Field Test of Cyanobacteria as Biofertilizer for The Cultivation of *Triticum Aestivum* (Wheat) Plant

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Abstract:
Wheat is rich in carbohydrates, Protein and essential Vitamins and Minerals such Vitamins B and E, Calcium and Iron, as well as fibre. Wheat is an important cereal crop in achieving global Food security. It is cultivated worldwide and is second highest in production. Wheat production is affected mainly by biotic and abiotic stresses all over the world. Maximum yield of wheat can be attained over balanced consumption of all plant nutrient resources. In recent past due to increased cost of chemical fertilizer and with moto of tumbling environmental pollution, creation of amplified awareness on the use of organic sources with bio-fertilizers which are the sources of macro, micro and secondary nutrients to endure both the soil fertility and productivity. The combined nutrient supply contemplates to collective use of inorganic and organic sources of plant nutrient and bio-fertilizers for crop productivity apart from keeping up inspiring soil health. Since past few years, bio-fertilizers like *Azotobacter*, PSB, (Phosphate solubilizing bacteria). *Azospirillum* and liquid bio-fertilizers have shown fabulous potential to improve yield of wheat as these are eco-friendly and low-cost agriculture inputs. Managing Nitrogen inputs in wheat production systems is a significant issue in order to attain maximum profitable production, and minimum negative environmental influence. Wheat production can be enhanced to two to three fold by implementing the cyanobacteria based bio fertilizer. Current study indicates a boost in crop growth, height, thickness of stem, increase in number of grain pods and quantity.

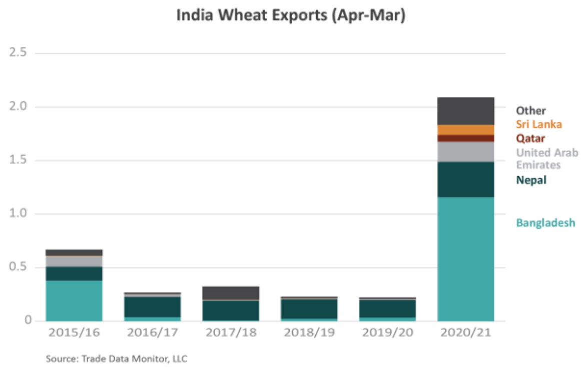
Keywords: biofertilizer, cyanobacteria, wheat, sustainable agriculture, crop yield, etc.

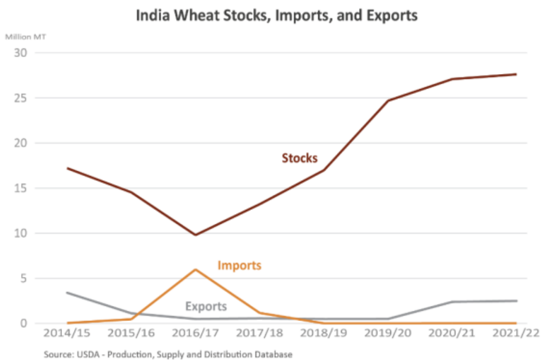
Introduction:

Wheat (*Triticum aestivum*) is one of the most important agricultural commodities. It is a staple food for people across the world. History of wheat consumption can be traced back to 11,000 BCE (Hancock, 2022). According to the Ministry of Agriculture and Farmers Welfare, in India, wheat is cultivated in almost every state with major producers being Uttar Pradesh, Bihar, Madhya Pradesh, Haryana and Rajasthan with total area under cultivation in the financial year 2022-23 being approximately 3.19 crore hectares. As of January 2023, India exported wheat worth ₹11,728.36 crore with total production being 112.18 million tons. The yield of wheat stands at roughly 3,190 kilograms per hectare.

Global prices for wheat and rice have seen steep rise during the past financial year due to decreased production, export restrictions, and strong demand from foreign countries. India witnessed bumper crop production for both the commodities in the financial year 2020-21 and has remarkably increased its exports starting in July 2020.

While the rice exports from India have steadily risen during the past several years, India's wheat exports have expanded in the financial year 2020-21 after being a net importer only a few years ago.





Wheat consumes about 24% of the total fertilizers used in India, majority of them being chemical fertilizer (Usama and Khalid, 2018). Excessive Use of chemical fertilizers can lead not only to soil health deterioration but also pose sever health risks to human health and overall ecology of the area (Bisht and Chauhan, 2020; Kumar *et al.*, 2019) causing possible pollution of water, land and air (Savci, 2012). Recently chemical fertilizers have been linked to various harmful effects on environment and human health (Savci, 2012; Dhankhar and Kumar, 2023, Sharma and Singhvi, 2017).

The goal of sustainable agricultural production can be accomplished without any variation in soil health and productivity of crop by collective application of farm organic manure, chemical fertilizers and bio-fertilizers. Use of bio-fertilizers such as biological nitrogen fixative and phosphate solubilizing micro-organisms is also gaining reputation since bio-fertilizers are cost effective, eco-friendly and renewable source of plant nutrient to supplementary chemical fertilizers. Above all, the role of balanced fertilizer is the application of essential plant nutrients in light proportion and in optimum quantity for a specific soil crop condition in lightening the yield, quality and its features of wheat production is important.

Impact of nitrogen levels on growth and yield of wheat: Nitrogen is one of the chief nutrients which diminish the yield of wheat if not supplied in proper amount as it is needed for vigorous growth of plants and to get high production per hectare. Nitrogen plays imperative role in all the metabolic progressions of plants. Nitrogen is the main constituent and major constituent of plants especially in living tissues establishment. Nitrogen is needed for chlorophyll synthesis as a part of the chlorophyll particle, tangled in photosynthesis and component of all amino acids and protein which are considered responsible for quality of wheat. Nitrogen application rate and timing are very important for yield and quality of wheat. The yield retorts of different cultivars differ widely under diverse nitrogen management. But nitrogen is one of the most significant and posh input in wheat cultivation (Ullah *et al*., 2018).

Organic fertilizers are being used in agriculture for several years. Cyanobacteria based biofertilizer can be used in place of chemical fertilizers. Cyanobacteria comprise of gram negative, photoautotrophic prokaryotes having large heterogeneous and polyphyletic assembly of simple plants which perform oxygenic photosynthesis. Cyanobacteria as Nitrogen–fixing bio-fertilizers. Certain cyanobacterial species like *Nostoc sp*., *Anabaena sp*., *Leptolyngbya sp*. etc. posses Nitrogen fixing genes called *Nif* genes that code for enzymes like Nitrogenase that fix the inorganic atmospheric Nitrogen (N_2) into organic forms like ammonia that can be utilized by plants for their growth and development (Latyseva *et al*., 2012; Kallas *et al*., 1985).

Ever increasing prices of Nitrogen fertilizers and risk of environmental pollution and groundwater corruption, warn for their careful and well-organized use. Many cyanobacteria species have been previously tested for their ability to be used as biofertilizer (Karthikeyan *et al*., 2007). Field trials of cyanobacteria as biofertilizer are being carried out for a long time for different crops (Vaishampayan *et al*., 2001; Abd-Alla et al., 1994). In case of cyanobacteria trial with rice cultivation in different states of India has resulted very positive results. The crop yield has increased by up to 32% in the soil supplemented with cyanobacteria (Kaushik, 2014). Mixed use of cyanobacteria based biofertilizer along with chemical fertilizer has also resulted in decreased impact on soil with increase in yield and thus average income of the farmers (Bhooshan *et al*., 2020).

Material and Methods:

Cyanobacteria pure culture was obtained from the cyanobacteria culture facility of Aakriti Biotechnology, Ranchi, Jharkhand, India. Pure strain of cyanobacteria was inoculated into BG11 media, pH – 7.5 and incubated at 25°C in light condition with 12 hours light and dark interval. Regular microscopic observations were taken to ensure purity of culture.

Poly House and Soil Preparation: Poly house of dimension 2.5 ft. width and 2.5 ft. height was prepared with sterilized soil bed of depth 10 cm.

Soil collection: Soil was collected from fertile field and sterilized by autoclaving.

Sterilization of Seed: Seed was Surface sterilized using detergent followed by formalin treatment for 30 minutes. Detergent solution was used to remove contaminants from the seed coat. This process ensures clean and disease-free seeds, promoting better germination and plant growth.

30 gram grains were planted on both the sides i.e. test soil bed and control soil bed.

Preparation of Bio fertilizer: Cyanobacteria were harvested from 2 litre culture, cleaned with water to eliminate any salt previously added. Cleaned cyanobacteria were re-dissolved in distilled water uniformly. During the experiment, wheat seeds were divided into two groups: one group was treated with the cyanobacterium, while the other group received no treatment and served as the control.

Observations for different parameters like shoot length, root length, thickness of primary root, thickness of stem, and numbers of grain pods were taken for up to 90 days till the development of wheat pods. All the readings were taken as average of 50 readings.

Results:

Inoculation of wheat with cyanobacteria resulted in enhanced growth parameters as compared to the control group. Cyanobacteria inoculation increased plant height, shoot width, increased root length, gain pods, thickness of primary root and overall health of plant.

Table-1: Data showing the difference between plant length and shoot length of test and control wheat plants.

Duration	Average length of whole plants		Average Shoot Length	
	Test	Control	Control	Test
15th days	20.25 cm	16 cm	23.62 cm	25.62 cm
30th days	32.25 cm	20.75 cm	26.07 cm	38.62 cm
75th days	43.25 cm	22.25 cm	29.75 cm	54.25 cm
90th days	49.5 cm	24.67 cm	28.47 cm	56.32 cm

Table-2: Data showing the difference between root length and primary root thickness of test and control wheat plants.

Duration	Average Root Length		Average thickness of primary roots	
	Test	Control	Test	Control
15th days	5.37 cm	7.62 cm	0.65 mm	0.15 mm
30th days	6.12 cm	5.32 cm	0.65 mm	0.3 mm
75th days	11 cm	2.92 cm	1.2 mm	1.02 mm
90th days	6.9 cm	3.8 cm	2.25 mm	1.07 mm

Table-3: Data showing the difference between stem thickness and number of grain pods in test and control wheat plants.

Duration	Average thickness of stem		Average no. of grain pods	
	Test	Control	Test	Control
15th days	1.17 mm	1.7 mm	-	-
30th days	1.27 mm	1.8 mm	-	-
75th days	2.75 mm	1.65 mm	8.75	3.25
90th days	3 mm	1.6 mm	9.5	4.5

Figure - 2: Different between growth of wheat with cyanobacteria inoculation (left) and without cyanobacteria (right) after 90 days.

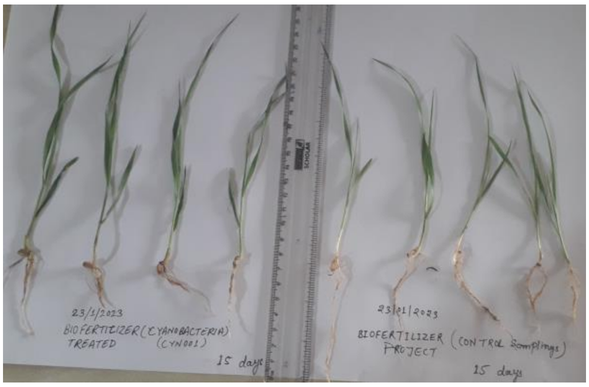


Figure - 1: Different between growth of wheat with cyanobacteria inoculation (left) and without cyanobacteria (right) after 15 days.

Discussion:

Cyanobacteria were cultured in BG11 media for 60 days to prepare Biofertilizer was dilutions was carefully prepared to ensure the introduction of a sufficient concentration of beneficial bacteria to the treated group. Over 90 days, several growth parameters were measured and compared between the two groups. The purpose was to evaluate whether the cyanobacterial treatment had any significant impact on wheat growth compared to the control group. The results of the study showed noticeable differences between the cyanobacteria-treated wheat and the non-treated wheat. The cyanobacteria-treated wheat exhibited enhanced growth in multiple aspects. Firstly, the treated plants displayed increased plant height, with taller and more root hair compared to the control group. This indicated improved elongation and development of the wheat plants. Moreover, the root length of the cyanobacteria-treated wheat was found to be significantly longer compared to the non-treated wheat. This indicated improved root system development, potentially leading to better nutrient and water absorption from the soil.

Furthermore, the biomass accumulation in the cyanobacteria-treated wheat was higher, indicating increased overall plant growth and productivity. The treated wheat plants showed greater shoot and root biomass compared to the control group, suggesting improved nutrient utilization and assimilation. Overall, the study demonstrated that the application of cyanobacteria as a biofertilizer for wheat plants had a positive impact on their growth and development. The treated wheat plants exhibited improved height, leaf area, root length and biomass accumulation compared to the non-treated plants. These findings suggest the potential of cyanobacteria as a beneficial and sustainable approach to enhance wheat production and improve crop performance. Further research is warranted to explore the underlying mechanisms and optimize the application methods for cyanobacterial treatments in wheat cultivation.

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