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Impact of Combining Bio-Inoculants along with Inorganic Fertilizers on Soil Biological Characteristics in Transplanted Rice (*Oryza sativa* L.) Crop

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A field experiment was carried out during the Kharif season in the year 2022 to assess the impact of inorganic fertilizers along with Bio-inoculants on soil biological properties in rice (*Oryza sativa* L.). The experiment consists of seven treatments viz., T_1 Control, T_2 100% RDF (150, 60, 40 N_2, P_2O_5, K_2O

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kg ha^{-1}), T_3 80% RDF + 10 kg BGA ha^{-1} Soil application + PSB (Phosphate solubilizing bacteria) 3kg/ha, T_4 60% RDF + 10kg BGA ha^{-1} Soil application + PSB, T_5 80% RDF + 500kg Azolla ha^{-1} Soil application +PSB, T_6 60% RDF + 500kg Azolla ha^{-1} Soil application +PSB and T_7 10kg BGA ha^{-1} Soil application + 500kg Azolla ha^{-1} Soil application + PSB were comprised in Randomized Block Design with three replication. The incorporation of inorganics, Azolla PSB, and BGA (bio-inoculants) actively activated microbial activities, resulting in a greater increase in the populations of total bacteria, actinomycetes, and fungi was observed throughout all growth stages, regardless of the treatments applied, when compared to their initial densities. This phenomenon supports the notion of a stronger rhizosphere effect. This trend aligns with the rise in organic carbon content. Moreover, soil dehydrogenase, alkaline phosphatase activities, along with microbial biomass carbon, displayed an upward trend in tandem with the progressive growth phases of the rice plant.

Keywords: Alkaline phosphatase; azolla, dehydrogenase; soil microbial biomass carbon; total microbial count; PSB; BGA.

1. INTRODUCTION

Rice, scientifically known as *Oryza sativa* L., belongs to the Poaceae family and is an important cereal crop cultivated under aquatic (anaerobic) conditions. It is grown in diverse climatic conditions and is considered the second most important cereal crop worldwide, serving as a major food source for 50% of the global population due to its nutritional value (Baroudy et al., 2020). The total cultivated area for rice globally in 2018 was approximately 164.19 million hectares, with a total annual grain production of 756.74 million tonnes and an average yield of 4.60 tonnes per hectare (FAOSTAT, 2021). Notably, around 90% of the world's rice is produced and consumed in the Asian region. Rice is a high-calorie food, containing approximately 75% starch, 6-7% protein, 2-2.5% fat, 0.8% cellulose, and 5-9% ash. In India, rice is cultivated across an area of approximately 45 million hectares, with an annual production of 111.76 million tonnes during 2021-2022 (Alexander, 1965; Chakrabarti et al., 2000). The productivity of rice in Indian plain regions is recorded at 2705 kg ha^{-1}. Uttar Pradesh, a state in India, has an area of 5.6 million hectares dedicated to rice cultivation, including both irrigated and rainfed (Singh & Giri, 2021). The cultivated area in the state has fluctuated between 5.2 and 6.1 million hectares over the past seven years. The average productivity in the state is approximately 2 tonnes ha^{-1}.

Azolla is commonly used as a cover crop in rice cultivation and is inoculated and grown to be incorporated into the soil. It has been historically recognized as a beneficial plant in agriculture, particularly in Southern China and Northern Vietnam, where it is used as a bio-fertilizer and green manure due to its nitrogen-fixing abilities (Hove and Lejeune, 1996). Azolla has also been utilised as poultry feed (Gouri et al., 2012). By applying Azolla in every planting season, the need for artificial fertilizers can be significantly reduced as it directly provides nutrients to the soil (Maftuchah et al., 1998; Kumari et al., 2017). Blue-green algae (BGA) are known to contribute up to 80 kg of nitrogen per hectare per season in the rice ecosystem. Under waterlogged conditions in rice fields, blue-green algae play a crucial role in maintaining soil fertility and crop yield, even in the absence of additional agro-chemical inputs (Kowalenko, 1978) The application of microbial phosphatic fertilizers has been shown to reduce synthetic P levels by 25-50% in agricultural practices. Direct application of Phosphate Rock (PR) is often ineffective for annual crops, but its availability can be enhanced by the application of acid-producing microorganisms capable of solubilizing phosphate rock. Various strains of PSB, such as *Pseudomonas striata*, *Bacillus polymyxa*, and *Pseudomonas fluorescens*, have been found to solubilize significant amounts of P from different sources (Krishnakumar et al., 2005).

Lenhard, 1956 proposed the idea of measuring dehydrogenase activity to ascertain the metabolic activity of microorganisms in soil and other ecosystems. Compared to other components, soil microbial biomass is more susceptible to agricultural management. Soil microbial biomass can be used to estimate the short-term impact of soil management on soil characteristics (Subbarao et al., 2009). In organically treated soil, dehydrogenase activity and soil biomass are substantially associated (Garcia et al., 2008). The transport of protons and electrons from substrates to acceptors, significantly contributes to the biological oxidation of soil organic matter (Glinski et al., 1986;Mallikarjun & Maity, 2018). With crop root development and crop rooting density, soil microbial biomass increased. Better crop production as a result of better fertilizer management results in the incorporation of more organic matter through root biomass, leaf fall, root exudates, etc., which affects the dynamics of C in the soil (Lynch and Panting, 1980;Kuttimani et al., 2017). The soil microbial diversity is the most significant functional component of the soil biota. Microbial adaptation to environmental circumstances allows microbial analyses to be discriminating in soil health evaluation, and changes in microbial populations and activities may therefore operate as a good indication of change in soil health (Pankhurst et al., 1995).

2. MATERIALS AND METHODS

The experiment was conducted at the Agronomy Research Field, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (UP) for Kharif, 2022. The experimental site falls under the subtropical climatic zone of Indo-Gangetic plains situated 26.47 0N latitude, 82.12 0E longitude and with an altitude of 113 meters above mean sea level. The actual soil used for the experiment was silty loam in nature with a bulk density of 1.42, alkaline in reaction pH 8.61, and available N, P, and K were 150.20, 13.8, and 256.3 kg ha-1 respectively. The initial microbial count of experimental soil was recorded as bacteria, actinomycetes, and fungi were recorded as 0.9, 3.32, and 1.21CFU/gm respectively. The value of soil microbial biomass carbon in the original soil sample was 183.6µg SMBC g-1 soil. The initial status of enzymes in terms of dehydrogenase and alkaline phosphatase is deduced as 78.80 and 136.41 µg g p-nitrophenol soil-1 h-^{1} respectively. Numbers for seven different treatments were designed, aiming to observe their influence on crop improvement in terms of growth and yield enhancement. The first one considered as T_1 was a control, Treatment T_2 consisted of RDF N:P: K150:60:40, In T_3, 80% RDF with an addition of 10 kgs. of bio-growth agent (BGA) ha-^{1} and 3 kg of PSB per hectare was applied. T_4 consisted of a 60 % RDF with the addition of 10 kg BGA ha-^{1} and 3 kgs of PSB ha-^{1}. Proceeding to T_5, this treatment consisted of 80% RDF together with the addition of 500 kg Azolla ha-^{1} and 3 kg of PSB ha-^{1}. Meanwhile, T_6 contained 60% RDF in addition to 500 kg of Azolla ha-^{1} and 3 kg of PSB ha-^{1}. Finally, T_7 consisted of the collective addition of 10 kg of BGA ha-^{1}, 500 kg of Azolla ha-^{1}, and 3 kg of PSB ha-^{1}. Each of the following treatments was designed in such an articulate manner as to sort out a main set of nutrient supplementation and organic inputs with bacterial assistance in optimizing crop productivity and nutrient use efficiency within the agricultural context.

3. RESULTS AND DISCUSSION

3.1 Soil Microbial Population (Bacteria, Fungi, Actinomycetes) and Organic Carbon

The data shows in Table 1 and Fig. 1 that successive application of different fertilizer treatments and biofertilizers has a significantly higher impact on soil microbial populations and organic carbon content. However, no significant difference in the OC content is evident among the treatments; amongst the bodies, there is a slightly increasing order with the enriching of the treatments, for which the trend in T_7 seems to have the highest value with 4.49 g/kg. All the microbial populations - bacteria, actinomycetes, and fungi - increased significantly in the biofertilizer treatments, with most increases occurring in RDF treatments and those with added BGA, Azolla and PSB. T_7, the combination of both BGA and Azolla with PSB, had the highest microbial count among all groups, especially bacteria, as high as 15.4 × 10^6 cfu, and actinomycetes, as high as 12.4 × 10^4 cfu. This proves that the application of biofertilizers has tremendous effects on improving soil microbial activities. the observed increase in microbial growth could be attributed to the incorporation of organic inputs, such as phosphate-solubilizing bacteria, blue-green algae (BGA), and Azolla, into the soil, which enhanced its physical properties. These findings are consistent with the results reported by Mandal et al. (2017).

3.2 Microbial Biomass Carbon (SMBC) and Dehydrogenase Activity

The data shown in Table 2 and results indicate that SMBC, as well as TPF of soil dehydrogenase activity, increased upon the application of different nutrient management treatments over the control. T_7 received the combined application of 10 kg BGA @ 500 kg Azolla + PSB, which resulted in the highest recorded values for the two parameters in that treatment: SMBC reached 215.02 µg g^{-1} soil and dehydrogenase activity became 156.45 µg TPF g^{-1} soil day^{-1} in. Treatments with 60% RDF and biofertilizers like BGA, Azolla, and PSB also exhibit increasing microbial activities. Hence, the integrated application of chemical fertilizers with biofertilizers enhances soil biological activities more effectively compared to the application of chemical fertilizers alone. The significant differences among treatments, as evident from the CD value at p = 0.05, might indicate the positive contribution of biofertilizer applications to soil health and fertility. Soil dehydrogenase is regarded as an indicator of microbial activity, facilitating the biological oxidation of organic biomass generated by Azolla, blue-green algae (BGA), and phosphate-solubilizing bacteria (PSB). It also plays a role in the transfer of hydrogen from organic substrates to inorganic acceptors. The findings of this study align with the observations reported by (Philip et al. 2018), (Mandal et al. 2017), and (Meena et al. 2014).

Table 1. Effect of bio-inoculants along with inorganic fertilizers on soil microbial population and organic carbon

Num	Treatments	OC (g/kg)	Bacteria ($\times 10^6$ cfu)	Actinomycetes ($\times 10^4$ cfu)	Fungi ($\times 10^3$sfu)
T_1	Control	4.20	6.9	8.1	6.09
T_2	100% RDF (150, 60, 40 N, P_2O_5, K_2O kg ha^{-1})	4.31	7.2	8.9	6.3
T_3	80% RDF + 10 kg BGAha^{-1}Soil application + PSB	4.39	8.6	9.2	6.8
T_4	60% RDF + 10kg BGA ha^{-1} Soil application + PSB	4.43	11.2	10.6	7.4
T_5	80% RDF + 500kg Azolla ha^{-1} Soil application +PSB	4.42	9.2	10.2	7.2
T_6	60% RDF + 500kg Azolla ha^{-1} Soil application +PSB	4.45	13.3	11.2	7.8
T_7	10kg BGA ha^{-1} Soil application + 500kg Azolla ha^{-1} Soil application + PSB	4.49	15.4	12.4	8.2
	C.D. (p=0.05)	NS	0.46	0.33	0.35
	SE(m) ±	0.082	0.14	0.10	0.112

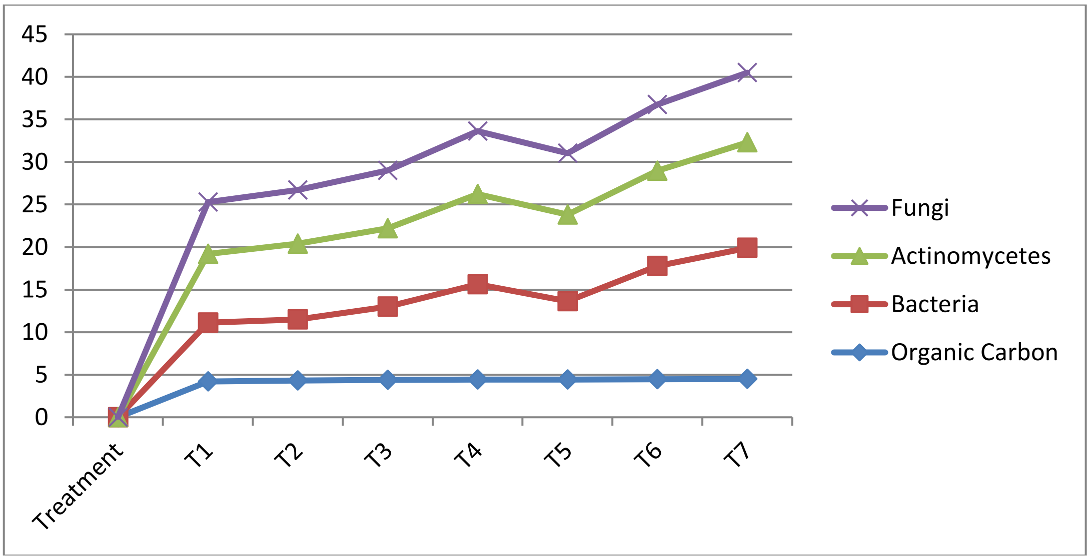

Fig. 1. Effect of bio-inoculants along with inorganic fertilizers on soil microbial population (bacteria, fungi, actinomycetes) and organic carbon

Table 2. Effect of bio inoculants along with inorganic fertilizer on soil dehydrogenase and soil microbial biomass carbon

Number	Treatments	Microbial Biomass Carbon (µg SMBC g^{-1} soil)	Soil Dehydrogenase (µg TPF g $soil^{-1} day^{-1}$)
T_1	Control	184.3	79.40
T_2	100% RDF (150, 60, 40 N, P_2O_5, K_2O kg ha^{-1})	186.00	86.32
T_3	80% RDF + 10 kg BGAha^{-1}Soil application + PSB	192.06	118.86
T_4	60% RDF + 10kg BGA ha^{-1} Soil application + PSB	198.03	132.45
T_5	80% RDF + 500kg Azolla ha^{-1} Soil application +PSB	196.32	123.45
T_6	60% RDF + 500kg Azolla ha^{-1} Soil application +PSB	205.00	137.24
T_7	10kg BGA ha^{-1} Soil application + 500kg Azolla ha^{-1} Soil application + PSB	215.02	156.45
	C.D. (p=0.05)	9.96	6.11
	SE(m) ±	3.19	1.96

Table 3. Effect of bio-inoculants along with inorganic fertilizers on alkaline phosphatase

Number	Treatments	Alkaline Phosphatase (µg p-nitrophenol g $soil^{-1}$ h^{-1})
T_1	Control	137.3
T_2	100% RDF (150, 60, 40 N, P_2O_5, K_2O kg ha^{-1})	129.56
T_3	80% RDF + 10 kg BGAha^{-1}Soil application + PSB	146.47
T_4	60% RDF + 10kg BGA ha^{-1} Soil application + PSB	155.84
T_5	80% RDF + 500kg Azolla ha^{-1} Soil application +PSB	149.21
T_6	60% RDF + 500kg Azolla ha^{-1} Soil application +PSB	159.54
T_7	10kg BGA ha^{-1} Soil application + 500kg Azolla ha^{-1} Soil application + PSB	167.32
	C.D. (p=0.05)	3.57
	SE(m) ±	1.14

3.3 Alkaline Phosphatase Activity

Results indicated that biofertilizer treatment with BGA, Azolla, and PSB along with variable percent of RDF has significantly influenced the soil alkaline phosphatase activity. Minimum enzyme activity, 137.3 µg p-nitrophenol g $soil^{-1}$ h^{-1}, was recorded in control (T_1), while biofertilizer treatments tended to have higher activities in soil enzymes. The highest activity, namely 167.32 µg, was recorded in T_7 with the application of only BGA and Azolla without RDF, indicating that the application of biofertilizers alone can lead to increased soil enzyme activity. Even the lower doses of RDF application with biofertilizers have shown higher activity, viz., T_6 with 159.54 µg and T_4 with 155.84 µg, respectively, than the full RDF treatment of T2, with 129.56 µg. The C.D. While the mean difference was 3.57 with SE(m) ± 1.14, it showed that biofertilizers are efficient in improving soil health and microbial activity under reduced levels of chemical fertilizers, these result is also supported by Li, J. Xie et al. *(*2021).

4. CONCLUSION

The comprehensive study presented a compelling case for the positive impact of sustainable agricultural practices on soil health and microbial populations. The research demonstrated that the incorporation of organic amendments, such as Azolla, along with the introduction of beneficial microorganisms like blue-green algae (BGA) and phosphate-solubilizing bacteria (PSB), significantly enhanced soil properties. Notably, the highest organic carbon content was observed in Treatment T7, which combined Azolla, BGA, and PSB, showcasing the potential of these combined practices to improve soil organic matter. Furthermore, the study revealed substantial increases in bacterial, actinomycetes, and fungal populations in treatments that integrated organic amendments and beneficial microbes, emphasizing the importance of microbial diversity in nutrient cycling and organic matter decomposition. These findings underscore the importance of adopting eco-friendly agricultural strategies that reduce reliance on synthetic fertilizers while promoting soil biodiversity and fertility.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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