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EFFECT OF *AZOSPIRILLUM BRASILENSE, AZOTOBACTER CHROOCOCCUM,* ON THE GROWTH AND YIELD OF ORYZA SATIVA UNDER BIOFERTILIZERS

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Abstract
The application of biofertilizers has drawn interest recently as a sustainable substitute for chemical fertilisers in agriculture. Azotobacter and Azospirillum, two nitrogen-fixing bacteria, are essential for increasing soil fertility and fostering plant growth. The purpose of this study is to assess how inoculating rice cultivars with Azotobacter and Azospirillum as biofertilizers affects plant development, yield, nitrogen fixation, and soil health. According to the findings, inoculation with these advantageous bacteria considerably increases soil fertility and rice yield, offering a sustainable substitute for traditional fertilisers.

1. Introduction

A staple crop and essential to the world's food security is rice (Oryza sativa). Nitrogen availability frequently limits rice yield, and excessive use of chemical nitrogen fertilisers has resulted in soil erosion, pollution, and higher production costs. A sustainable method of enhancing crop output and soil health in this situation is the use of biological nitrogen fixation via inoculation with two well-known nitrogen-fixing bacteria, Azotobacter and Azospirillum. This study examines how inoculating several rice cultivars with Azotobacter and Azospirillum affects growth metrics, yield, soil nitrogen content, and other agronomic characteristics. With the potential to counteract many of the drawbacks of traditional chemical-based agriculture, biofertilizers bring a fresh perspective to Indian agriculture. Microbial inoculants, commonly referred to as "biofertilizers," are carrier-based preparations that contain beneficial microorganisms in a viable state and are intended for application as seeds or soil. Their purpose is to increase the number and biological activity of desired microorganisms in the root environment, thereby improving soil fertility and promoting plant growth.In crop cultivation, Rhizobium, Azotobacter, Azospirillum, phosphate-solubilizing bacteria, and fungi are the most often employed biofertilizers. Trichoderma sp. and Pseudomonas sp. are arbuscular mycorrhizal (AM) fungi that are thought to be both potential biocontrol agents and plant growth enhancers. Biofertilizers are inexpensive, renewable sources of plant nutrients, and their application in agriculture has gained particular importance in the current context of integrated farming, organic farming, and nutrient management techniques. revealed that biofertilizers are a cost-effective way to boost crop output when combined with chemical fertilisers. The availability of nitrogen is one of the main factors limiting crop productivity. Because chemical nitrogenous fertilisers are limited in their manufacture, availability, and use, biologically fixed nitrogen will be crucial to boosting agricultural yields. Because each gramme of biofertilizer contains at least 10 million viable cells of a particular strain, a minimal dose of biofertilizer is enough to yield desired outcomes.

1.1 Role of Biofertilizers

Biofertilizers on the other hand are cost-effective and renewable source of plant nutrients to supplement the parts of chemical fertilizers. Biofertilizers are known to play an important role in increasing availability of nitrogen and phosphorus besides improving biological fixation of atmospheric nitrogen and enhance phosphorus availability to crop. Dual inoculation of vermi compost and bacteria biofertilizers proved more effective in increasing the growth of different crop plants. Biofertilizers are products containing living cells of different types of micro-organisms which when, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen, phosphorus) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock phosphate.

1.2 Role of Azotobactor and Azospirillum

Azotobacter and Azospirillum are the most predominant and important ones. Both are known to provide a nitrogen economy of 20-30 kg nitrogen ha-1 , coupled with production of growth promoting substance, besides improving growth yield quality attributes of fruit and thus, leading to the improvement of crop. The favorable effect of Azotobacter and mineral nitrogen fertilizer on growth, chemical composition of leaves, and yield was reported on pea indicated that both inoculation with Azotobacter and application of N increased seed yield. Azotobacter is free living nitrogen fixer, however in plant rhizosphere due to availability of various readily utilizable carbon compounds, the bacteria are considered to be advantage for plant growth and yield. Azotobacter and Azospirillum are the two most important non-symbiotic N-fixing bacteria in nonleguminous crops. Under appropriate conditions, Azotobacter and Azospirillum can enhance plant development and promote the yield of several agricultural important crops in different soils and climatic regions. These beneficial effects of Azotobacter and Azospirillum on plants are attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake by roots, displacement of fungi and plant pathogenic bacteria and to a lesser extent, biological nitrogen fixation. Role of PSB PSB in agricultural practice would not only offset the high cost of manufacturing phosphate fertilizers but would also mobilize insoluble in the fertilizers and soils to which they are applied.

2. Objectives:

1. To compare the efficacy of Azotobacter and Azospirillum in enhancing rice growth and productivity across different rice cultivars.

3. Methods

3.1. Cultivars: Certified seeds of two cultivars, viz., IR – 64 and NDR – 359 were procured from the State Agriculture Research Farm of the Bilaspur (C. G.)

3.2. Chemicals for various experiments: All the chemicals required for various biochemical estimations were of analytical grade procured from manufactures such as E.
Merck, Sigma-Aldrich, SRL, Thy Media etc.

3.3 Chemical fertilizers: The source of nitrogen, phosphorous and potassium
Fertilizers were: (i) Urea (NH2-CO-NH2), (ii) Single super phosphate [P2O5 in the form of [Ca (H2PO4). 2H2O], and (iii) Muriate of potash (KCl) respectively.

3.4 **Inoculants**: Two bacterial microbes, viz., *Azospirillum brasilense*, *Azotobacter chroococcum*, were procured from the Institute of Bilaspur C. G.

3.5 Cultivation practices: Studies were conducted both in paddy fields and in earthenware pots. In paddy fields, either traditional practices of cultivation or System of Rice Intensification (SRI) technique prescribed by Laulanie (1993) were followed. The pot experiments included cultivation on sterilized sand as well as both on sterilized and un-sterilized paddy soils.

3.1.3 Experimental design in paddy field: Except co-inoculation experiments in fields, all the field experiments (both in traditional practices and in SRI techniques) were laid out in split-plot design with

Composition of the medium for *Azospirillum brasilense*
(Medium No.MTCC-446, according to Catalogue of Strains,2000, IMTECH)

Beef extract …….1.0g Yeast
Extract…….2.0g
Peptone……..5.0g
NaCl……....5.0g
Distilled Water…….1.0 litre

The above ingredients were homogenized in 1 litre conical flask and from this 200 ml contents were kept in 5 number of 250 ml conical flasks and autoclaved for 35 min at 15 lb pressure.

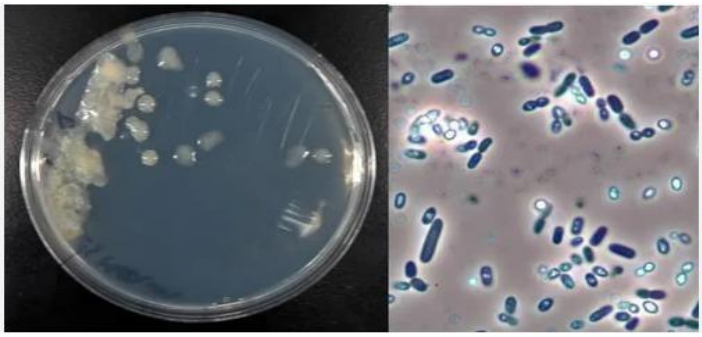
Composition of the medium for *Azotobacter chroococcum*
(Medium No. MTCC-46, according to Catalogue of Strains, 2000, IMTECH)

K2HPO4……1.0g
MgSO4.7H2O……0.2g
NaCl……...0.2gFeSO4.
7H2O…….……..5.0mg*Soil extract:……...100.0ml
Agar:…….....15.0gDistilled
water.....900.0 ml

Added 2% mannitol to the above medium and adjusted to pH 6.
Preparation of Soil extract
Ingredients: Soil - 77.0 g, Na2C03
- 0.2 g, Distilled water 200.0 ml

Procedure: Approximately 100 gram soil from paddy was taken and spread on a glass plate or in a large Petri dish and allowed to dry for 1 or 2 days under natural condition. From this, 77.0 g soil was weighed and mixed with 200 ml distilled water in a 500 ml conical flask, autoclaved for 1 hour, cooled and filtered it through cheese cloth. To get a clear extract, the filtered soil extract was centrifuged at 5000 rpm in an ordinary centrifuge. The supernatant was collected and 100 ml from this was used for preparation of the mediun for *Azotobacter chroococcum*.

4. Results And Discussion Morphological identification of Azotobacter
Bacterial colonies appeared on the culture medium showed similar morphological characters as that of *Azotobacter chroococcum.*



Chlorophyll (a, b and total) in leaves of Oryza sativa L.: Chlorophyll (a, b and total) in leaves of both the cultivars (cv. IR – 5 and cv. NDR –12) were estimated in sand culture and of plants raised on sterilized paddy soil in pots to study the effect of inoculation of *Azospirillum brasilense* and *Azotobacter chroococcum* in integration with different levels of urea-N. Results of experiment on chlorophyll in the leaves of Oryza sativa L. grown in sand culture : Sand culture experiment conducted in rainy autumn (aman) season. Both the rice cultivars were inoculated with *A. brasilense* and *A. chroococcum* in integration with different levels of urea-N. The treatment combinations were as follows:

N0B4 = no N + *Azotobacter chroococcu*m (seed inoculated) + P and K
N1B2 = *Azospirillum brasilense* (root dip) + 20 kg N ha-1 + P and K
N2B4 = 40 kgNha-1 + *Azotobacter chroococcum* (seed inoculated) +P and K
N3B1 = *Azospirillum brasilense* (seed inoculation) +60 kg Nha-1+ P and K
N4B5 = 80 kg N ha-1 + *Azotobacter chroococcum* (root dip) + P and K

The level of chlorophyll a was found maximum with N4B0, i.e., no bioinoculants + full dose of Nitrogen + P and K, Chlorophyll b with N2B4, i.e., *Azotobacter chroococcum* (seed inoculated) + 50% Nitrogen + P and K, total chlorophyll in N4B5, i.e., root dip of *Azotobacter chroococcum* + 80 Kg N ha-1 + P and K. In case of inoculation of A.brasilense, total chlorophyll was maximumwith N1B2, i.e., *Azospirillum brasilense* (root dip) + 25% Nitrogen + P and K and with N3B1, i.e., *Azospirillum brasilense* (seed inoculated) + 75% Nitrogen + PK. In case of *Azotobacter chroococcum* chlorophyll a, chlorophyll b and total chlorophyll were found maximum with N4B5, i.e., root dip of *Azotobacter chroococcum* + 80 kg N ha-1 + P and K.

Table – 1: Effect of *A.brasilense* and *A.chroococcum* in integration with urea-N in different levels on chlorophylls (a, b and total) in the leaves of Oryza sativa L. (cv. IR -5). Plants were raised in sand culture.

Treatments	**Chlorophyll (mg/g leaf fr. wt.) on 60 DAT**		
	Chlorophyll a	**Chlorophyll b**	**Total Chlorophyll**
N0B4	0.54 ± 0.033	0.33 ± 0.072	0.88 ± 0.099
N1B4	0.62 ± 0.025	0.44 ± 0.089	1.06 ± 0.102
N2B4	0.64 ± 0.013	0.55 ± 0.100	1.19 ± 0.102
N3B1	0.64 ± 0.021	0.54±0.132	1.18 ±0.118
N4B5	N4B5 0.65± 0.007	0.62 ± 0.105	1.26 ± 0.100

Fig. 1 data on total chlorophyll of treatments without diazotrophic inoculation and of only of root dipping inoculation of *A. brasilense* and *A. chroococcum* in integration with no urea-N and two in levels (0, 50 and 75 per cent) on cv. IR –5 have been compared and it was observed that integration resulted better in terms of total chlorophyll in leaves, where urea-N was 50 per cent

Conclusion- Plant growth and biomass parameters recorded in the cv. IR – 64 and cv. NDR – 359 in the present study indicated that, due to inoculation of *Azospirillum brasilense* and *Azotobacter chroococcum* both individually and on integration with urea – nitrogen increased yield parameters significantly in certain combinations, which were in agreement with findings of many researchers.

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