoot, spike and root length Hussain & Hasnain (2011).

Chittapun et al., (2017) used *Nostoc carneum* and *Nostoc commune* as cyanobacterial biofertilizers for cultivating rice plants. He found that these cyanobacterial biofertilizers helped in increasing the root length, plant growth, yield and grain quality. According to Xue et al., (2016) lettuce crops showed highest plant yield, when treated with cyanobacterial biofertilizers containing *Anabaena cylindrica* and *Nostoc sp*.. Maurya et al., (2016) found that, when maize crops were treated with lipid extracted from algae biomass as a biofertilizer, it helped in increasing the yield of the plants.

Aggarwal et.al., (2009) reported that application of fly ash in combination with nitrogen had some advantageous effect on grain and biomass yield of wheat crop irrespective of the variety though the positive effect was non-significant. Biofertilizer with carriers is

essential to improve crop growth and production and soil fertility status because of rich organic carbon source. Maize straw and sugar cane husk enriched with IAA have been proved a better source of organic matter for improving growth and yield of maize (Mahimairaja et al., 2008) (Ahmed et al., 2011) reported that most cyanobacterial isolates increased wheat root length, which increased lengths of shoot, while *Phormidium molle* and *N. muscorum* isolates showed non-significant effect in length of shoot. Also, *Phormidium molle* and *N. passeriniamum* caused a significant increase in fresh weights of root and shoot of wheat plant. Results also showed cyanobacterial inoculation clearly increased plant shoot and root dry weight, while *Phormidium molle* did not exhibited such effects.

Studies have demonstrated the positive effects of straw carrier-based biofertilizers on wheat growth and yield parameters. Sharma et al., (2019) showed that application of biofertilizers containing *Azotobacter* and phosphate-solubilizing bacteria significantly increased wheat grain yield, plant height, and root length compared to control treatments. Similarly, Gupta et al., (2021) reported improvements in wheat yield attributes following inoculation with straw-based biofertilizers, including higher number of tillers and increased grain weight. Straw carrier-based biofertilizers contribute to enhanced nutrient uptake by wheat plants, particularly nitrogen and phosphorus. The microbial activity within the biofertilizers promotes the conversion of atmospheric nitrogen into plant-available forms and solubilizes bound phosphorus in the soil, making it more accessible to wheat roots (Nautiyal et al., 2013). Moreover, the application of biofertilizers improves soil health by increasing organic matter content, microbial biomass, and soil enzymatic activity (Singh et al., 2018).

Our results showed that the application of *Nostoc* and carrier based biofertilizers promoted the seed germination **(Fig 14, 16 and Table 1a, 1b)** in comparison to the control and chemical fertilizer but neem individually and combination with *Nostoc* shows decline in germination rate. The increased germination rate could be due to secretion of certain or higher concentrations of cyanobacterial hormones, auxin and cytokinin which have a positive response to the plant growth and seed germination Hussain & Hasnain (2011).

But decline in germination rate by neem individually and combination with *Nostoc* may be due to study that both the neem and eucalyptus plants possess remarkable allelopathic effects on the growth and the germination of the wheat. It may be due to the phytotoxic chemicals released by the leaves of neem. The allelochemicals responsible for allelopathic effect have been shown to be toxic to germination and plant growth (Rao & Mamta, 2013).

We reported increase in the photosynthetic efficiency and photosynthetic pigments in plants treated with different carrier based biofertilizers **(Fig. 19, 20, 21, 22, 23 and 24 and Table 3a, 3b ,4a, 4b)**. 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 improvement in nutrient uptake in plants treated with biofertilizer is correlated with better or higher chlorophyll and protein content. Shah & Ahmad (2006) also reported similar results. Abd-Alla et al., (1994) revealed that incoulating plants with live or killed cyanobacteria either alone or with K, P and S increased chloroplylls and carotenoids. The significant increase in dry weight, total nitrogen and pigments content

of plants inoculated with live or killed inoculum alone or with K, P and S could be attributed to nitrogenase activity of nitrogen fixing organisms in the surface of the soil.

Biofertilizers, such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR), promote the biosynthesis and accumulation of photosynthetic pigments in wheat plants. AMF enhance chlorophyll synthesis by improving nitrogen and phosphorus availability, stimulating gene expression related to chlorophyll biosynthesis, and enhancing chloroplast development (Porcel et al., 2012; Smith & Read, 2008).

PGPR produce phytohormones and metabolites that regulate chlorophyll content and chloroplast structure, enhancing light capture and utilization efficiency in wheat leaves (Glick, 2012). In addition to chlorophylls, biofertilizers stimulate the synthesis of secondary pigments, such as carotenoids and flavonoids, in wheat plants. Carotenoids serve as photoprotective pigments, scavenging reactive oxygen species and dissipating excess light energy to prevent photooxidative damage (Cazzonelli & Pogson, 2010). Flavonoids act as antioxidants and UV-absorbing compounds, shielding plant tissues from oxidative stress and UV radiation (Mierziak et al., 2014). Biofertilizer-induced accumulation of secondary pigments enhances wheat plants' resilience to environmental stresses and improves their adaptation to fluctuating light and temperature conditions.

Experimental studies and field trials have demonstrated the efficacy of biofertilizers in increasing pigment content in wheat plants. For example, Hussain et al., (2020) reported

significant enhancements in chlorophyll and carotenoid levels in wheat leaves following inoculation with AMF and PGPR-based biofertilizers.

Similarly, Sharma et al., (2018) observed elevated concentrations of flavonoids and anthocyanins in wheat tissues treated with microbial inoculants under field conditions. These findings underscore the practical relevance of biofertilizers as a sustainable strategy to improve the photosynthetic performance of wheat crops.

We also reported an increase in sugar content, protein content and glycolipid content in wheat plants **(Fig 25, 26, 27, 28 , 29 and 30, table 5a ,5b)** due to the treatment with different carrier based biofertilizers. Biofertilizers containing beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR), facilitate nutrient acquisition by wheat plants through various mechanisms. AMF form symbiotic associations with plant roots, extending their hyphae into the soil and enhancing the uptake of water, phosphorus, and micronutrients (Smith & Read, 2008).

PGPR promote plant growth and nutrient availability through nitrogen fixation, phosphate solubilization, and production of phytohormones and enzymes (Glick, 2012). The increased availability of essential nutrients stimulates metabolic pathways involved in lipid biosynthesis and accumulation in wheat tissues (Smith et al., 2011). Furthermore, biofertilizer application stimulates the production of secondary metabolites, including phytochemicals and bioactive compounds, which contribute to lipid synthesis and storage in wheat grains (Vahdati & Hoseini, 2019).

Several studies have demonstrated the potential of biofertilizers to increase lipid content in wheat plants under controlled conditions and field trials. For example, research by Hussain et al., (2020) reported significant enhancements in lipid accumulation and fatty acid composition in wheat grains following inoculation with AMF and PGPR-based biofertilizers. Similarly, Sharma et al., (2018) observed higher lipid content and improved oil quality in wheat seeds treated with microbial inoculants under field conditions. These findings highlight the practical relevance of carrier based biofertilizers as a sustainable strategy to enhance lipid productivity in wheat cultivation.

The improved nutrient status stimulates metabolic pathways involved in protein synthesis and sugar metabolism, leading to enhanced accumulation of these compounds in wheat grains (Sharma et al., 2018). Experimental studies and field trials have demonstrated the efficacy of biofertilizers in increasing protein and total sugar content in wheat plants. For example, Hussain et al., (2020) reported significant enhancements in protein and sugar accumulation in wheat grains following inoculation with AMF and PGPR-based biofertilizers.

Similarly, Sharma et al., (2018) observed elevated levels of protein and soluble sugars in wheat seeds treated with microbial inoculants under field conditions. These findings underscore the practical relevance of carrier based biofertilizers as a sustainable strategy to improve the nutritional value and economic viability of wheat production. Using straw and ash as carrier for biofertilizers treatment with *Nostoc commune* provided significant increase in nitrogen uptake and enhanced the yield of wheat plant with better morphological, 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. The utilization of carrier-based biofertilizers represents a sustainable approach to agricultural nutrient management. By reducing reliance on chemical fertilizers, minimizing nutrient runoff and leaching, and enhancing soil fertility and ecosystem resilience, biofertilizers contribute to environmental conservation and long-term agricultural sustainability. Moreover, the cost-effectiveness and scalability of biofertilizer production and application make them viable options for smallholder farmers and large-scale agricultural operations.

6. CONCLUSION

Our study suggests that all the applied carrier based nostoc biofertilizers and chemical fertilizers caused changes in the morphological, physiological and biochemical parameters of wheat plants. Compared to all the treatments, relative water content was increased in plants grown in Hoagland solution containing all nutrients treated with chemical fertilizer. However, plants grown in nitrate absence with combination of *Nostoc* and straw as biofertilizer showed greater RWC than other biofertilizers and chemical treatments. The shoot biomass increased in plants grown in Hoagland solution containing all nutrients treated with combination of Ash +*Nostoc* and straw +*Nostoc* as carrier based biofertilizers.

The seed germination rate increased in seeds treated with biofertilizer combination of straw+*Nostoc* in Hoagland solution containing all nutrients. However, seeds treated with Hoagland solution (absence of nitrates) with biofertilizer and chemical fertilizer showed a increased germination rate as compared control plants (absence of nitrates) also seed treated with neem as carrier material showed reduced rate of germination in both the treatments with and without nitrate.

The photosynthetic efficiency increased in plants grown in Hoagland solution containing all nutrients with *Nostoc*, a combination of ash +*Nostoc* and straw +*Nostoc* compared to control plants. Plants treated with neem and neem + *Nostoc* showed the lowest Fv/Fm values compared to control plants. In plants grown in the absence of nitrates with *Nostoc* and a combination of Ash+*Nostoc* and straw +*Nostoc* the

photosynthetic efficiency increased compared to its control plants. Plants grown in Hoagland solution containing all nutrients with *Nostoc*, ash + *Nostoc*, straw+*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 straw +*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 ash +*Nostoc* and straw +*Nostoc* showed an increase in total sugar content. Plants grown in Hoagland solution (absence of nitrates) treated with a combination of straw +*Nostoc* showed lower total sugar content compared to its control. Plants treated with *Nostoc*, ash +*Nostoc* and straw+ *Nostoc* showed higher protein content than to all the treatments containing complete nutrients. Plants treated with straw+ *Nostoc* showed more protein content than all the treatments grown in Hoagland solution containing no nitrates. The glycolipid content in plants treated with Ash + *Nostoc* and straw + *Nostoc* was high compared to all the treatments grown in Hogland solution containing all nutrients. Whereas in plants treated with a combination of Ash+*Nostoc* the glycolipid content increased compared to all the treatments grown in Hoagland solution (absence of nitrates).

Biofertilizers treatment with *Nostoc* in combination of carriers like ash and straw provided a significant increase in nitrogen uptake and enhanced the yield of wheat plants with better physiological and biochemical attributes even in the absence of inorganic nitrogen fertilizer application. The results indicated that the use of biofertilizer with combination of carriers would be a great substitute for inorganic fertilizer.

In conclusion, the promotive effect of carrier-based biofertilizers on wheat plants holds significant promise for sustainable agriculture. Through their ability to enhance nutrient availability, stimulate root growth, and induce stress tolerance mechanisms, biofertilizers contribute to improved wheat productivity, grain quality, and soil health. The findings presented in this paper underscore the practical relevance of biofertilizers as eco-friendly alternatives to conventional fertilization practices, aligning with the principles of resource conservation, environmental stewardship, and food security. By integrating biofertilizers into wheat production systems, farmers can mitigate the negative environmental impacts associated with chemical fertilizers while enhancing agricultural sustainability and resilience in the face of changing climatic conditions and evolving market demands. Overall, the promotive effect of carrier-based biofertilizers on wheat plants represents a promising avenue for advancing towards more sustainable and productive agricultural systems.

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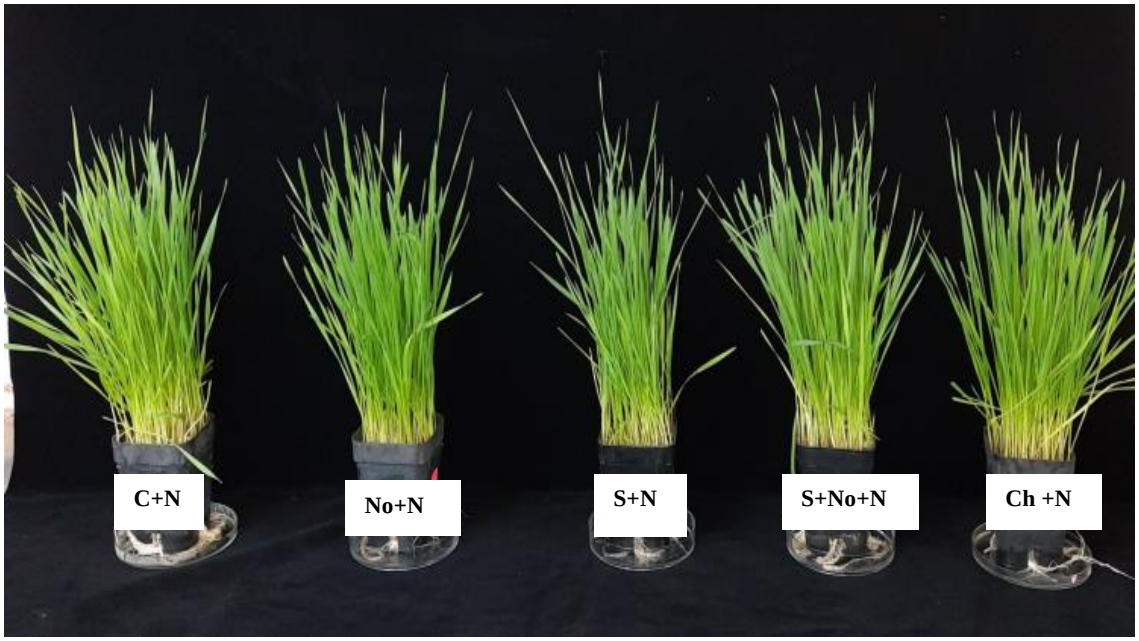

Fig. .5 .Effect of (No+N);*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc* and (Ch+N);chemical on *Triticum aestivum* in presence of nitrates

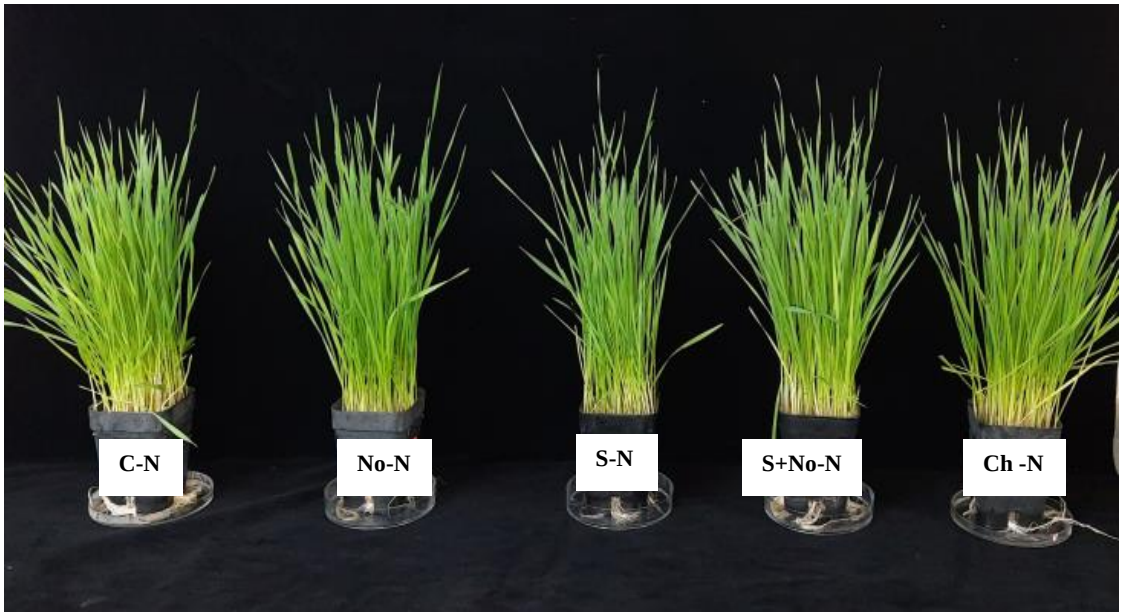

Fig .6.Effect of (No-N);*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*, and (Ch-N);chemical fertilizer on *Triticum aestivum* in absence of nitrates

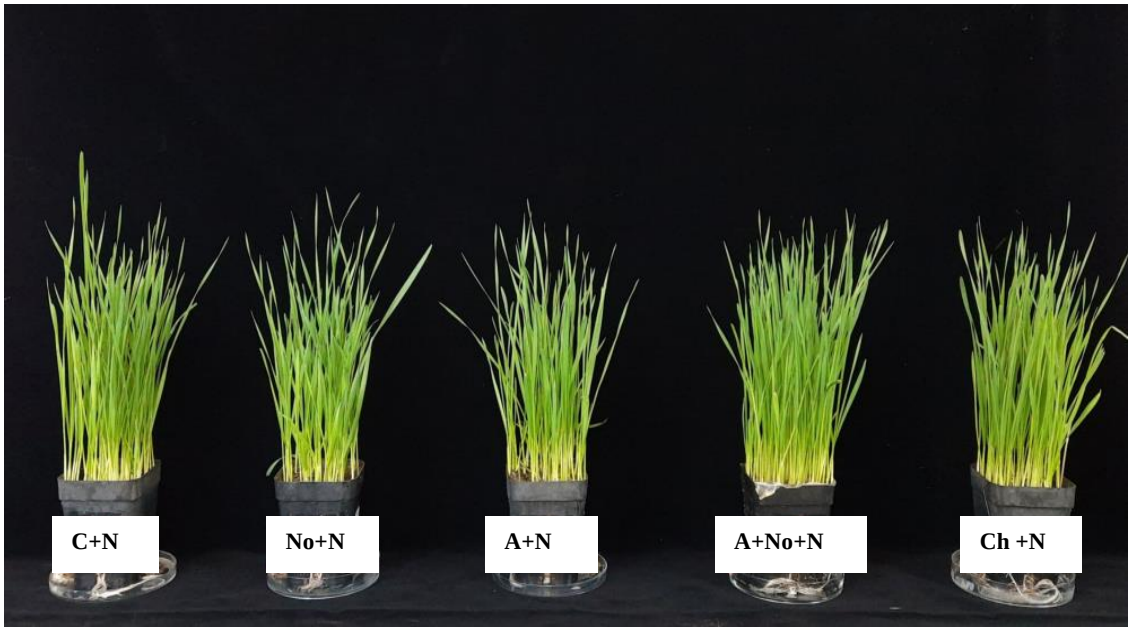

Fig.7 Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc* and (Ch+N);chemical on *Triticum aestivum* in presence of nitrates

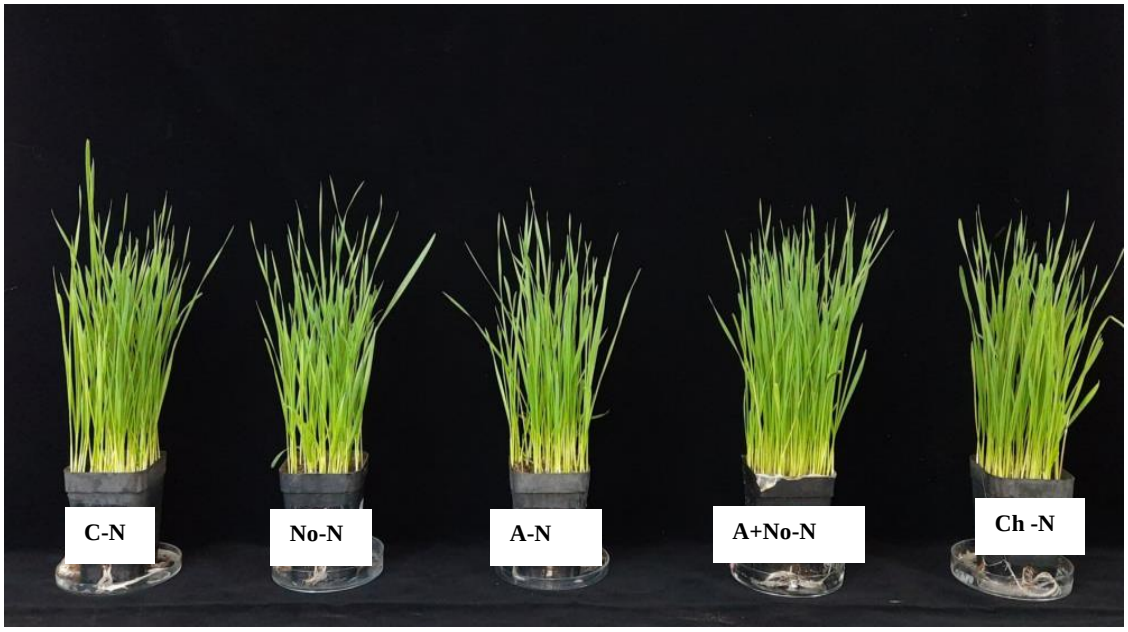

Fig . 8 Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc* and (Ch-N);chemical fertilizer on *Triticum aestivum* in absence of nitrates

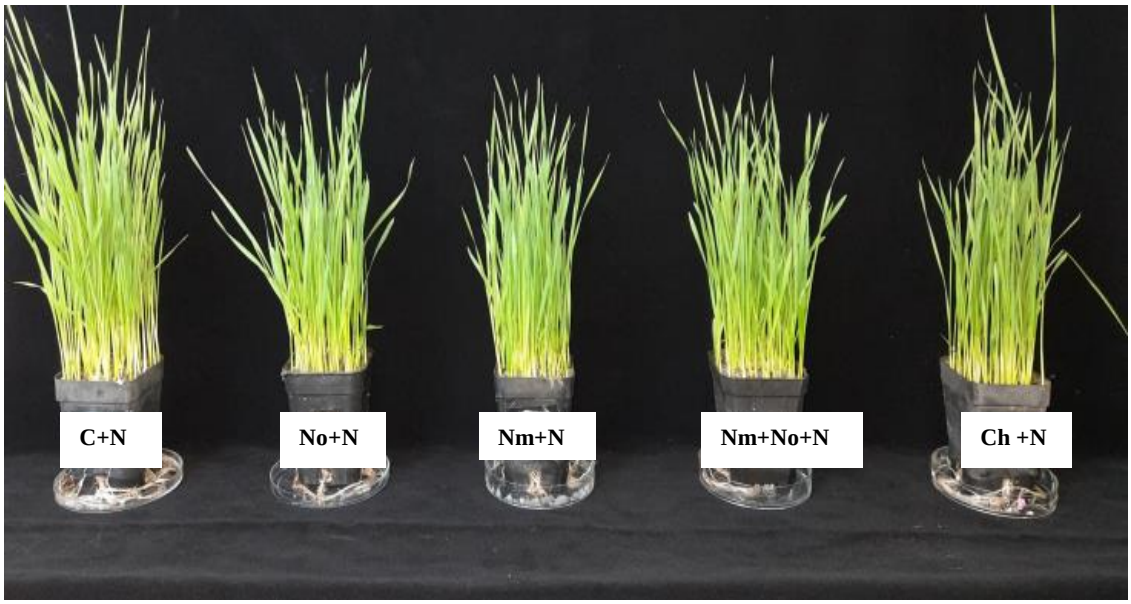

Fig. 9 Effect of(No+N);*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc* and (Ch+N);chemical on *Triticum aestivum* in presence of nitrates

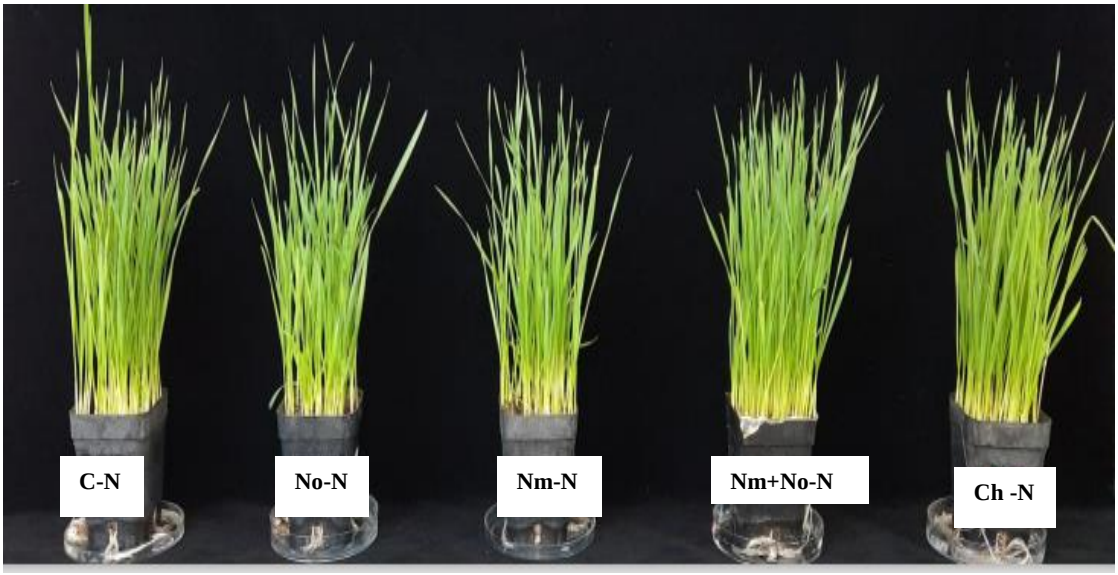

Fig. 10 Effect of (No-N);*Nostoc* (Ne-N);Neem, (Ne+No-N);Neem+*Nostoc* and (Ch-N);chemical fertilizer on *Triticum aestivum* in absence of nitrates

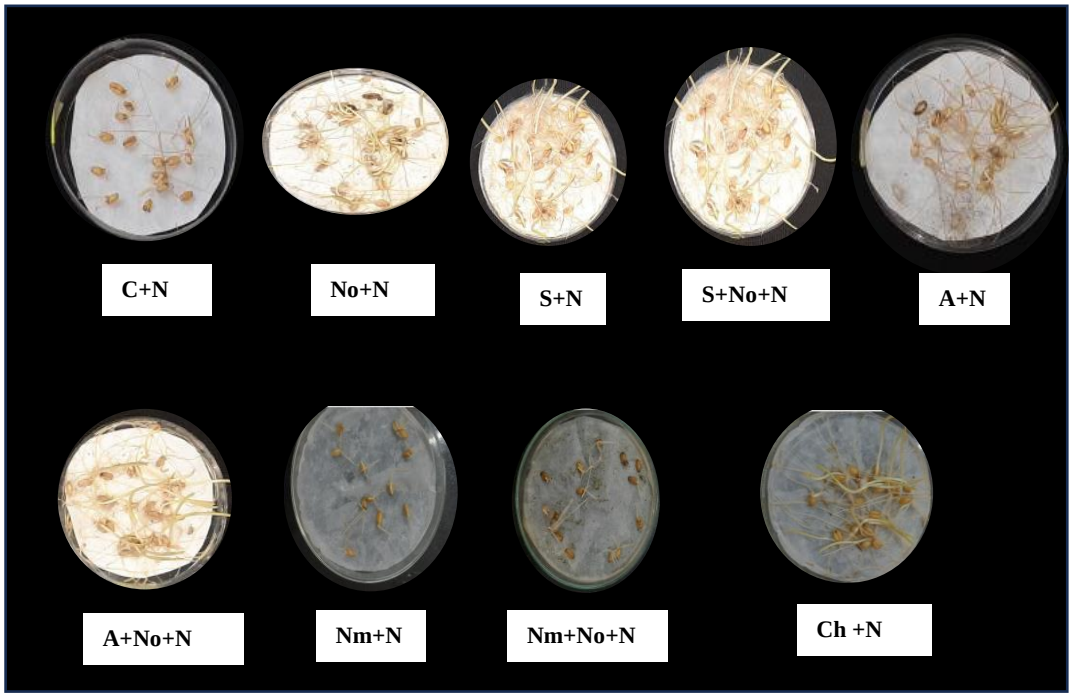

Fig.11 Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical on seed germination in *Triticum aestivum* in presence of nitrates.

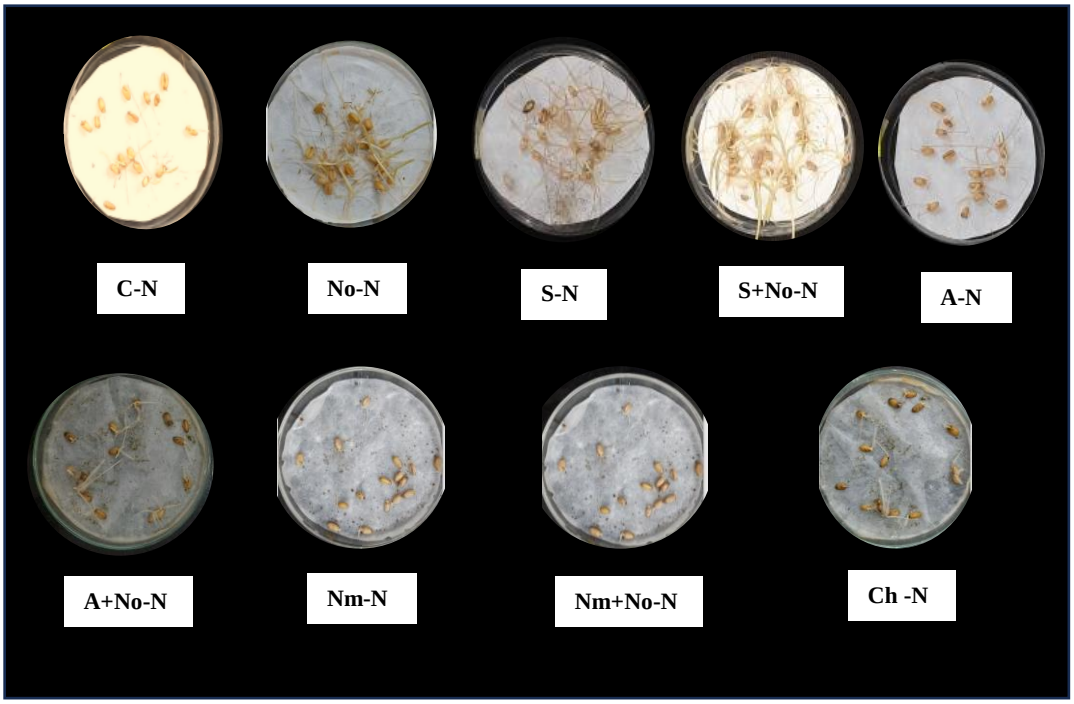

Fig .12 Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*,(Ne-N);Neem,(Ne+No-N);Neem+*Nostoc*and(ChN);chemical fertilizer on seed germination in *Triticum aestivum* in absence of nitrates

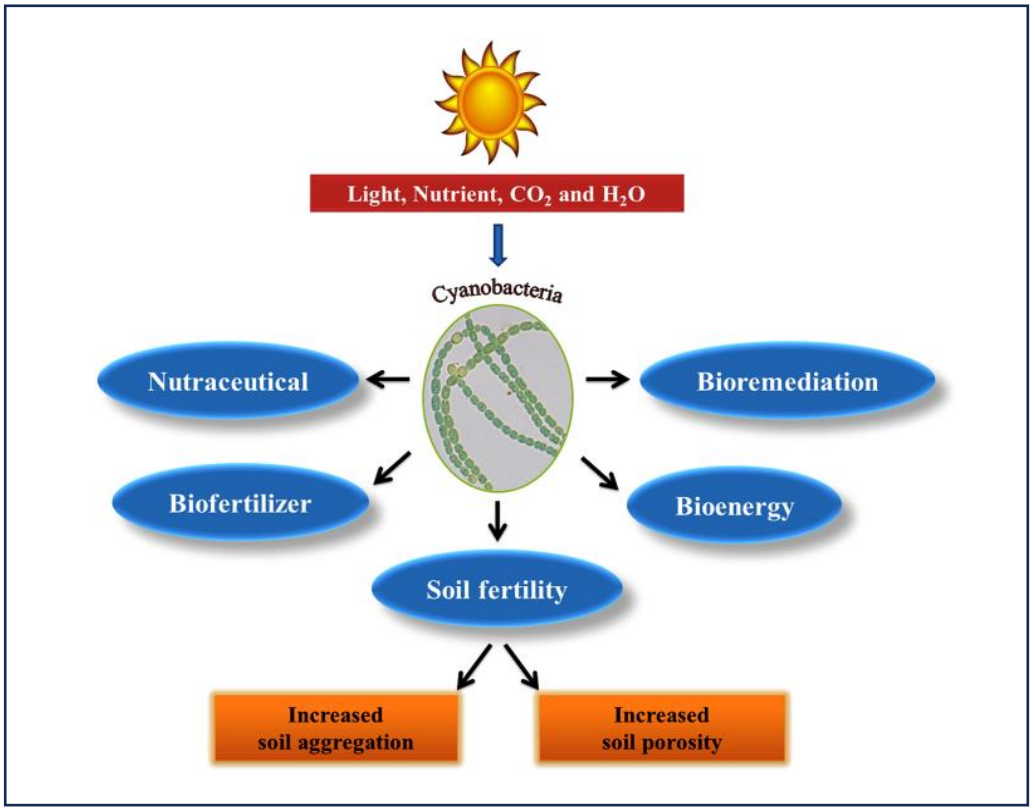

Fig 1. Role of Cyanobacteria for the development of sustainable agriculture and environment

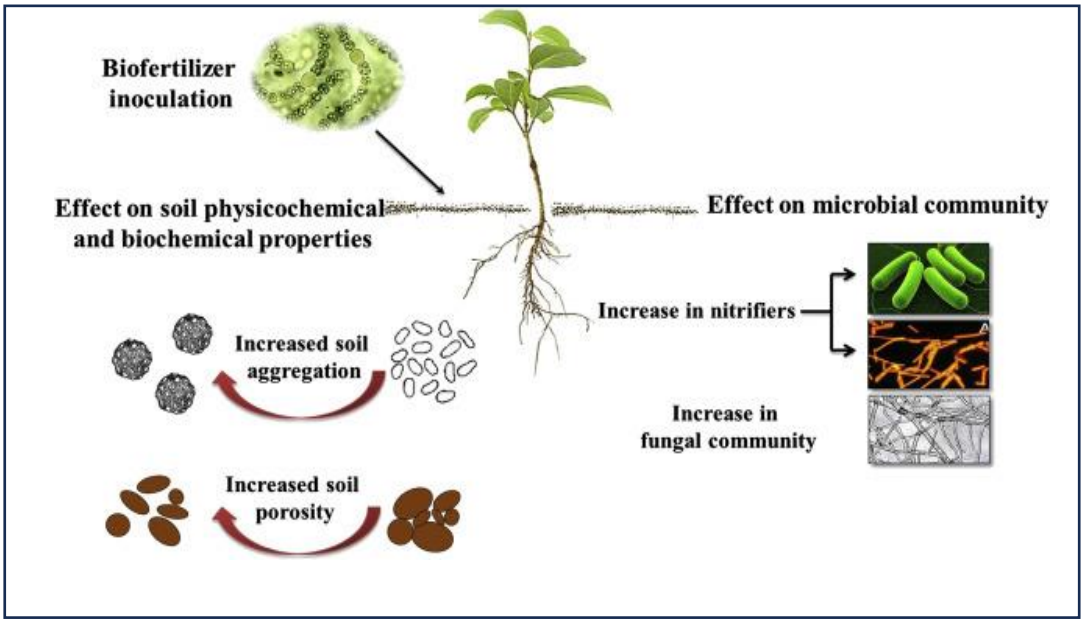

Fig.2. Role of Biofertilizers in a sustainable environment

Fig 3 . Collection of Nostoc commune from the rocks of the Goa University plateau

Fig 4. carrier materials used in biofertilizer preparation

Table 1.a. Effect of biofertilizer treatments on Relative water content (RWC) and Percent germination of *Triticum aestivum*. (+N): presence of N; where ± indicates standard deviation, n=3

Treatment	Relative water content (RWC) (%)	% change	Germination (%)	% change
Control	90.65 ± 1.012	0	57.5 ± 0.701	0
Nostoc	92.39 ± 1.013	1.73	67.5 ±0.701	10
Ash	89.95± 1.011	-0.70	62.5 ±0.703	5
Ash + *Nostoc*	93.27 ±1.012	2.61	72.5 ±0.701	15
Straw	91.50 ±1.012	0.84	65 ±0.702	7.5
Straw + *Nostoc*	95.30 ±1.013	4.64	77.5 ±0.703	20
Neem	84.31 ±1.011	-6.34	52.5 ± 0.710	-5
Neem + *Nostoc*	86.27±1.010	-4.38	60 ± 1.302	2.5
Chemical	89.31 ± 1.012	-1.34	62.5 ± 1.201	5

Table 1.b. Effect of biofertilizer treatments on Relative water content (RWC) and Percent germination of *Triticum aestivum*. (-N): absence of N; where ± indicates standard deviation, n=3

Treatment	Relative water content (RWC) (%)	% change	Germination (%)	% change
Control	92.69± 1.012	0	60± 0.703	0
Nostoc	94.22±1.011	1.52	72.5±0.701	12.5
Ash	90.22± 1.012	-2.47	70± 0. 1.201	10
Ash + *Nostoc*	94.22±1.011	1.52	75± 0.701	15
Straw	92.21± 1.012	-0.48	62.5± 0.701	2.5
Straw + *Nostoc*	97.57±1.011	4.87	80± 1.401	20
Neem	86.57±1.013	-6.12	52.5 ±0.704	-7.5
Neem + *Nostoc*	88.57±1.012	-4.12	57.5 ±0.702	-2.5
Chemical	91.57±1.012	-1.12	67.5 ± 0.707	7.5

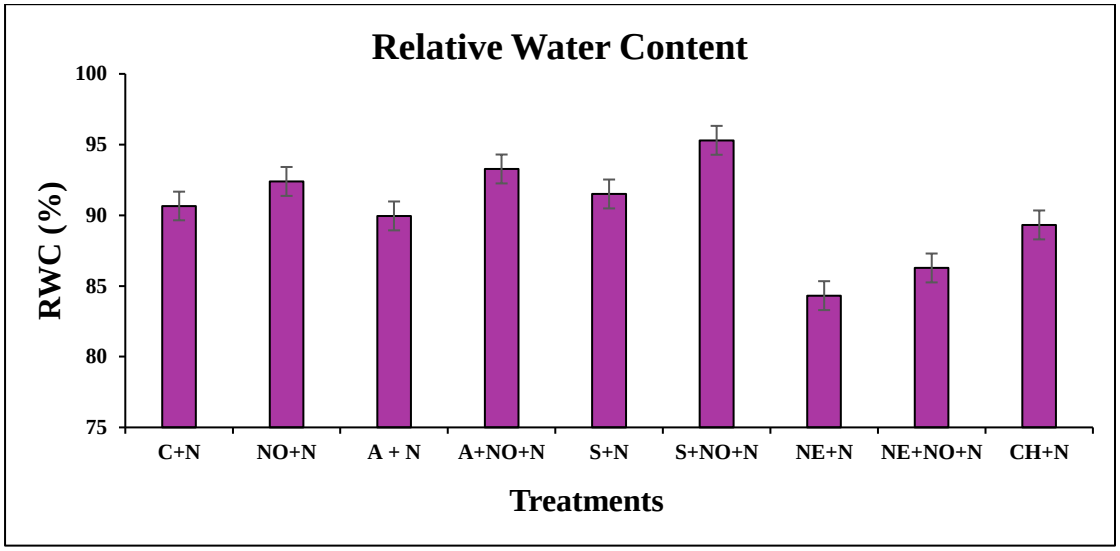

Fig. 13. Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical fertilizer on RWC in *Triticum aestivum* in presence of nitrates.

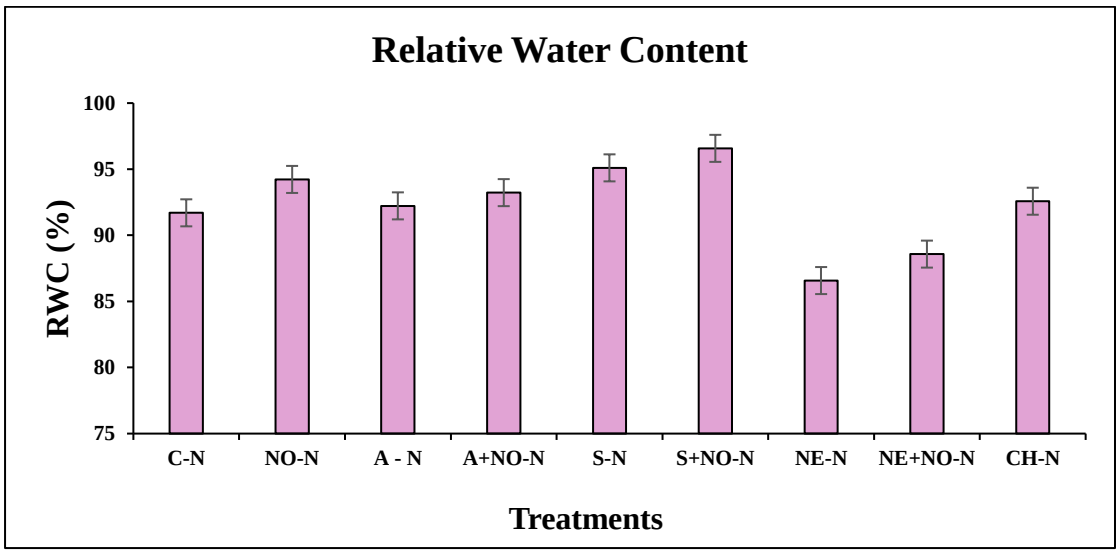

Fig. 14 . Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*, (Ne-N);Neem, (Ne+No-N);Neem+*Nostoc* and (Ch-N);chemical fertilizer on RWC in *Triticum aestivum* in absence of nitrates.

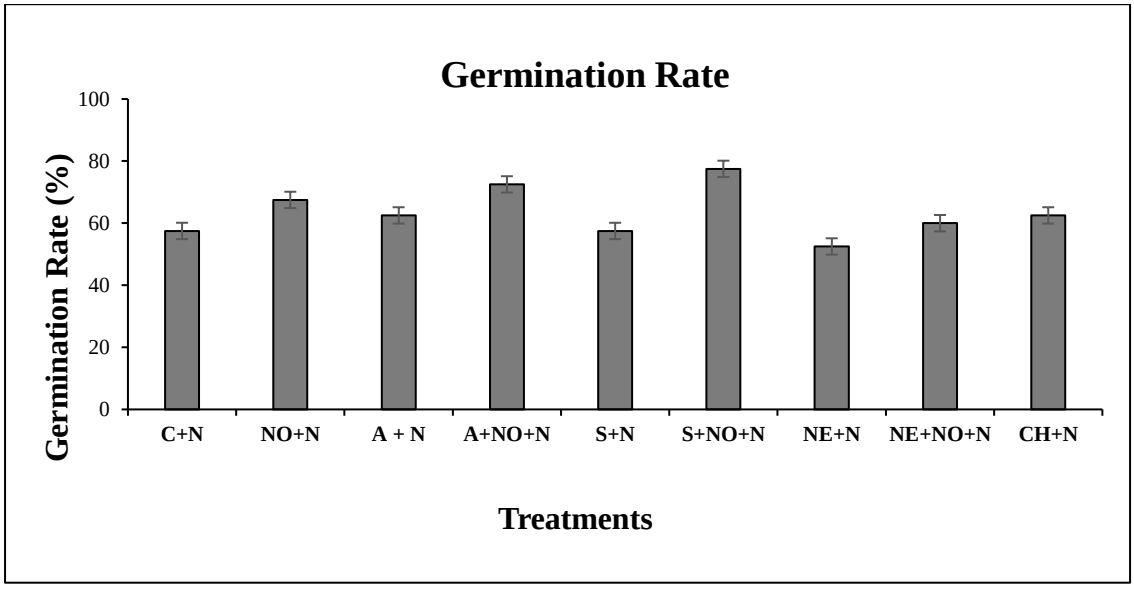

Fig. 15 . Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical on seed germination in *Triticum aestivum* in presence of nitrates.

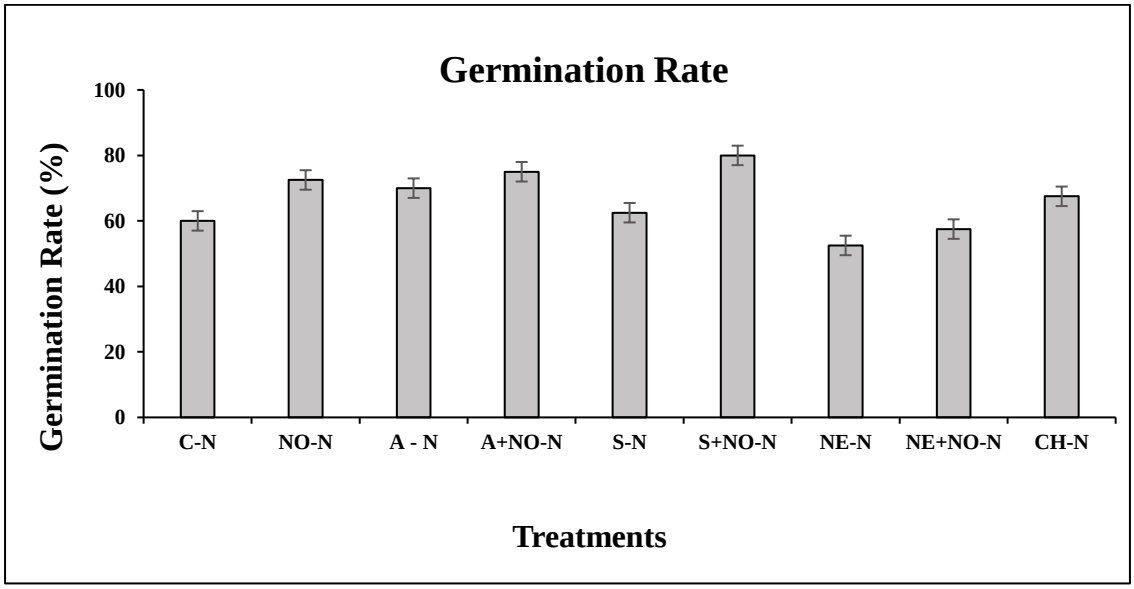

Fig. 16. Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*,(Ne-N);Neem,(Ne+No-N);Neem+*Nostoc* and (Ch-N);chemical fertilizer on seed germination in *Triticum aestivum* in absence of nitrates.

Table 2.a. Effect of biofertilizers treatment on Biomass (root and shoot) of *Triticum aestivum*. (+N): presence of N; where ± indicates standard deviation, n=3

Treatment	Biomass			
	Shoot	% change	Root	% change
Control	0.103 ± 0.004	0	0.047 ± 0.008	0
Nostoc	0.114 ± 0.005	0.11	0.053 ± 0.004	0.7
Ash	0.109 ±0.015	0.6	0.045 ±0.015	-0.1
Ash + *Nostoc*	0.131 ±0.018	2.8	0.067 ± 0.007	2.1
Straw	0.121 ±0.026	1.8	0.041 ± 0.021	-0.6
Straw + *Nostoc*	0.151 ±0.022	4.8	0.079 ±0.030	3.2
Neem	0.109 ±0.006	0.6	0.047 ± 0.005	0.1
Neem + *Nostoc*	0.102 ±0.005	-0.1	0.055 ± 0.023	0.9
Chemical	0.135 ±0.008	3.3	0.063 ±0.014	1.6

Table 2.b. Effect of biofertilizer treatments on Biomass of *Triticum aestivum*. (-N): absence of N; where ± indicates standard deviation, n=3

Treatment	Biomass			
	Shoot	% change	Root	% change
Control	0.116 ± 0.010	0	0.051 ±0.006	0
Nostoc	0.130 ±0.013	1.4	0.060 ± 0.005	0.9
Ash	0.112 ± 0.026	-0.4	0.052 ±0.016	0.1
Ash + *Nostoc*	0.149 ±0.012	3.3	0.074 ±0.009	2.3
Straw	0.131 ±0.024	1.6	0.061 ±0.013	1
Straw + *Nostoc*	0.166 ±0.039	5	0.080 ± 0.025	2.9
Neem	0.110 ±0.011	-0.4	0.044 ±0.005	-0.8
Neem + *Nostoc*	0.111 ±0.024	-0.5	0.050 ±0.010	-0.1
Chemical	0.146 ±0.023	3	0.065 ±0.005	1.4

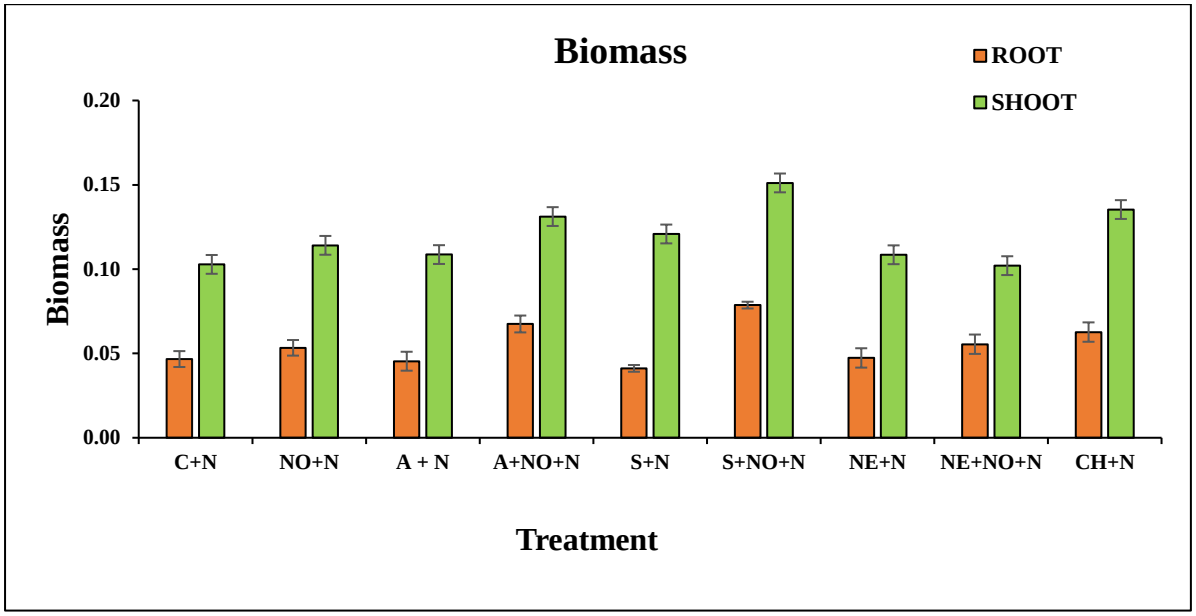

Fig. 17. Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical fertilizer on biomass in *Triticum aestivum* in presence of nitrates.

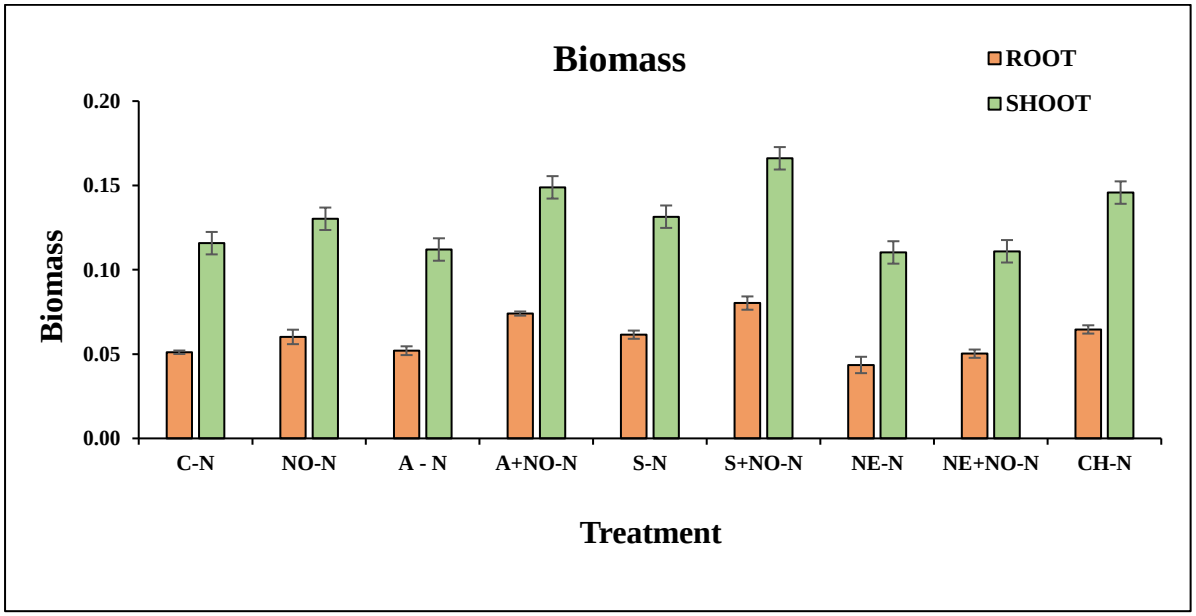

Fig. 18. Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*, (Ne-N);Neem, Ne+No-N);Neem+*Nostoc* and (Ch-N);chemical fertilizer on biomass in *Triticum aestivum* in absence of nitrates.

Table 3.a. Effect of biofertilizer treatments on Photosynthetic pigments in *Triticum aestivum*. (+N): presence of N; where ± indicates standard deviation, n=3.

Treatment	Photosynthetic pigments (mg/g FW)					
	Chl a	% change	Chl b	% change	Carotenoids	% change
Control	13.74 ±0.256	0	4.62 ± 0.34	0	1.212±0.102	0
Nostoc	16.91 ± 0.263	3.17	5.18 ± 0.38	0.55	1.492±0.104	0.28
Ash	12.55 ± 0.234	-1.18	3.814 ±0.41	-0.81	1.41 ±0.110	0.19
Ash + *Nostoc*	18.43 ± 0.233	4.69	7.517 ±0.43	2.89	2.288±0.101	1.07
Straw	14.82 ±0.213	1.07	4.950 ±0.31	0.32	2.27 ±0.201	1.05
Straw +*Nostoc*	19.23 ± 0.234	5.49	6.893 ±0.32	2.26	2.41 ±0.208	1.20
Neem	13.82 ± 0.221	0.08	2.360 ±0.31	-2.26	1.298±0.110	0.08
Neem +*Nostoc*	14.77 ± 0.201	1.03	3.344 ±0.43	-1.28	1.378±0.112	0.16
Chemical	17.87 ± 0.211	4.12	4.582 ±0.46	-0.04	1.87 ±0.210	0.66

Table 3.b. Effect of biofertilizer treatments on Photosynthetic pigments in *Triticum aestivum*. (-N): absence of N; where ± indicates standard deviation, n=3.

Treatment	Photosynthetic pigments (mg/g FW)					
	Chl a	% change	Chl b	% change	Carotenoids	% change
Control	15.72±0.213	0	5.63 ±0.31	0	1.35± 0.112	0
Nostoc	17.80 ±0.221	2.08	6.28 ± 0.34	0.65	1.89 ±0.105	0.53
Ash	14.15 ±0.302	-1.57	4.55 ±0.38	-1.08	1.63 ±0.109	0.27
Ash + *Nostoc*	20.71 ±0.331	4.99	6.95 ±0.34	1.32	2.01 ±0.102	0.65
Straw	16.04 ±0.234	0.37	5.95 ±0.31	0.32	1.05 ±0.107	-0.30
Straw + *Nostoc*	21.90 ±0.216	6.18	7.95 ±0.41	2.32	2.88 ±0.212	1.53
Neem	12.01 ±0.189	-3.71	2.95 ±0.45	-2.68	1.27 ±0.124	-0.08
Neem + *Nostoc*	14.30 ±0.190	-1.41	3.95 ±0.33	-1.68	1.45 ±0.101	0.09
Chemical	18.43 ±0.214	2.71	4.95 ±0.32	-0.68	2.19 ±0.134	0.84

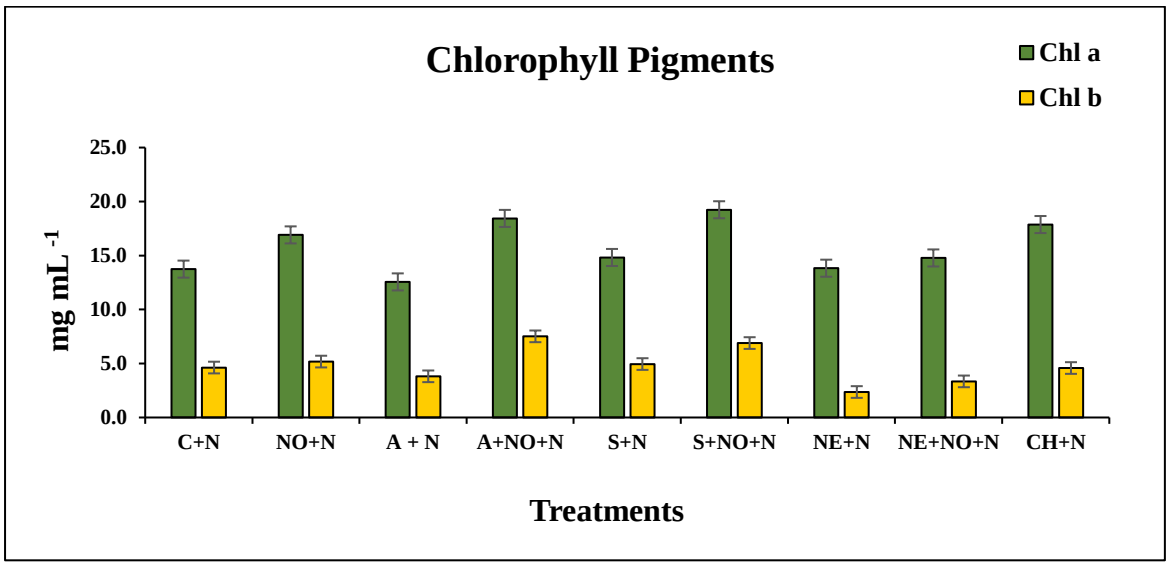

Fig. 19. Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical on chlorophyll pigments in *Triticum aestivum* in presence of nitrates.

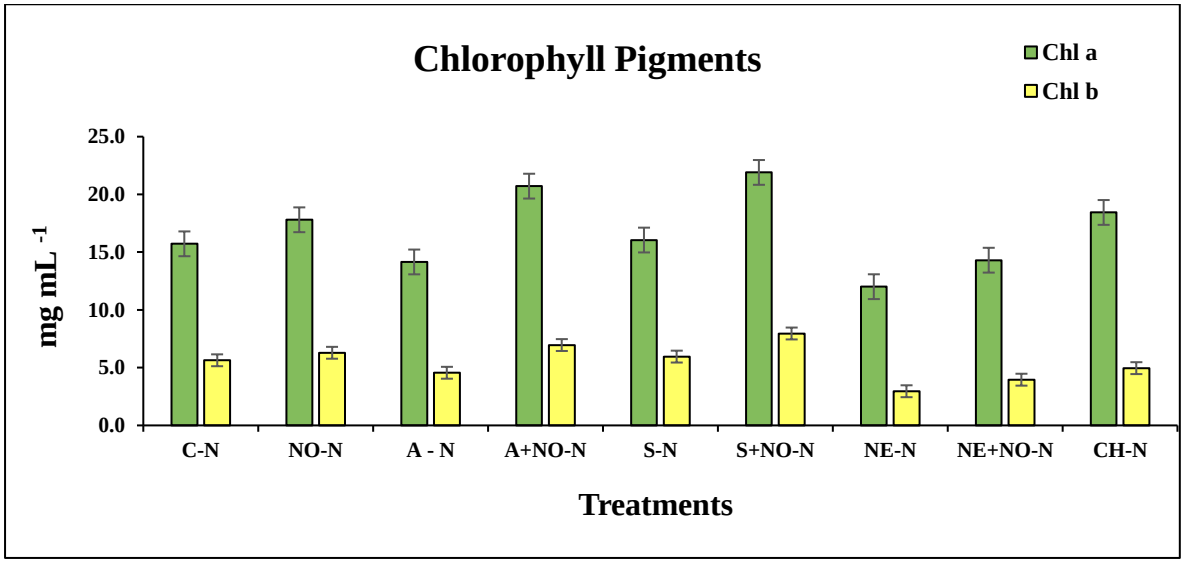

Fig. 20. Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*, (Ne-N);Neem, (Ne+No-N);Neem+*Nostoc* and (Ch-N);chemical fertilizer on chlorophyll pigments in *Triticum aestivum* in absence of nitrates.

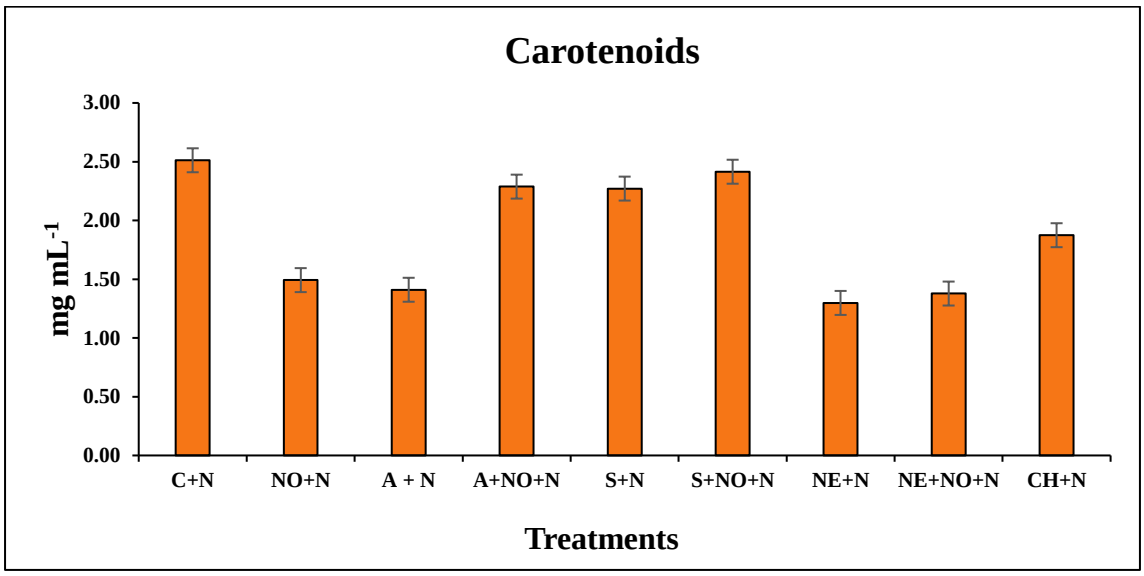

Fig. 21 Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical on carotenoid in *Triticum aestivum* in presence of nitrates.

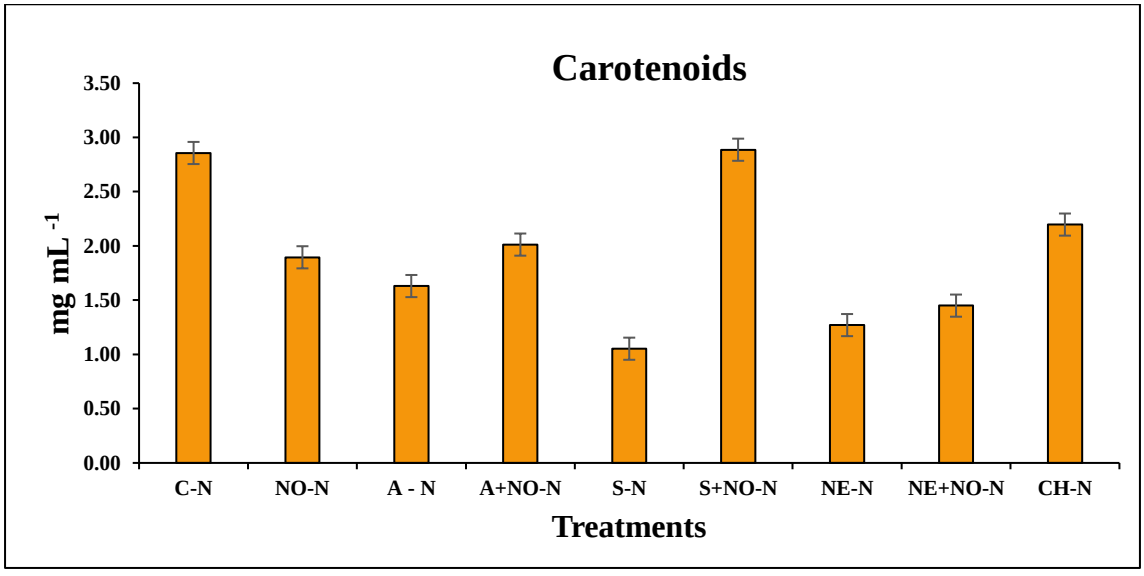

Fig. 22 Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*, (Ne-N);Neem, (Ne+No-N);Neem+*Nostoc* and(Ch-N);chemical fertilizer on carotenoid in *Triticum aestivum* in absence of nitrates.

Table 4.a. Effect of biofertilizer treatments on Photosynthetic efficiency of *Triticum aestivum*. (+N): presence of N; where ± indicates standard deviation, n=3.

Treatment	Photosynthetic efficiency Fv/Fm ratio	% change
Control	0.525 ±0.012	0
Nostoc	0.612 ± 0.012	0.35
Ash	0.549 ±0.013	0.22
Ash + *Nostoc*	0.552 ±0.011	0.34
Straw	0.574 ±0.014	0.51
Straw + *Nostoc*	0.586 ± 0.013	0.62
Neem	0.467 ± 0.010	-0.61
Neem + *Nostoc*	0.413 ±0.011	-0.11
Chemical	0.589 ±0.012	0.65

Table 4.b. Effect of biofertilizer treatments on Photosynthetic efficiency of *Triticum aestivum*. (-N): absence of N; where ± indicates standard deviation, n=3.

Treatment	Photosynthetic efficiency Fv/Fm ratio	% change
Control	0.476 ± 0.012	0
Nostoc	0.506 ± 0.010	0.21
Ash	0.612 ± 0.012	0.14
Ash + *Nostoc*	0.622 ± 0.013	0.15
Straw	0.602 ± 0.011	0.13
Straw + *Nostoc*	0.634 ± 0.014	0.16
Neem	0.452 ±0.010	-0.2
Neem + *Nostoc*	0.509 ± 0.012	-0.3
Chemical	0.540 ±0.013	0.62

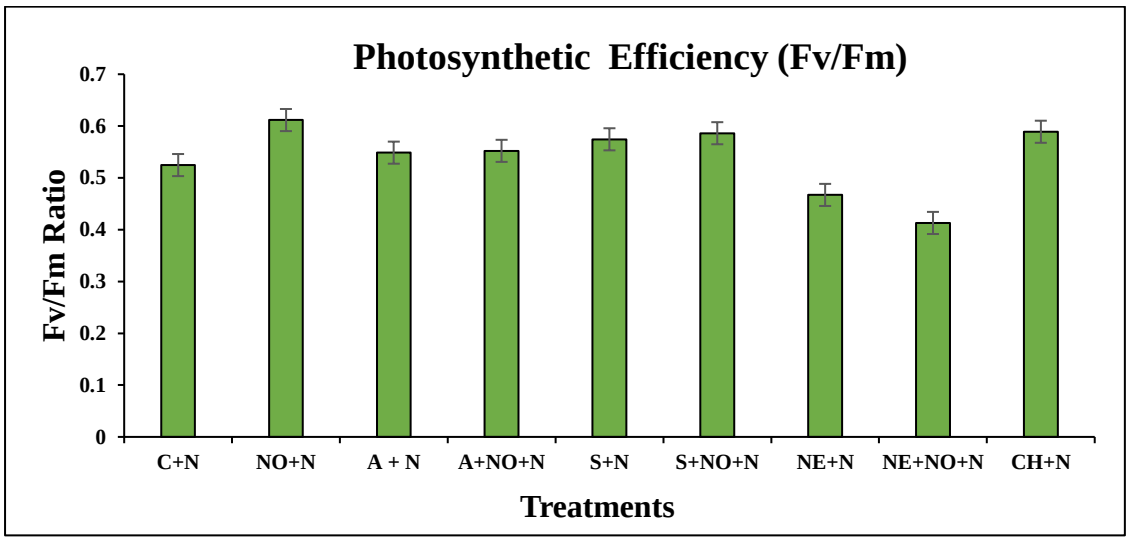

Fig. 23. Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical on Fv/Fm ratio in *Triticum aestivum* in presence of nitrates.

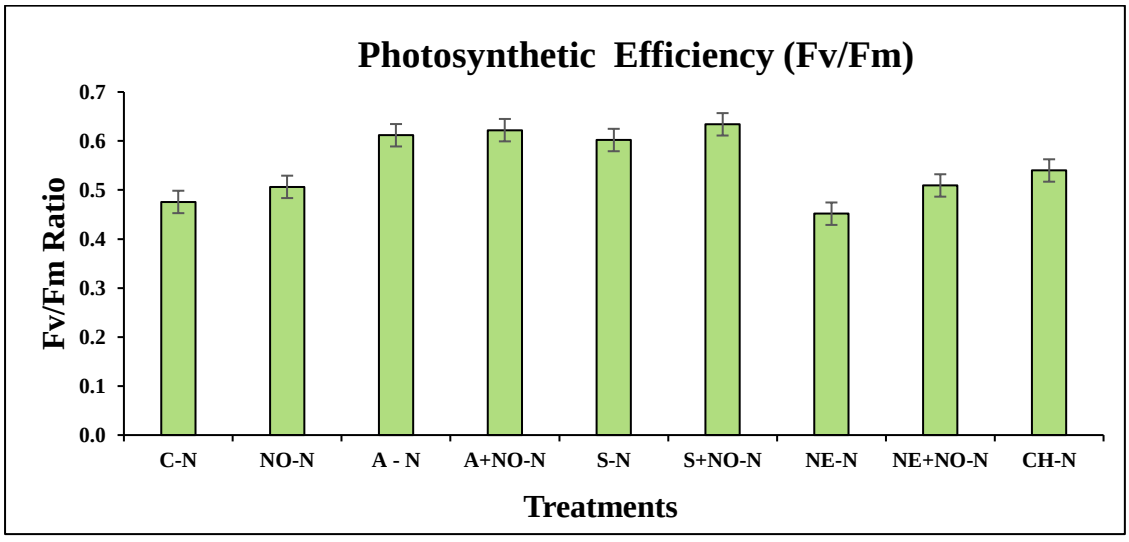

Fig. 24. Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*, (Ne-N);Neem, (Ne+No-N);Neem+*Nostoc* and (Ch-N);chemical fertilizer on Fv/Fm ratio in *Triticum aestivum* in absence of nitrates.

Table 5.a. Effect of biofertilizer treatments on Total sugars (mg/mL), Protein content (mg/mL), Glycolipid content (mg/mL) of *Triticum aestivum*. (+N): presence of N; where ± indicates standard deviation, n=3

Treatment	Total sugar content (mg/mL)	% change	Protein content (mg/mL)	% change	Glycolipid content (mg/mL)	% change
Control	36.19 ±0.013	0	34.10 ± 0.018	0	69.06 ±1.062	0
Nostoc	40.00 ±0.008	3.81	49.50± 0.016	15.43	108.73 ±1.058	39.7
Ash	35.21 ±0.006	-0.98	45.68±0.017	11.57	102.46 ±1.060	33.4
Ash + *Nostoc*	47.42 ±0.011	11.23	55.46±0.018	21.32	139.80 ±1.071	70.7
Straw	39.56 ±0.010	3.37	51.61±0.019	17.50	110.60 ±1.082	41.5
Straw + *Nostoc*	49.16 ±0.007	12.96	62.03±0.016	27.93	132.73 ±1.068	63.7
Neem	28.19 ±0.002	-8.00	28.98 ±0.017	-5.12	68.60 ±1.089	-0.5
Neem + *Nostoc*	32.33 ±0.011	-3.86	37.89 ±0.015	3.78	85.53 ±1.067	16.5
Chemical	42.72 ±0.008	6.53	50.82 ±0.018	16.71	112.06 ±1.087	43.0

Table 5.b. Effect of biofertilizer treatments on Total sugars (mg/mL), Protein content (mg/mL), Glycolipid content (mg/mL) of *Triticum aestivum*. (-N): absence of N; where ± indicates standard deviation, n=3

Treatment	Total sugar content (mg/mL)	% change	Protein content (mg/mL)	% change	Glycolipid content (mg/mL)	% change
Control	40.25 ± 0.007	0	38.02 ±0.017	0	86.33±1.067	0
Nostoc	43.44 ± 0.006	3.19	58.45 ±0.019	20.43	82.60 ±1.078	-3.7
Ash	38.51 ± 0.065	-1.74	47.69 ±0.016	9.62	96.33 ±1.067	10.0
Ash + *Nostoc*	46.32 ±0.009	6.08	55.46 ±0.017	17.43	114.93 ±1.062	28.6
Straw	42.92 ±0.020	2.67	50.03 ±0.016	12.02	92.20±1.087	5.9
Straw + *Nostoc*	51.47 ±0.044	11.23	68.08 ±0.018	30.09	107.86 ± 1.09	21.5
Neem	30.84 ±0.057	-9.40	28.93 ±0.019	9.01	61.26 ±1.056	-25.1
Neem + *Nostoc*	36.11 ±0.006	-4.14	40.19 ±0.017	-2.19	85.66 ±1.067	-0.7
Chemical	44.33 ±0.012	4.09	51.95 ±0.018	13.91	95.87 ±1.064	9.5

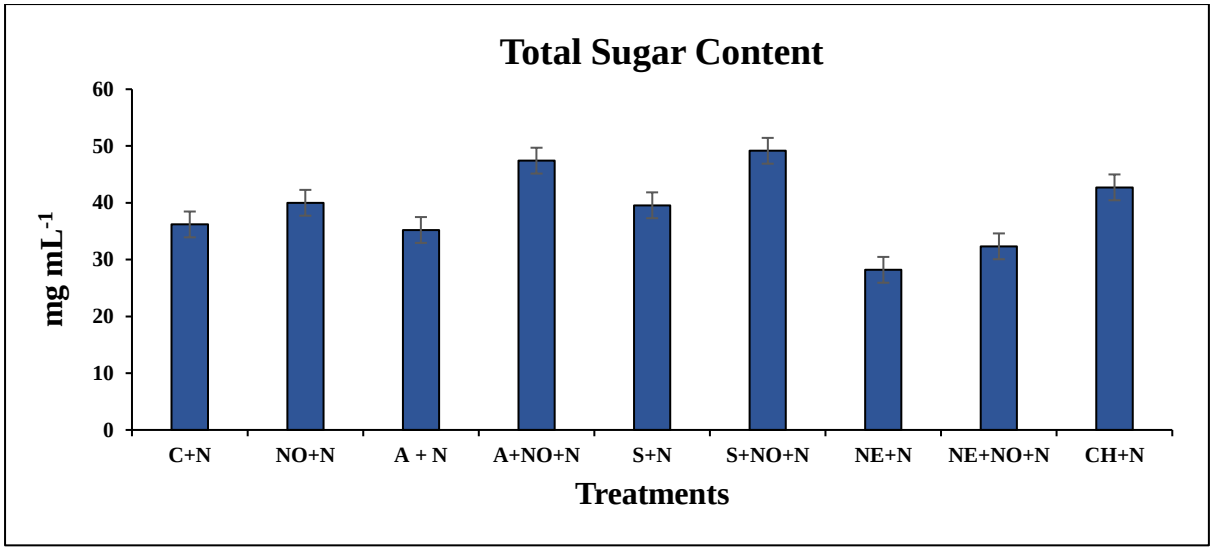

Fig. 25. Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical on total sugars in *Triticum aestivum* in presence of nitrates.

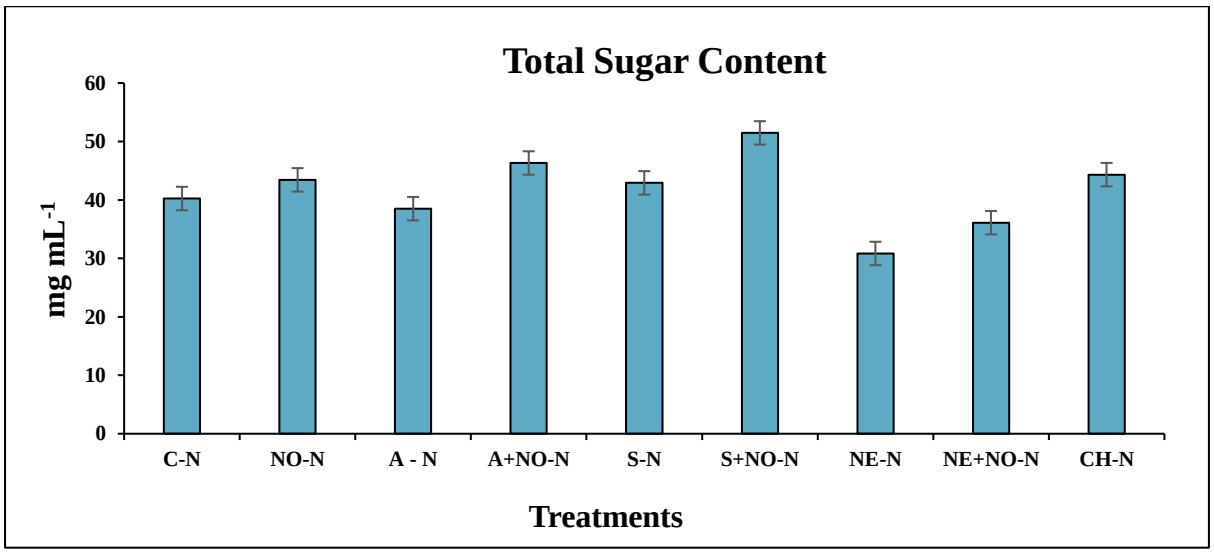

Fig. 26 Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc,* (Ne-N);Neem, (Ne+No-N);Neem+*Nostoc* and(Ch-N);chemical fertilizer on total sugars in *Triticum aestivum* in absence of nitrates.

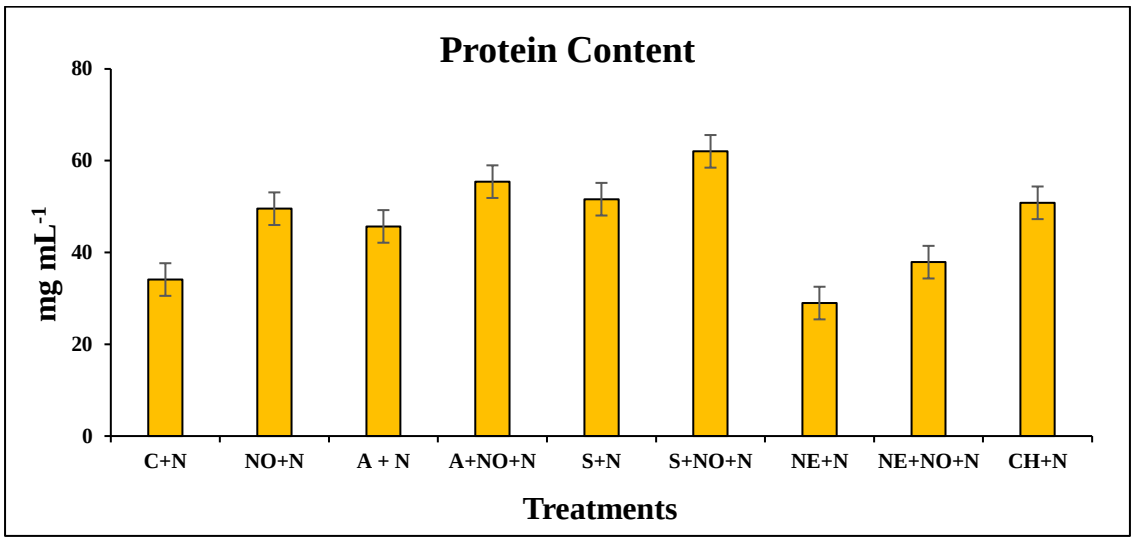

Fig. 27 Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc*,(Ne+N);Neem,(Ne+No+N);Neem+*Nostoc*and (Ch+N);chemical on protein content in *Triticum aestivum* in presence of nitrates.

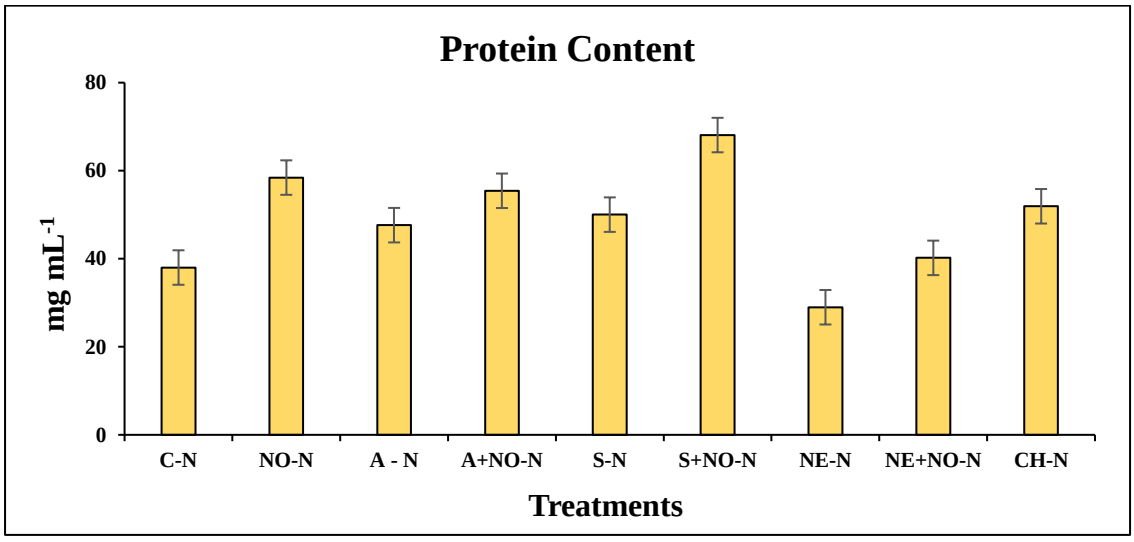

Fig. 28 Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*, (Ne-N);Neem, (Ne+No-N);Neem+*Nostoc* and Ch-N);chemical fertilizer on protein content in *Triticum aestivum* in absence of nitrates.

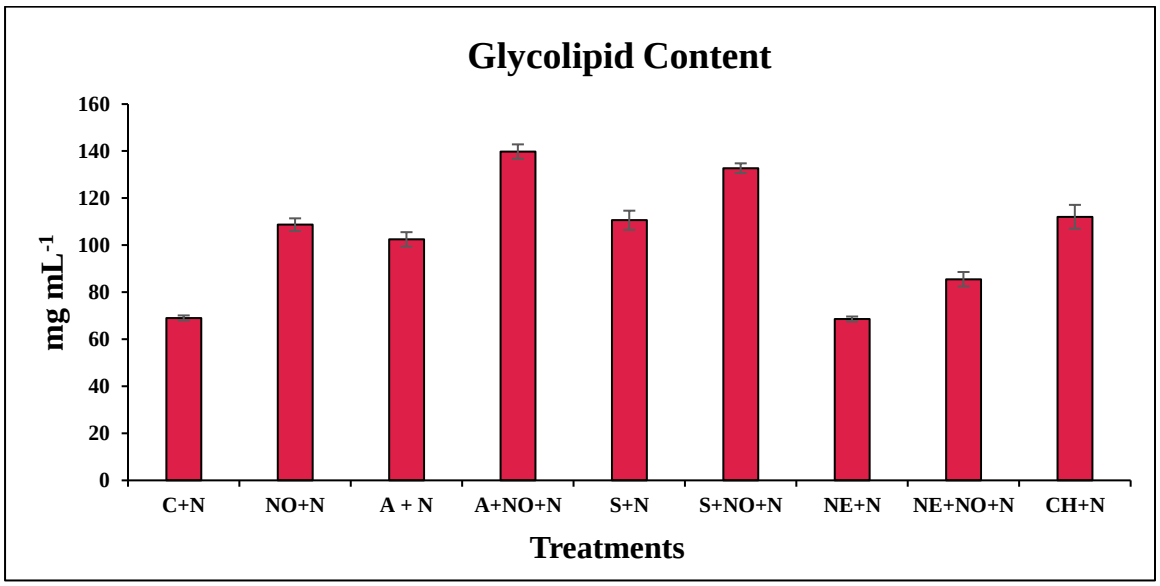

Fig. 29. Effect of (No+N);*Nostoc*, (A+N);Ash, (A+No+N);Ash+*Nostoc*, (S+N);Straw, (S+No+N);Straw+*Nostoc* ,(Ne+N);Neem, (Ne+No+N);Neem+*Nostoc*and(Ch+N);chemical on glycolipid content in *Triticum aestivum* in presence of nitrates.

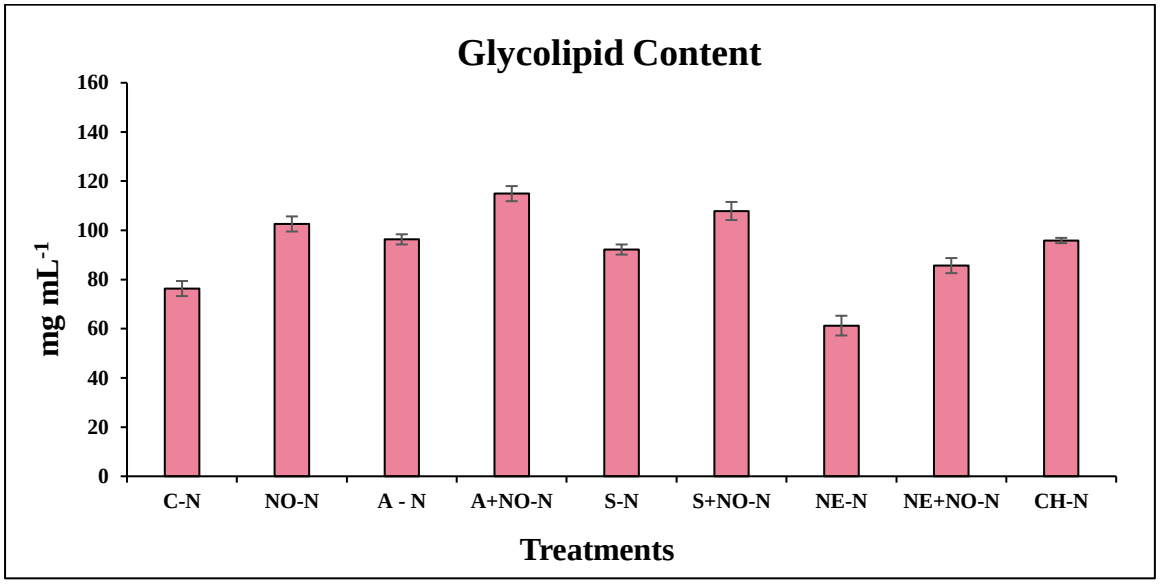

Fig. 30 Effect of (No-N);*Nostoc*, (A-N);Ash, (A+No-N);Ash+*Nostoc*, (S-N);Straw, (S+No-N);Straw+*Nostoc*, (Ne-N);Neem, (Ne+No-N);Neem+*Nostoc*and(Ch-N);chemical fertilizer. on glycolipid content in *Triticum aestivum* in absence of nitrates.